UNITED NATIONS

EP

UNEP/MED WG.509/24

Mediterranean Action Plan
Barcelona Convention

30 August 2021
Original: English

Meeting of the MED POL Focal Points

Teleconference, 27-28 May and 6-7 October 2021

Agenda item 12: Harmonization and standardization of IMAP Pollution Cluster Monitoring

Monitoring Guidelines/Protocols for Sample Preparation and Analysis of Marine Biota for IMAP Common Indicator 17: Heavy and Trace Elements and Organic Contaminants

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UNEP/MAP
Athens, 2021

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Annex XV: Background Assessment Criteria recommended to be used to assess concentrations in Mediterranean sediments, mussel (Mytilus galloprovincialis) and fish (Mullus barbatus) (UNEP(DEPI)/MED WG.444/12, 6th Meeting of the Ecosystem Approach Coordination Group 2017) (5.2.6);
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Note by the Secretariat

In line with the Programme of Work 2020-2021 adopted by COP21 the MED POL Programme has prepared the Monitoring Guidelines related to IMAP Common Indicators 13, 14, 17 and 20 for consideration of the Integrated Meeting of the Ecosystem Approach Correspondence Groups on Monitoring (December 2020), whilst the Monitoring Guidelines for Common Indicator 18, along with the Monitoring Guidelines related to data quality assurance and reporting are under finalization for consideration of the Meeting on CorMon on Pollution Monitoring planned to be held in April 2021.

These Monitoring Guidelines present coherent manuals to guide technical personnel of IMAP competent laboratories of the Contracting Parties for the implementation of the standardized and harmonized monitoring practices related to a specific IMAP Common Indicator (i.e. sampling, sample preservation and transportation, sample preparation and analysis, along with quality assurance and reporting of monitoring data). For the first time, these guidelines present a summary of the best available known practices employed in marine monitoring by bringing integrated comprehensive analytical practices that can be applied in order to ensure the representativeness and accuracy of the analytical results needed for generation of quality assured monitoring data.

The Monitoring Guidelines/Protocols build upon the knowledge and practices obtained over 40 years of MED POL monitoring implementation and recent publications, highlighting the current practices of the Contracting Parties' marine laboratories, as well as other Regional Seas Conventions and the EU. A thorough analysis of presently available practices of UNEP/MAP, UNEP and IAEA, as well the HELCOM, OSPAR and European Commission Joint Research Centre was undertaken to assist an innovative approach for preparation of the IMAP Monitoring Guidelines/Protocols.

The Monitoring Guidelines/Protocols also address the problems identified during realization of the Proficiency testing being organized by UNEP/MAP-MEDPOL and IAEA for two decades now, given that many unsatisfactory results within inter-laboratory testing may be connected to inadequate laboratory practices of the IMAP/MEDPOL competent laboratories.

In order to support national efforts, this Monitoring Guidelines provides two Technical Note for sample preparation and analysis of marine Biota for IMAP Common Indicator 17: a) Technical Note for the analysis of biota samples for heavy metals, which includes the following Protocols: i) Protocol for biota tissues digestion using nitric acid (microwave assisted digestion in closed systems and digestion on hot plate); ii) Protocol for the analysis of heavy metals with Flame Atomic Absorption Spectroscopy (F-AAS); iii) Protocol for the analysis of heavy metals with Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS); iv) Protocol for the analysis of heavy metals with Inductive Coupled Plasma – Mass Spectroscopy (ICP-MS); v) Protocol for the analysis of Total Hg with thermal decomposition, amalgamation and Atomic Absorption Spectroscopy (AAS); vi) Protocol for the analysis of Total Hg with Cold Vapour Atomic Absorption Spectrometry (CV-AAS); b)Technical Note for the analysis of biota samples for organic contaminants, which includes the following Protocols: i) Protocol for the analysis of organochlorine pesticides and PCBs in biota using Gas Chromatography-Electron Capture Detector (GC-ECD); ii) Protocol for the analysis of organochlorine pesticides and PCBs in biota using Gas Chromatography – Mass Spectrometry (GC-MS); iii) Protocol for the analysis of PAHs in biota using High Performance Liquid Chromatography – Fluorescence (HPLC-UVF); iv) Protocol for the analysis of PAHs in biota using Gas Chromatography – Mass Spectrometry (GC-MS); v) Protocol for the normalization or organic contaminants concentrations using the lipid content.

The Monitoring Guidelines/Protocols, including this one related to sample preparation and analysis of marine biota for IMAP Common Indicator 17 establish a sound ground for further regular update of monitoring practice for the purpose of successful IMAP implementation.

In accordance with the Conclusions and Recommendations of the Integrated Meetings of the Ecosystem Approach Correspondence Groups on IMAP Implementation (CORMONs) (Videoconference, 1-3 Dec. 2020), and in particular paragraph 22, this Meeting requested the

Secretariat to amend this Monitoring Guideline by addressing agreed technical proposals that were described in the Report of the Meeting in line with its agreement to proceed with submission of this document to the Meeting of MEDPOL Focal Points. Requested amendments included technical written suggestions that were provided by several Contracting Parties up to 10 days after the Integrated Meeting of CORMONs. The amended document was shared by the Secretariat on 19 February 2021 for a period of 2 weeks for the non-objection by the Integrated Meetings of CORMONs on the introduced changes. Further to no objection from the Integrated Meeting of CORMONs, this Monitoring Guideline is submitted for consideration of present Meeting of MEDPOL Focal Points.

List of Abbreviations / Acronyms

CI	Common Indicator
COP	Conference of the Parties
CORMON	Correspondence Group on Monitoring
EcAp	Ecosystem Approach
EEA	European Environmental Agency
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organization of the United Nation
HELCOM	Baltic Marine Environment Protection Commission - Helsinki Commission
IAEA	International Atomic Energy Agency
IOC	International Oceanographic Commission
IMAP	Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria
MAP	Mediterranean Action Plan
MED POL	Programme for the Assessment and Control of Marine Pollution in the Mediterranean Sea
MED QSR	Mediterranean Quality Status Report
OECD	Organisation for Economic Co-operation and Development
OSPAR	Convention for the Protection of the Marine Environment for the North-East Atlantic
PoW	Programme of Work
QA/QC	Quality Assurance/Quality Control
QSR	Quality Status Report
US EPA	United States Environmental Protection Agency

1. Introduction

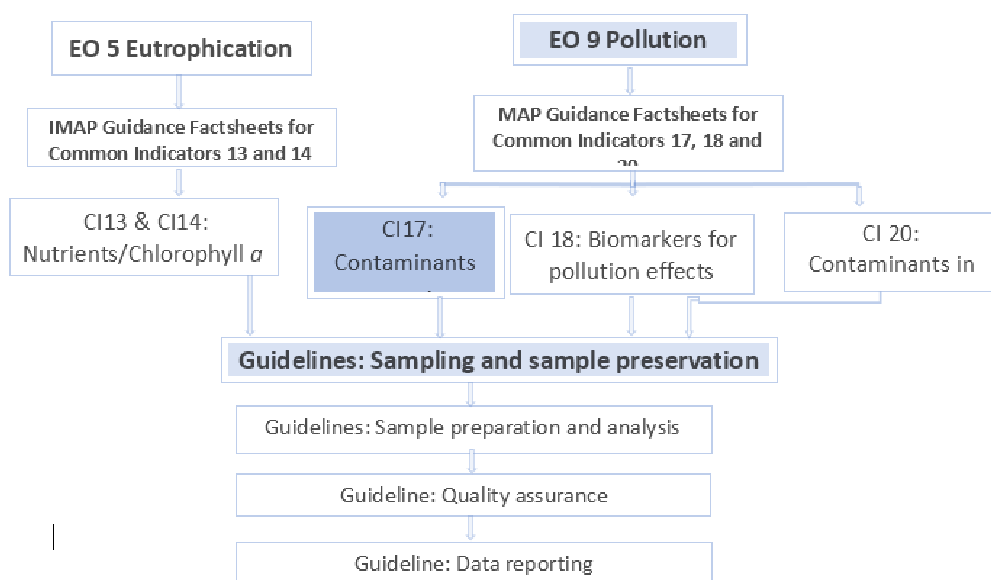
1. Marine biota uptake contaminants from the marine environment through food and the water medium (breathing, skin exchange). Depending on their physicochemical properties and the organism's metabolism, contaminants may be bioaccumulated in the organism's body and, in some cases (such as Hg and persistent organic pollutants), they may be biomagnified in the top levels of the marine food chain. Since the establishment of the UNEP/MAP – MED POL Monitoring programme in 1981 (MED POL Phase II), the benthic fish *Mullus barbatus* and the bivalve *Mytilus galloprovincialis* have been used as sentinel species to assess the accumulation of contaminants in marine organisms of the Mediterranean Sea. In the framework of the Integrated Monitoring and Assessment Programme (IMAP) Common Indicator 17 (CI17), the same organisms are recommended for analysis, namely the benthic fish *Mullus barbatus* (muscle tissue) and the bivalves *Mytilus galloprovincialis* or *Donnax trunculus* (whole body). Parties may decide to include in their national monitoring programmes the collection and analysis of additional species of national interest. In all cases, contaminants data, along with relevant metadata, have to be reported to the UNEP/MAP Secretariat using the appropriate format.
2. Both organisms are encountered in the coastal marine environment and are absent in the offshore marine areas. Therefore, they cannot be used as sentinel organisms to assess the impact of contaminants in the offshore marine environment. For such offshore areas, appropriate sentinel species to be used for pollution assessment, will be designated by Parties at a later stage.
3. In line with IMAP requirements (UNEP 2019a[1], UNEP 2019b[2]), mandatory contaminants to be analysed in the muscle of fish and the whole body of bivalves include: heavy metals (Cadmium (Cd), Lead (Pb) and Mercury (Hg)), organochlorinated compounds (PCBs, hexachlorobenzene, lindane and ΣDDTs) and Polycyclic Aromatic Hydrocarbons (US EPA 16 Reference PAHs compounds). Also, additional parameters should be measured, such as: length, sex, and total wet weight of organism, as well as lipid content of the tissue to be analysed.
4. Heavy metals and organic contaminants are encountered in marine biota at trace levels (ng/kg - mg/kg); therefore it is of paramount importance to avoid cross contamination from the laboratory environment (dust particles and the analyst), from sample containers or packing materials, from instruments used during sample pre-treatment and sample preparation, and from the chemical reagents used for analysis. Accordingly, sample handling and analysis should be made in a clean laboratory, to eliminate cross contamination and to control the total analytical blank. To that purpose, if the laboratory is not specifically designed as "clean lab" (class 100 US Federal Standard 209), it has to be equipped with appropriate laminar flow rooms, clean benches, and fume hoods, specifically designed for trace metal analysis.
5. The UNEP/MAP Proposed assessment criteria (Background Assessment Criteria - BAC and Environmental Assessment Criteria - EAC) for targeted heavy metals and organic contaminants in fish and bivalves are presented in the Annex XIV.
6. The Protocols on of this Guidelines, as provided here-below, aim at streamlining marine biota sample preparation and analysis for heavy metals and organic contaminants, including step-by-step guidance on the methods to be applied in the Mediterranean area for sample preparation and analysis of marine biota tissues for the determination of heavy metals and organic contaminants, in a view of assuring comparable quality assurance of the data, as well as comparability between sampling areas in different national monitoring programmes. They are not intended to be analytical training manuals, but guidelines for Mediterranean laboratories, which should be tested and modified in order to validate their final results.
7. In order to avoid unnecessary repetitions, reference is also made to the protocols already published and publicly accessible, which can also be used by the Contracting Parties' competent

[1] (UNEP 2019a). UNEP/MED WG.467/5. IMAP Guidance Factsheets: Update for Common Indicators 13, 14, 17, 18, 20 and 21: New proposal for candidate indicators 26 and 27;;

[2] UNEP (2019b). UNEP/MED WG.463/6. Monitoring Protocols for IMAP Common Indicators related to pollution; UNEP/MAP (2019)

laboratories participating in IMAP implementation. Namely, here-below elaborated IMAP Protocols build on previous UNEP/MAP - IAEA Recommended Methods, for the analysis of heavy metals and organic contaminants, such as: IAEA (2011a) IAEA (2011) Recommended method on microwave digestion of marine samples for the determination of trace element content (Annex I); IAEA (2011b) Recommended method for the determination of selected trace element in samples of marine origin by flame atomic absorption spectrometry (Annex III); IAEA (2011c) Recommended method for the determination of selected trace element in samples of marine origin by atomic absorption spectrometry using graphite furnace (Annex IV); IAEA (2012a) Recommended method on the determination of Total Mercury in marine samples by thermal decomposition, amalgamation and Atomic Absorption Spectrophotometry (Annex VI); IAEA (2012) Recommended method on the determination of Total Hg in samples or marine origin by Cold Vapour Atomic Absorption Spectrometry (Annex VII); UNEP/IAEA (2011d) Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment: Reference Methods for Marine Pollution Studies No 71 (Annex IX);, which were prepared in the framework of the MED POL monitoring programme. They are also streamlined with similar Guidelines/Protocols for marine biota sample preparation and analysis, which were developed by other Regional Seas Organisations, such as OSPAR (Annex XI and XIII) and HECLOM (Annexes II, VIII, X, XII). The analytical method developed by US EPA is also considered (Annex V). Given the suitability of any of these Guidelines in the context of IMAP, they can be further used by competent Mediterranean laboratories for developing their lab-specific sampling and sampling processing methodologies. The Parties' laboratories should accommodate and always test and modify each step of the procedures to validate their results.

8. The below flow diagram informs on the category of this Monitoring Guideline related to sample preparation and analysis of marine biota for IMAP Common Indicator 17 within the structure of all Monitoring Guidelines prepared for IMAP Common Indicators 13, 14, 17, 18 and 20.



Flow Diagram: Monitoring Guidelines for IMAP Ecological Objectives 5 and 9

2. Technical note for the analysis of biota samples for heavy metals

9. Analysis of marine biota samples for the determination of heavy metals include: i) digestion of biota tissues and ii) analysis of the digested sample for heavy metals.

10. National laboratories may decide to use any validated analytical method they consider appropriate, which meets specific performance criteria (LOD, LOQ, precision, recovery and specificity). However, in order to assist analytical laboratories of Mediterranean Parties, a non-

exclusive list of Protocols has been drafted to be used as guidelines for the analysis of heavy metals in marine biota samples. Analytical laboratories should accommodate, test and modify each step of the procedures presented in the Protocols in order to validate their final results. The list of methods and analytical equipment is not exhaustive, and laboratories are encouraged to use their own equipment/methods that consider adequate for the required analyses.

11. Regardless of the analytical method used, heavy metal analysis follows some procedures common to all analytical methodologies, such as the calibration of the analytical equipment and the cleaning and handling procedures to avoid the contamination of the samples from the laboratory's environment and the tools and containers used in the analysis.

a) Calibration

12. Calibration standards prepared from single standard stock solutions or multielement standards, by dilution of the stock solution using dilute acid, as required. All standard solutions have to be stored in polyethylene, borosilicate or quartz volumetric flasks, depending on the best suitability for the respective analytes. Standard solutions with lower concentrations, if prepared correctly and controlled in a QA system (checking of old versus new, and checking with standards from a different source), can be kept for a period no longer than one month.

13. The calibration procedure has to meet some basic criteria in order to give the best estimate of the true element concentration of the sample analysed (HELCOM, 2012a[3]):

i) The concentrations of standards for the preparation of the calibration curve should cover the range of anticipated concentrations;
ii) The required analytical precision should be known and achievable throughout the entire range of concentrations;
iii) The measured value at the lower end of the range has to be significantly different from the procedural analytical blank;
iv) The chemical and physical properties of the calibration standards must closely resemble those of the sample under investigation;
v) The analytical instruments should be recalibrated regularly (every 10-20 samples) to correct for instrumental drift and analytical efficiency.

b) Avoiding contamination

14. To avoid metal contamination in the laboratory all glassware and plastic vessels used should be carefully cleaned. The general cleaning guidelines include:

i) The vessels are allowed to soak overnight in a plastic container in an alkaline surfactant solution (e.g. Micro solution 2% in tap or even better distilled water).
ii) Vessels are rinsed thoroughly first with tap or even better distilled water then with ultrapure deionised water (18 MΩ cm,).
iii) Vessels are left to stand in 10% (v/v) concentrated HNO_3 solution (analytical grade) at room temperature for at least 6 days.
iv) Vessels are thoroughly rinsed with ultrapure deionised water (at least 4 times).
v) Vessels are allowed to dry under a laminar flow hood.
vi) Vessels are stored in closed plastic polyethylene bags (e.g. zip-lock variety) to prevent the risk of contamination prior to use.

15. This procedure should be used for all plastic ware use in the laboratory as tips, cup for auto-sampler, plastic containers.

16. Under this Technical Note, this Guideline sfor sample preparation and analysis of marine biota for IMAP Common Indicator 17 provides the following IMAP Protocols for the analysis of heavy metals in marine biota samples:

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[3] HELCOM (2012a). Manual for marine monitoring in the COMBINE programme. Annex B-12, Appendix 4: Technical note on the determination of trace metallic elements in biota.

- Protocol for biota tissues digestion using nitric acid (microwave assisted digestion in closed systems and digestion on hot plate);
- Protocol for the analysis of heavy metals with Flame Atomic Absorption Spectroscopy (F-AAS);
- Protocol for the analysis of heavy metals with Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS);
- Protocol for the analysis of heavy metals with Inductive Coupled Plasma – Mass Spectroscopy (ICP-MS);
- Protocol for the analysis of Total Hg by thermal decomposition, amalgamation and Atomic Absorption Spectrophotometry;
- Protocol for the analysis of Total Hg with Cold Vapour Atomic Absorption Spectrometry (CV-AAS).

17. These Protocols are based on Analytical Methods developed by IAEA (Annex I: IAEA (2011a). IAEA (2011) Recommended method on microwave digestion of marine samples for the determination of trace element content; Annex III: IAEA (2011b) Recommended method for the determination of selected trace element in samples of marine origin by flame atomic absorption spectrometry; Annex IV: IAEA (2011c) Recommended method for the determination of selected trace element in samples of marine origin by atomic absorption spectrometry using graphite furnace; Annex VI: IAEA (2012a) Recommended method on the determination of Total Mercury in marine samples by thermal decomposition, amalgamation and Atomic Absorption Spectrophotometry (AAS); Annex VII: Recommended method on the determination of Total Hg in samples or marine origin by Cold Vapour Atomic Absorption Spectrometry), HELCOM (Annex II: Manual for marine monitoring in the COMBINE programme: Technical note on the determination of trace metallic elements in biota; Annex VIII: COMBINE Programme: Technical note on the determination of Total Mercury in marine biota by Cold Vapour Atomic Absorption Spectroscopy) and US EPA (Annex V: US-EPA Method 200.8: Determination of trace elements in waters and wastes by inductively coupled plasma-mass spectrometry).

2.1. Protocol for biota tissues digestion using nitric acid

18. Biota tissues samples have to be digested (wet ashing) prior to analysis. The rate of digestion and the efficiency of acid decomposition increase substantially with elevated temperatures and pressure, therefore microwave digestion in closed vessels is the preferred method. However, in case no such equipment is available, sample digestion in open vessels over a hot plate is an alternative method. Biota samples can be digested in wet or dried condition, however regardless of the method applied, it is of paramount importance to secure the complete destruction of all organic material of the sample, as well as to avoid metals losses and the contamination of the sample (HELCOM, 2012a).

19. The existence of residual dissolved organic carbon compounds in the digested sample would change the viscosity of the solution and therefore may lead to erroneous results when calibration of the AAS instrument is made using aquatic calibration standard solutions. Also, in the GF-AAS, residual organic carbon may undergo secondary reactions with the analyte prior to or during the atomization process causing matrix interferences (Harms, 1985[4]).

a) Microwave acid digestion in closed systems (for heavy metals analysis with AAS, GFAAS and ICP-MS analysis)

20. Biota tissue digestion can be performed in Teflon, or equal quality vessels of pure material, which are metal free and resistant to strong acids, therefore loss of elements through volatilisation and contamination by desorption of impurities from the vessel surface are significantly reduced. Also, since only small quantities of high-purity nitric acid are used, extremely low analytical blanks can be obtained. Microwave systems enable a very fast energy transfer to the sample and a very rapid build-

[4] Harms, U. 1985. Possibilities of improving the determination of extremely low lead concentrations in marine fish by graphite furnace atomic absorption spectrometry. Fresenius Journal of Analytical Chemistry, 322: 53-56.

up of high internal vessel temperature and pressure, with the advantage of an enormous reduction in digestion time occurs (HELCOM, 2012a)

Digestion reagents for the analysis of Cd, Pb and other heavy metals analysis

21. The following reagents are required :
- i) HNO_3 (65%, e.g., Suprapur);
- ii) H_2O_2 (analytical grade) to be kept in the fridge after opening;
- iii) Ultrapure deionised water (> 18MΩ cm, e.g. Millipore).

22. Dried biota tissue samples (approximately 0.2. g) are weighted in the microwave vessel and placed in a laminar hood compatible with acid fume. Approximately 5 ml of nitric acid (HNO_3) are added and each vessel and left to react for at least 1hour (or more if possible). After the room temperature pre-digestion, 2ml of hydrogen peroxide (H_2O_2) are added carefully, the vessels are closed and placed in the microwave apparatus and digestion steps are followed. Detailed methods for biota tissues microwave digestion with strong acids are presented in Annex I ((IAEA 2011a[5])and Annex II (HELCOM 2012a).

23. All chemicals used in the analysis should be kept extremely clean once opened. Double bagged and only to be opened in a clean bench or clean room. It is also strongly advised not to use any pipettes or other devices to take out chemicals from the main container, but to subsample the chemicals into pre-cleaned containers for daily use. This is paramount to avoid contamination of the very expensive ultra clean chemicals needed for this analysis.

Digestion reagents for Mercury analysis

24. For Mercury analysis the following reagents are required:
- i) HNO_3 (65%, analytical grade, certified low in mercury);
- ii) Ultrapure deionised water (> 18MΩ cm,);
- iii) 10% $K_2Cr_2O_7$ (w/v) solution (e.g. 10 g $K_2Cr_2O_7$ analytical grade diluted into 100 ml with ultrapure deionised water).
- iv) V_2O_5 analytical grade

25. Dried biota tissue samples (approximately 0.2. to 1.5 g depending of the expected concentration) are weighted in the microwave vessel and placed in a laminar hood compatible with acid fume. If processing high weight of bivalve (> 1g), add 40 mg of V_2O_5 to each tube (including blanks). Five ml of concentrated nitric acid (HNO_3) are added and let to react for at least 1hour. If large amount of sample is used more acid should be added until the mixture becomes liquid. To control the performance of the digestion procedure, at least 2 blanks should be prepared in a similar manner as the samples for each batch of analysis. Also at least one Certified Reference Material should be used and prepared in duplicate for each digestion batch. These digestions are prepared in a similar manner as the samples. A reference material of similar composition and concentration range should be used. After digestion, the vessels are removed from the microwave apparatus and placed in a ventilated fume hood to cool. When the pressure is adequate, the vessels are opened 1 ml of $K_2Cr_2O_7$ solution is added (final concentration should be 2% v/v) and their content is transferred to a volumetric flask, preferably of Teflon, but glass is also good, and made to a known volume.

b) Acid digestion in open systems

26. In case no microwave digestion system is available, it is possible to perform a digestion over a programmable heating plate placed inside a specially designed fume hood, allowing acid treatment. However, for the complete destruction of the organic matter, large quantities of reagents and voluminous apparatus with large surfaces are usually needed and the method is subject to

[5] IAEA (2011a). Recommended method on microwave digestion of marine samples for the determination of trace element content (IAEA/Marine Environmental Studies Laboratory in co-operation with UNEP/MAP MED POL)

contamination problems (too high blank values) if insufficiently purified acids are used. Also, the rate of reaction and efficiency of acid decomposition in open vessels is lower than in closed vessels under pressure. Therefore, digestion over a hot plate is not a recommended method and should be avoided if possible.

27. Dried biota tissue samples (approximately 0.2. g) are weighted in the microwave vessel and placed in a laminar flow hood compatible with acid fume. Approximately 5 ml of concentrated nitric acid (HNO_3) are added to each vessel and let to react at room temperature for at least 1 hour. The tubes are closed and placed in an aluminium block on a hot plate at 90°C for 3hrs. The samples are allowed to cool to room temperature, and the tubes are opened carefully, and the samples are transferred to the labelled 50 ml polypropylene graduated tubes or volumetric flasks.

28. A method for biota tissues digestion in open systems, using aqua regia, HNO_3 / $HClO_4$ can be found in Black et al, (2013[6]).

2.2. Protocol for the analysis of heavy metals with Flame AAS

29. Flame Atomic Absorption Spectroscopy (F-AAS) has adequate sensitivity for the determination of a wide range of metals in marine biota tissues. The sample solution is aspirated into a flame and atomized. In case of flame-AAS, a light beam is directed through the flame, into a monochromator, and onto a detector that measures the amount of light absorbed by the element in the flame. Each metal has its own characteristic wavelength, so a source hollow cathode lamp composed of that element is used. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.

30. A detailed analytical protocol for the analysis of heavy metals in biota tissue samples prepared by IAEA (2011b[7]) is presented in the Annex III .

2.3. Protocol for the analysis of heavy metals with GF-AAS

31. In marine biota tissues Cd, Pb, as well as other heavy metals, can be determined by Graphite Furnace Atomic Absorption Spectroscopy (GF-AAS), which has adequate sensitivity for these determinations. For GF-AAS analysis, after the digestion of the biota sample, an aliquot of sample solution (10-50 µl) is introduced into a graphite tube of the GF-AAS and atomized by rapid heating at high temperature. A light beam is directed through the graphite tube, into a monochromator, and onto a detector that measures the amount of light absorbed by the atomized element in the tube. Each metal has its own characteristic wavelength, so a source hollow cathode lamp composed of that element is used. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.

32. The AAS software generally gives typical electrothermal programs for each element for 10 µl of sample in diluted HNO_3 (0.1%) and indications concerning maximum ashing and atomization temperatures. More specific information may also be found in the literature, such as recommendations regarding matrix modifiers and the use of partition tubes or tubes with platform. When a program is optimized for the determination of an element in a specific matrix, all information should be reported in the logbook of methods of the laboratory.

33. For some elements and some matrices, the results obtained are still not satisfactory (e.g. maximum ashing temperature is not sufficient to eliminate the background), this procedure should be redone with the addition of a matrix modifier. Different matrix modifiers could be tried before finding the best solution.

34. A detailed analytical protocol for the analysis of heavy metals in sediments by GF AAS prepared by IAEA (2011c[8]) is presented in the Annex IV. .

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[6] Black, K., Kalantzi, I., Karakassis, I., Papageorgiou, N., Pergantis, S., Shimmield, T. (2013). Heavy metals, trace elements and sediment geochemistry at four mediterranean fish farms, Science of the Total Environment. Elervier, 444, 128–137.

[7] IAEA (2011b) Recommended method for the determination of selected trace element in samples of marine origin by flame atomic absorption spectrometry

[8] IAEA (2011c) Recommended method for the determination of selected trace element in samples of marine origin by atomic absorption spectrometry using graphite furnace

2.4. Protocol for the analysis of heavy metals with ICP-MS

35. Inductive Coupled Plasma – Mass Spectroscopy (ICP-MS) is currently state-of-the-art instrumentation for metal analysis, with the possibility to determine at sub-µg L^{-1} concentrations of a large number of elements in acid digested biota tissue samples. ICP-MS allows a rapid analysis of a wide range of heavy metals. Most routine instruments utilize a quadrupole mass spectrometer, so mass resolution is not high enough to avoid overlap of double charged elements or multi-element ions (mainly hydrides, oxides and hydroxides) formed in the plasma. The main concern is for the Ar interferences as the plasma is usually an argon plasma, overlapping with As. Some elements are prone to memory effects (particularly Hg) and needs extra precautions to avoid carry over effects. Modern ICP-MS instruments software includes all the tuning and correction formulas needed and described above to perform the analysis (HELCOM 2012).

36. A multi-elemental determination of heavy metals by ICP-MS in water and solid samples after acid digestion, is described in the US EPA Method 200.8 (1994[9]). The method was initially intended for inorganic solid samples (soils and sediments) but can also be directly applied to organic samples. According to Enamorado-Baez et al. (2015[10]), for biota tissues the digestion step could use only nitric acid (similar to the US-EPA 3051 method established for sediments, sludge, soils, and oils) but increasing the sample mass to acid volume ratio.

37. Metal species originating in a liquid are nebulized and the resulting aerosol is transported by argon gas into the plasma torch. The ions produced by high temperatures are entrained in the plasma gas and introduced, by means of an interface, into a mass spectrometer. The ions produced in the plasma are sorted according to their mass-to-charge ratios and quantified with a channel electron multiplier. Interferences must be assessed, and valid corrections applied. Interference correction must include compensation for background ions contributed by the plasma gas, reagents, and constituents of the sample matrix. The US EPA Method 200.8 is presented in Annex V.

2.5. Protocol for the analysis of Total Mercury with by thermal decomposition, amalgamation and AAS

38. Total mercury in biological tissues can be analysed by solid Hg analyser, which has adequate sensitivity for this determination. A detailed method describing the protocol for the determination of total mercury (inorganic and organic) in sediment prepared by IAEA (2012a[11]) ("Recommended method on the determination of Total Mercury in marine samples by thermal decomposition, amalgamation and Atomic Absorption Spectrophotometry" Annex VI). With this method, Total Hg is determined without any chemical pre-treatment of the sample, minimising possible contamination and/or additional errors due to sample handling. The method is based on the US EPA 7473 method (US EPA, 2007[12]).

39. The sample is dried and then chemically decomposed under oxygen in the decomposition furnace. The decomposition products are carried out to the catalytic section of the furnace, where oxidation is completed (halogens and nitrogen/sulfur oxides are trapped). The mercury present in the remaining decomposition products is selectively trapped on an amalgamator. After flushing the system with oxygen, the mercury vapour is released by rapid heating of the amalgamator, and carried through the absorbance cell in the light path of a single wavelength atomic absorption spectrophotometer. The absorbance is measured at 253.7 nm as a function of mercury quantity (ng). The typical working range

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[9] US EPA (1994) US-EPA Method 200.8: Determination of trace elements in waters and wastes by inductively coupled plasma-mass spectrometry.

[10] Enamorado-Báez, S.M., Abril, JM and Gómez-Guzmán, JM (2013) Determination of 25 Trace Element Concentrations in Biological Reference Materials by ICP-MS following Different Microwave-Assisted Acid Digestion Methods Based on Scaling Masses of Digested Samples. Hindawi Publishing Corporation, ISRN Analytical Chemistry, Volume 2013, Article ID 851713, 14 pages. http://dx.doi.org/10.1155/2013/851713

[11] IAEA (2012a) Recommended method on the determination of Total Hg in marine samples by Thermal Decomposition Amalgamation and Atomic Absorption Spectrometry

[12] US EPA (2007). U.S. Environmental Protection Agency, EPA method 7473, Mercury in solids and solutions by thermal decomposition, amalgamation and atomic absorption spectrophotometry Rev 0. http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/7473.pdf

is 0.1–500 ng. The mercury vapour is carried through a long (first) and a short path length absorbance cell. The same quantity of mercury is measured twice with different sensitivity resulting in a dynamic range that spans four orders of magnitude. The typical detection limit is 0.01 ng of mercury.

2.6. Protocol for the analysis of Total Hg in samples of marine origin by CV-AAS

40. The **Cold Vapour Atomic Absorption Spectrometry** (CV-AAS) method is widely used for the determination of total mercury in biological tissues and it is simple, rapid and applicable to a large number of environmental samples. The inorganic mercury is reduced to its elemental form with stannous chloride. The cold mercury vapour is then passed through the quartz absorption cell of an AAS instrument where its concentration is measured. The light beam of Hg hallow cathode lamp is directed through the quartz cell, into a monochromator and onto a detector that measures the amount of light absorbed by the atomized vapour in the cell. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.
41. The typical working range is 0.25–100 ng mL^{-1} for direct injection of cold vapour, using "batch" system (IAEA, 2012b[13]). CV-AAS analysis can be performed manually using batch CV-AAS or automatically using flow injection (FIAS) techniques. FIAS is a very efficient approach for introducing and processing liquid samples in atomic absorption spectrometry, reduces sample and reagent consumption, and has a higher tolerance of interferences, lower determination limits and improved precision compared with conventional cold vapour techniques (HELCOM, 2012b[14]).
42. A recommended method describing the protocol for the determination of total mercury in biota prepared by IAEA (2012b) is presented in Annex VII. (Recommended method on the determination of Total Hg in samples or marine origin by Cold Vapour Atomic Absorption Spectrometry). A method for the determination of Total Hg in marine biota using CV-AAS is also proposed by HELCOM (2012b) (Annex VIII)

3. Technical note for the analysis of organic contaminants in marine biota

43. In line with IMAP requirements (UNEP/MAP, 2019; UNEP/MAP 2019a), the mandatory organic contaminants to be monitored in marine biota in the framework of the IMAP are: Organochlorinated compounds (PCBs [28, 31, 52, 101, 105, 118, 138, 153, 156, 180], Hexachlorobenzene, Lindane and ΣDDTs) and polycyclic aromatic hydrocarbons (US EPA 16 individual PAHs congeners – Acenaphene, Acenaphthylene, Anthracene, Benz(a)anthracene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Benzo(ghi)perylene, Chrysene, Dibenzo(a,h)anthracene, Fluoranthene, Indeno(1,2,3-cd)pyrene, Naphthalene, Phenanthrene, Pyrene). However, Contracting Parties to the Barcelona Convention may decide to include in their national monitoring programmes the analysis of additional heavy organic compounds according to their national priorities.
44. Analysis of marine biota samples for the determination of organic contaminants include: i) extraction; ii) concentration; iii) clean-up; iv) fractionation; and v) quantification of contaminants.
45. National laboratories may decide to use any validated analytical method they consider appropriate, which meets specific performance criteria (LOD, LOQ, precision, recovery and specificity). However, in order to assist analytical laboratories of the Contracting Parties, the IMAP Protocols have been drafted to be used as guidelines for the analysis of organic compounds in marine biota samples. Analytical laboratories should accommodate, test and modify each step of the procedures presented in the IMAP Protocols in order to validate their final results. The list of methods and analytical equipment is not exhaustive, and laboratories are encouraged to use their own equipment/methods that consider adequate for the required analyses.
46. Under this Technical note, this Guideline sfor sample preparation and analysis of marine biota for IMAP Common Indicator 17 provides the following five IMAP Protocols:

[13] IAEA (2012b). Recommended method on the determination of Total Hg in samples or marine origin by Cold Vapour Atomic Absorption Spectrometry.
[14] HELCOM (2012b). COMBINE Annex B-12, Appendix 4, Attachment 1. Technical note on the determination of Total Mercury in marine biota by Cold Vapour Atomic Absorption Spectroscopy.

- Protocol for the analysis of organochlorine pesticides and PCBs in biota using Gas Chromatography-Electron Capture Detector (GC-ECD);
- Protocol for the analysis of organochlorine pesticides and PCBs in biota using Gas Chromatography – Mass Spectrometry (GC-MS);
- Protocol for the analysis of PAHs in biota using High Performance Liquid Chromatography–Fluorescence (HPLC –UVF);
- Protocol for the analysis of PAHs in biota using Gas Chromatography – Mass Spectrometry GC-MS;
- Protocol for the normalization or organic contaminants concentrations using the lipid content **.**

47. These protocols are based on Analytical Methods developed by UNEP/IAEA (Annex IX: Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment. Reference Methods for Marine Pollution Studies No 71;), HELCOM (Annex X: Manual for marine monitoring COMBINE programme. Annex B-12, Appendix 3. Technical note on the determination of chlorinated biphenyls and organochlorine pesticides in biota; Annex XII: Manual for marine monitoring in the COMBINE programme. Annex B-12, Appendix 2. Technical Note on the determination of Polycyclic Aromatic Hydrocarbons in Biota) and ICES/OSPAR (Annex XI : CEMP Guidelines for monitoring contaminants in biota and sediments. Technical Annex 8: Determination of chlorobiphenyls in biota; Annex XIII: CEMP Guidelines for monitoring contaminants in biota and sediments. Technical Annex 3: Determination of parent and alkylated PAHs in biological materials).

3.1. Protocol for the analysis of organochlorine pesticides and PCBs in marine biota using GC-ECD

48. The analysis of PCBs and organochlorine pesticides (OCPs) in marine biota samples (fish muscle and bivalve whole body) involves extraction from the matrix with organic solvents, followed by clean-up and gas chromatographic separation with electron capture (GC-ECD) or mass spectrometric (GC-MS) detection. To minimize systematic errors due to insufficiently optimized gas chromatographic conditions, determinant losses (evaporation, unsatisfactory extraction yield), and/or contamination from laboratory ware, reagents and the laboratory environment, it is essential that the sources of systematic errors are identified and eliminated as far as possible (HELCOM, 2012c[15]).

49. For analysis, the samples are prepared for solvent extraction. To achieve a satisfactory recovery of the chlorinated hydrocarbons, samples are dried by either desiccation with anhydrous sodium sulphate or by freeze-drying. Lipids are then Soxhlet extracted from biota using hexane or petroleum ether. Following initial clean-up treatments (treatment of biota extracts with concentrated sulphuric acid to destroy some interfering lipids), extracts are fractionated using column chromatography.

50. All reagents, including the distilled water should be of analytical quality. Commercially available solvents like acetone, acetonitrile, dichloromethane, hexane and pentane are invariably contaminated with ECD-active substances; their concentrations vary from batch to batch and with supplier. Therefore, reagent quality should be checked by injection of 2 µl of a 100 ml batch of solvent, after concentration to 50 µl in a rotary evaporator. No peak in the GC-ECD chromatogram (90 - 250 °C) should be larger than that for 1pg of lindane. Otherwise, the solvent must be distilled.

51. The laboratory used for organic trace analysis must be a dedicated facility, isolated from other projects that could be sources of contamination. It must be properly constructed with fume hoods and benches with electric sockets that are safe for use with flammable solvents. The laboratory must have extractors and rotary evaporators cooling water to run the stills. In tropical regions and in dry climates, a refrigerated re-circulating system should be used to reduce temperatures to the required levels and/or to conserve water. Stainless steel or ceramic tiles make good non-contaminating surfaces. If necessary, benches can be coated with a hard epoxy resin and walls can be painted with epoxy paint. A sheet of aluminium foil on the workbench provides a surface which can be cleaned with solvent. A vented storage facility for solvents is essential. Benches must be fitted with frames to hold stills, extractors,

[15] HELCOM (2012c). Manual for marine monitoring in the COMBINE programme. Annex B-12, Appendix 3. Technical note on the determination of chlorinated biphenyls and organochlorine pesticides in biota.

etc. The emergency cut-off switch should be accessible from both inside and outside the laboratory. Firefighting equipment should be mounted in obvious places and laboratory personnel trained in their use.

52. Quantitative analysis with Electron Capture Detector (ECD) is performed by comparing the detector signal produced by the sample with that of defined standards. Due to incomplete separation, several co-eluting compounds can be present under a single detector signal, therefore, the shape and size of the signal have to be critically examined. The relative retention time and the signal size should be confirmed on columns with different polarity of their stationary phases, or by the use of multi-dimensional GC techniques. The GC should be calibrated before each batch of measurements. Since the ECD has a non-linear response curve, a multilevel calibration is strongly advised. For the purpose of determining recovery rates, an appropriate internal standard should be added to each sample at the beginning of the analytical procedure. The ideal internal standard is a PCB which is not present in the sample and which does not interfere with other PCBs (HELCOM, 2012c).

53. A step-by-step method for the determination of organochlorine pesticides and polychlorinated biphenyls in biological samples is prepared by UNEP/IAEA (2011d[16]) (Annex IX.), including the list of reagents, the solvents, standards and examples for the preparation of the stock, intermediate and working solutions. A method for the analysis of organochlorine pesticides and PCBs in biota tissues is also proposed by HELCOM (2012c) (Annex X) and OSPAR (2018a[17]) (Annex XI).

3.2. Protocol for the analysis of organochlorine pesticides and PCBs in marine biota using GC-MS

54. The analysis of PCBs and organochlorine pesticides (OCPs) in marine biota samples (fish muscle and bivalve whole body) involves extraction from the matrix with organic solvents, followed by clean-up and gas chromatographic separation with mass spectrometric (GC-MS) detection. For analysis, the samples are prepared for solvent extraction. To achieve a satisfactory recovery of the chlorinated hydrocarbons, samples are dried by either desiccation with anhydrous sodium sulphate or by freeze-drying. Lipids are then Soxhlet extracted from biota using hexane or petroleum ether. Following initial clean-up treatments (treatment of biota extracts with concentrated sulphuric acid to destroy some interfering lipids), extracts are fractionated using column chromatography (UNEP/IAEA. 2011d).

55. Quantitative analysis is performed by comparing the detector signal produced by the sample with that of defined standards, using a mass spectrometer (MS). Often, due to incomplete separation, several co-eluting compounds can be present under a single detector signal. Therefore, the shape and size of the signal have to be critically examined. With a MS detector, either the molecular mass or characteristic mass fragments should be recorded for that purpose. The GC should be calibrated before each batch of measurements. Since the MS has a non-linear response curve, a multilevel calibration is advised. For the purpose of determining recovery rates, an appropriate internal standard should be added to each sample at the beginning of the analytical procedure..

56. A method for extraction, concentration, clean up and fractionation for the determination of organochlorine pesticides and polychlorinated biphenyls in biological samples is prepared by UNEP/IAEA (2011d) (Annex VIII.), including the list of reagents, the solvents, standards and examples for the preparation of the stock, intermediate and working solutions. The analysis of PCBs and organochlorinated pesticides can be done by GC-ECD followed by confirmation using GC-MS. A method for the analysis of organochlorine pesticides and PCBs in biota tissues using GC-MS is also proposed by HELCOM (2012c) (Annex X) and ICES/OSPAR (2018a) (Annex XI).

3.3. Protocol for the analysis of PAHs in marine biota using HPLC-Fluorescence

57. PAHs emitted from combustion processes are predominantly parent (un-substituted) compounds, while PAHs from petroleum and its by-products contain a range of alkylated compounds

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[16] IAEA (2011d). Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment. Reference Methods for Marine Pollution Studies No 71

[17] ICES/OSPAR (2018a). CEMP Guidelines for monitoring contaminants in biota and sediments. Technical Annex 8. Determination of chlorobiphenyls in biota

in addition to the parent PAHs. HPLC has the capacity to determine parent PAHs but has not the required selectivity to be used for alkylated PAHs' determination. However, this is not a handicap for the analysis of the EPA 16 PAHs, which are parent compounds.

58. PAHs are lipophilic and so are concentrated in the lipids of an organism, therefore they have to be extracted with Soxhlet extraction, or alkaline digestion followed by liquid-liquid extraction with an organic solvent. For Soxhlet extraction, wet tissues should be dried by mixing with a chemical agent (e.g., anhydrous sodium sulphate). Non-polar solvents alone will not effectively extract all the PAHs from tissues when using Soxhlet extraction, and mixtures such as hexane/dichloromethane may be effective. Tissue extracts will always contain many compounds other than PAHs, and a clean-up is necessary to remove those compounds which may interfere with the subsequent analysis. In order to reduce the sample volume to 2 cm^3 solvents are evaporated using a rotary-film evaporator at low temperature (water bath temperature of 30 °C or lower) and under controlled pressure conditions, in order to prevent losses of the more volatile PAHs such as naphthalenes. Evaporation to dryness should be avoided. When reducing the sample to final volume, solvents can be removed by a stream of clean nitrogen gas. Solvents and adsorptive materials must all be checked for the presence of PAHs and other interfering compounds. If such compounds are found, then the solvents, reagents, and adsorptive materials must be purified or cleaned using appropriate methods (HELCOM, 2012d[18]).

59. If Soxhlet extraction was used residual lipids have to be removed before the analytical determination, with an additional clean-up stage, using column chromatography with silica and alumina.

60. Detailed guidelines for the determination of Petroleum Hydrocarbons in biological samples using HPLC are prepared by HELCOM (2012d) (Annex XII.) and ICES/OSPAR (2018b[19]) (Annex XIII).

3.4. Protocol for the analysis of PAHs in marine biota using GC-MS

61. GC-MS analytical method has the sufficient selectivity to determine the full range of PAHs including parent (unsubstituted) PAH compounds (combustion derived) and alkylated PAH compounds (petroleum spill derived). (ICES/OSPAR, 2018).

62. Samples are Soxhlet extracted using methanol. Tissue extracts will always contain many compounds other than PAHs, and a clean-up is necessary to remove those compounds which may interfere with the subsequent analysis. In order to reduce the sample volume to 2 ml solvents are evaporated using a rotary-film evaporator at low temperature (water bath temperature of 30 °C or lower) and under controlled pressure conditions, in order to prevent losses of the more volatile PAHs such as naphthalenes. Evaporation to dryness should be avoided. When reducing the sample to final volume, solvents can be removed by a stream of clean nitrogen gas. Solvents and adsorptive materials must all be checked for the presence of PAHs and other interfering compounds. If such compound are found, then the solvents, reagents, and adsorptive materials must be purified or cleaned using appropriate methods.

63. If Soxhlet extraction was used, residual lipids have to be removed before the analytical determination, with an additional clean-up stage, using column chromatography with silica and alumina.

64. Quantification is done by GC-MS. The two injection modes commonly used are splitless and on-column injection. Automatic sample injection should be used wherever possible to improve the reproducibility of injection and the precision of the overall method. If splitless injection is used, the liner should be of sufficient capacity to contain the injected solvent volume after evaporation. For PAH analysis, the cleanliness of the liner is also very important if adsorption effects and discrimination are to be avoided, and the analytical column should not contain active sites to which PAHs can be adsorbed. (HELCOM, 2012d).

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[18] HELCOM (2012d). Manual for marine monitoring in the COMBINE programme. Annxe B-12, Appendix 2. Technical Note on the determination of Polycyclic Aromatic Hydrocarbons in Biota.

[19] ICES/OSPAR (2018b). CEMP Guidelines for monitoring contaminants in biota and sediments. Technical Annex 3. Determination of parent and alkylated PAHs in biological materials.

65. Detailed methods for the determination of PAHs in biological samples using GC-MS are proposed by HELCOM (2012d) (Annex XII) and ICES/OSPAR (2018b) (Annex XIII.).

3.5. Protocol for the normalization of organic contaminants concentrations using the lipid content

66. Normalisation to the total lipid content of marine biota is a means to reduce the variability of pollution level. For organic contaminants that accumulate through hydrophobic partitioning into the lipids of organisms, measured concentrations of contaminants in biota can be normalised to fish with a lipid content of 5% (European Commission 2014[20]). This default lipid content of 5% has been incorporated in the OECD (1996[21]) 305 Guideline for bioconcentration to ensure comparability between results of bioconcentration tests. The rationale behind this lipid normalisation is that the whole body biota concentration is linearly correlated with the lipid content of the species (EC 2014). Other taxonomic groups, such as bivalves, have different lipid contents than fish. For marine bivalves a lipid content of approximately 1% is proposed (European Food Safety Authority, 2009[22]).

67. There is evidence that using lipid contents for normalization purpose may not always be appropriate, because it requires a linear correlation between the concentration of contaminant and the lipid content, which may not be the case for PAHs (León et al., 2013[23]). Normalization can be useful in specific areas with similar oceanographic conditions and/or for contaminants with a predominant diffuse input in the marine environment (such as PCBs), but not to compare areas subjected to different exposition to pollutants, food availability. Therefore, normalization to lipid content is not a mandatory parameter to be reported in the framework of IMAP, but is to the Contracting Parties to decide if such an exercise is useful in facilitating pollution detection in specific areas. However it is useful to include normalization procedures in the Guidelines, making clear that the method should be tested before being applied, using sufficient data from the area under investigation.

68. In case it is decided to apply normalise measured data to lipid content, a detailed procedure is described in the EC Guidance No 32 (EC 2014) (Annex XIV.). In such cases, European Commission's suggests that contaminant concentrations are normalised to lipid contents of 5% in fish and 1% in bivalves, or to dry weight contents of 26% in fish and 8.3% in bivalves, on the basis of the measured lipid content or dry weight, or on the basis of generic values for lipid content or dry weight for the relevant species obtained from FishBase Global Information System on Fish[24]

69. To calculate the normalised concentrations *concnorm, lipid* or *concnorm, dry weight* from measured concentrations *concmeas* for a fish species x, the following equations can be used (lipid content and dry weight content expressed as mass fractions):

$$concnorm,\ lipid = concmeas \cdot 0.05/lipid\ content_x$$

or

$$concnorm,\ dry\ weight = concmeas \cdot 0.26/dry\ weight_x$$

70. Similarly, to calculate the normalised concentrations *concnorm, lipid* or *concnorm, dry weight* from measured concentrations *concmeas* for a bivalve species x, the following equations can be used (lipid content and dry weight content expressed as mass fractions):

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[20] European Commission (2014). Common implementation strategy for the Water Framework Directive (2000/60/EC). Guidance Document No. 32 on Biota Monitoring (the Implementation of EQSbiota) under the Water Framework Directive. Technical Report - 2014 – 083.

[21] OECD (1996). OECD Guidelines for Testing Chemicals: Proposal for Updating Guideline 305. Bioconcentration: Flow-Through Fish Test Paris 1996.

[22] EFSA. 2009 Guidance Document on Risk Assessment for Birds and Mammals. Parma, Italy: European Food Safety Authority. Authority EFS.358 pp.

[23] León V.M., Martínez-Gómez, C., García, I., Campillo, J.A, Benedicto J. (2013). Spatial distribution and temporal trends of polycyclic aromatic hydrocarbons in Mytilus galloprovincialis from the Iberian Mediterranean coast. Environmental Monitoring and Assessment, 185, 2, 1055-1070.

[24] FishBase: A global information system on fishes (www.fishbase.in)

$$concnorm, lipid = concmeas \cdot 0.01/lipid\ content_x$$

or

$$concnorm, dry\ weight = concmeas \cdot 0.083/dry\ weight_x$$

71. It is also noted that using the exact lipid or dry weight content of the biota samples is always preferred over generic values for the species (such as those available from FishBase).

72. The total lipid content of fish or bivalves can be determined using the method of Bligh and Dyer (1959[25]) using chloroform/methanol extraction techniques (OECD, 1996) or, alternatively the method proposed by Smedes (1999[26]), which has a comparable efficiency of extraction and high accuracy, but is using less toxic organic solvents (propan-2-ol–cyclohexane–water (8 + 10 + 11 v/v/v) mixture to avoid the use of chloroform).

[25] Bligh EG, Dyer WJ: A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959, 37:911-917.
[26] Smedes F (1999). Determination of total lipid using non-chlorinated solvents. Analyst, 124:1711-1718.

Annex I:

Recommended Method on

MICROWAVE DIGESTION OF MARINE SAMPLES

FOR THE DETERMINATION

OF TRACE ELEMENT CONTENT

REPORT

Recommended Method on
MICROWAVE DIGESTION OF MARINE SAMPLES FOR THE DETERMINATION OF TRACE ELEMENT CONTENT

IAEA/NAEL

Marine Environmental Studies Laboratory in co-operation with

UNEP/MAP MED POL

November 2011

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Recommended Method on

MICROWAVE DIGESTION OF MARINE SAMPLES
FOR THE DETERMINATION OF TRACE ELEMENT CONTENT

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NOTE: This method is not intended to be an analytical training manual. Therefore, the method is written with the assumption that it will be performed by formally trained analytical chemist. Several stages of this procedure are potentially hazardous, especially stages with HF; users should be familiar with the necessary safety precautions.

In addition, the IAEA's recommended methods are intended to be guidance methods that can be used by laboratories as a starting point for generating their own standard operating procedure. If performance data are included in the method, they shall not be used as absolute QC acceptance criteria.

1. SCOPE

The method here below describes the protocol for dissolution of samples from marine origin. Digests are suitable for analyses of total content of trace element in sediment and biological material.

The goal of this method is the total sample decomposition with the judicious choice of acid combinations this is achievable for most matrices. The selection of reagents which give the highest recoveries for the target analytes is considered the optimum method condition.

The recommended protocol is mainly based on the EPA 3052 method; users are encouraged to consult this document (EPA, 1996).

2. PRINCIPLE

The grinded and dried samples are solubilized in an acid mixture using microwave oven apparatus.

The use of hydrofluoric acid allows the decomposition of silicates by reaction of F with Si to form the volatile SiF4. The excess of hydrofluoric acid is either neutralized by boric acid, or digests are evaporated to dryness depending on the method used to analyze samples.

3. SAMPLE PRE-TREATMENT

Sediment samples are prepared following the recommendations of UNEP (2005).

Marine organisms are prepared following the recommendations of UNEP (1984, 1994).

4. REAGENTS

The reagents used shall meet the purity requirement of the subsequent analyses

4.1. ULTRAPUR WATER (type MilliQ).
4.2. NITRIC ACID 65%.
4.3. HYDROFLUORIC ACID.
4.4. HYDROCHLORIC ACID.
4.5. BORIC ACID.
4.6. HYDROGEN PEROXIDE.

5. MATERIAL

5.1. MICROWAVE APPARATUS

The microwave decomposition system should be temperature controlled. The temperature sensor should be accurate at ±2.5°C. The calibration of the temperature sensor should be done at least once a year, preferably by the maintenance service of the manufacturer.

The microwave unit should be corrosion resistant.

The unit cavity should be well ventilated and connected to fume cleaner or special neutralizing system.

The method requires microwave transparent and acid resistant material (i.e. PFA, TFM) to be used as reactor. The minimal volume of the vessels should be 45 ml and it should be able to work under the pressure of 800PSI. the reactor system should be equipped with a pressure relief system.

5.2. ANALYTICAL BALANCE with 0.001 g precision at least.
5.3. FUME HOOD.
5.4. LAMINAR FLOW HOOD.
5.5. VOLUMETRIC CONTAINERS of 50 ml or 100 ml in polypropylene.
5.6. WEIGHING CUP in polyethylene.
5.7. PLASTIC SPATULAS.

6. PROCEDURE

6.1. All PLASTIC MATERIAL (i.e. volumetric, weighing cup…) should be acid cleaned by soaking in laboratory soap (or 10% alcohol) for at least 24h, followed by 24h of soaking in 10% nitric acid. Stronger acid cleaning protocol could be applied depending on the requirement of the subsequent analyses.
6.2. MICROWAVE VESSELS should be at least cleaned after each use by running the same microwave program used for samples with 5 ml of HNO_3. If the risk of cross contamination is high (i.e. running sandy sediment after organic rich sediment) and/or in the case of long storage, the vessels should be cleaned twice. If available, an acid cleaner

(using acid vapors) can be used as a final cleaning stage. After cleaning, the vessels should be carefully rinsed with water and dried under a laminar flow hood. If a laminar flow hood is not available, vessels should be kept locked in double plastic bag; date of storage should be mentioned on the second bag.

6.3. Accurately weigh 0.1 to 0.5 g of well mixed sample in the microwave vessel.

6.4. In a fume hood, add 5 ml of nitric acid and 2 ml of hydrofluoric acid, close vessels with caps, then it is recommended to let samples react for at least 1 hour (or more if possible). Protect vessels by covering them with plastic bags or place them in a laminar flow hood compatible with acid fume. The quantity of hydrofluoric acid depends on the expected content of silicon dioxide, samples with low concentrations of silicon dioxide (< 10% like plant material to 0% like biological sample) may require less hydrofluoric acid (0.5 ml to 0 ml). Examples of acid quantities for different matrix are listed in table below.

	HF (ml)	HNO_3 (ml)	HCl (ml)	H_2O_2 (ml)	Boric (g)
Sediment	2	5	2 or 0	2	0.8
Fish	0	5	2 or 0	2	0
Sea plant	0.5	5	2 or 0	2	0

6.5. After room temperature pre-digestion, add 2 ml of hydrogen peroxide and close the reactors as recommended by the microwave manufacturer.

NOTE: The quantity and ratio of reagent can be adapted on a performance based judgment (i.e. visual total digestion, certified reference material results).

- In case of a sample containing high calcium carbonate, the hydrofluoric acid content can be set to 0 to avoid precipitation of insoluble CaF.
- A two stage digestion, using half of the hydrofluoric acid at the first stage and half at the second, could increase recovery and help achieving total decomposition.
- Additional reagent can be added depending on the sample composition to achieve complete dissolution. For example, 2±2 ml of HCl can be added to help the stabilization of As, Sb, Hg, Fe and Al at high level; however HCl might increase analytical difficulties for some techniques (i.e. ICP-MS) (Kingston 1997)
- Only one acid mixture or quantity should be used in a single batch, in the microwave, to insure consistent reaction conditions between all vessels and monitored conditions. This limitation is due to the current practice of monitoring a representative vessel, and applying a uniform microwave field to reproduce these reaction conditions within a group of vessels being simultaneously heated.

6.6. Place the closed reactor in the microwave apparatus, connect temperature and pressure control as specified by the manufacturer. The samples should be heated at 180°C

(minimum) in about 6 minutes and the temperature maintained for at least 10 minutes. The total decomposition is primarily controlled by maintaining samples at 180°C for 10 minutes. The ramping profile can be adapted, especially for safety purpose when very reactive samples are decomposed (i.e. biological material). In that case, it is recommended to increase the ramping time to 10 or 15 minutes. If possible, record temperature and pressure profile. In most samples matrices, pressure should peak between 5 and 15 minutes; profiles can be used to optimize temperature program.

6.7. At the end of the temperature profile, let the sample cool until the inside temperature goes down to 60°C, then remove the reactors from the microwave and place them in a ventilated fume hood. The pressure is carefully released following the manufacturer's instruction and reactors are opened.

6.8. In the case of removal of hydrofluoric acid excess with boric acid, 0.8 g of boric acid and 15 ml of water are added in the vessel. The quantity of boric acid is proportional to the quantity of hydrofluoric acid (usually 0.4 g for 1 ml should be sufficient). The vessels are closed again and run in the microwave with a program that heat samples at 170°C in 10 minutes and maintain this temperature for 10 minutes.

6.9. At the end of the temperature profile, let the sample cool until inside the temperature goes down to 60°C, then remove the reactors from the microwave and place them in a ventilated fume hood. The pressure is carefully released following the manufacturer's instruction and reactors are opened. Transfer the samples in a volumetric container and dilute them to a known volume (or a known weight, this requires to record the tare of each container before).

NOTE: An excess of boric acid will produce cloudy solutions, this might cause problem with sample introduction system of ICP. The use of boric acid will prevent measurement of boron, and possible bias introduced should be carefully investigated.

- If the use of boric acid is not possible, or if it is necessary to reduce the concentration of acid in final solutions, digest can be evaporated to incipient dryness on a hot plate at about 140°C. This stage should be performed in a controlled environment to avoid contamination and acid vapour should be treated. Some microwave oven apparatus can perform evaporation. The residue is then diluted to a known volume in nitric or hydrochloric diluted solution (usually 2% v/v) depending on the subsequent analytical method used.
- In case of insoluble precipitate or residue some extra steps can be performed like the addition of 2 ml of perchloric acid to the solution before evaporation, but this requires doing the evaporation under a specific hood for safety reason. Another option is the addition of 2 ml of concentrated hydrochloric acid, evaporation to near dryness, addition of concentrated nitric acid, evaporation to near dryness and dilution in known volume in 2% nitric acid solution.

Most samples will be totally dissolved by this method with the judicious choice of the acid combinations. A few refractory sample matrix compounds, such as TiO2, alumina, and other oxides may not be totally dissolved, and in some cases may sequester target analyte elements.

7. QUALITY CONTROL

7.1. Each microwave batch should contain at the minimum one certified reference material of representative matrix.

7.2. A duplicate or triplicate sample should be processed on a routine basis. A duplicate sample should be processed with each analytical batch or every 10 samples. A duplicate sample should be prepared for each matrix type (i.e. sediment, sea plant, etc.).

7.3. A spiked sample should also be included whenever a new sample matrix is being analyzed, especially if no certified reference material is available for that matrix.

7.4. Blank samples should be prepared using the same reagents and quantities used in sample preparation, placed in vessels of the same type, and processed with the samples. Each microwave batch should contain at least two blank samples.

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Annex II:

HELCOM Manual for marine monitoring in the COMBINE programme

ANNEX B-12, APPENDIX 4: TECHNICAL NOTE ON THE DETERMINATION OF TRACE METALLIC ELEMENTS IN BIOTA

HELCOM Manual for marine monitoring in the COMBINE programme

ANNEX B-12, APPENDIX 4: TECHNICAL NOTE ON THE DETERMINATION OF TRACE METALLIC ELEMENTS IN BIOTA

1. INTRODUCTION

Metallic elements appear in different marine biological matrices in trace concentrations, ranging from the mg/kg through the ƒÊg/kg to the ng/kg level. Stoeppler (1991) provided a comprehensive review of the most frequently used techniques for quantitative analysis of metallic trace elements, such as optical atomic absorption, fluorescence or emission spectrometry, anodic, cathodic or adsorptive stripping voltammetry, isotope dilution mass spectrometry and total reflection X-ray fluorescence, respectively. In spite of the powerful instrumental techniques presently in use, various analytical error sources have to be taken into consideration that may significantly influence the accuracy of the analytical data.

2. WORKING CONDITIONS

For each step of the analytical procedure, contamination of the sample may occur from the environment (laboratory air dust particles and the analyst), from sample containers or packing materials, from instruments used during sample pre-treatment and sample preparation, and from the chemical reagents used for analysis. The predominant purpose of the analytical clean laboratory is to eliminate contamination, which may be airborne or laboratory-induced, as far as possible and to control the total analytical blank.
Contamination by particles from the laboratory air may be controlled by a high-efficiency particulate filter. (A clean room is designed to maintain air with 100 particles per ft3 or 3.6.103 per m3 of 0.5 ƒÊm particles (class 100 of U.S. Federal Standards 209), or better, preferably with a minimum of activity in the room.) U.S. Federal Standards 209 describes designs for complete laminar flow rooms, clean benches, and fume hoods, and contains information on design, testing, and maintenance of clean rooms, and should be considered an essential reference for those interested in a clean laboratory.
To control the analytical blank for analysis of metallic trace elements, one must not only maintain good laboratory air quality, but also select the appropriate composition and type of construction materials used to build the laboratory. Principally, contaminants must be effectively removed at the source to minimize their uncontrolled distribution in the analytical clean laboratory. Accordingly, the laboratory's walls should be cleaned easily and therefore painted with special metal-free wipe-resistant paints. Surfaces of working areas should be protected with, for example, disposable plastic (polyethylene, PTFE) foils. The floors should, for example, be covered with adhesive plastic mats. Details of the design that are essential for obtaining a working laboratory with low trace element blanks are described by Moody (1982), Mitchell (1982a), Boutron (1990), and Schmidt and Gerwinski (1994).

3. PREATREATMENT OF LABORATORY WARE AND REAGENTS, CONTAMINATION CONTROL

Chemically resistant materials, used in the production of high-quality laboratory ware appropriate for metallic trace element analysis, include low- and high-density polyethylene (LDPE and HDPE), polypropylene (PP), polytetrafluorethylene (PTFE), perfluoralkoxy (PFA), ethylenetetrafluorethylene (ETFE), tetrafluorethyleneper- fluorpropylene (FEP), borosilicate and quartz glass, respectively. With appropriate pretreatment and handling, these materials meet the requirements of purity necessary for the required analytical investigations. Cleaning procedures for plastic and glass laboratory ware were comprehensively dealt with by Moody and Lindstrom (1977), Tschopel et al. (1980), Kosta (1982) and Boutron (1990). Generally, immersion in diluted (10-25 % v/v) high-purity nitric acid at room temperature for a period of one to three days, followed by repeated rinsing with high-purity water, is recommended. Steaming in high-purity acids (predominantly nitric acid) is also very effective to remove impurities from container surfaces and condition them for subsequent analysis.

The materials mentioned above for the production of laboratory ware exhibit some adsorptive or exchange properties. Boundary-surface interactions can be important, particularly when very dilute analytical solutions are being handled, since uncontrollable losses through sorption of element ions can occur (Tschopel et al., 1980; Harms, 1985). Based on this information, it is imperative that volumetric flasks, reagent vessels, pipette tips, etc., for handling samples, sample solutions and low-level reference or analyte solutions must never be used for transferring or processing stock calibration solutions, analytes solutions or concentrated reagents. Considerable quantities of analytes may be adsorbed from such solutions by the respective container surfaces, residuals of which may be leached later when dilute sample or analyte solutions are handled.
The availability of high-purity reagents is a key condition for reliable investigations of metallic trace element concentrations. For many analytical problems, the level of a specific contaminant can adequately be controlled only by applying specific purification methods. The first order of priority in regard to high-purity reagents is a sufficient supply of high-purity water. Ion-exchange units are universally accepted as an effective means of removing dissolved ionic species from water. Since high-purity water is frequently used in metallic trace element analysis, equipment for sustainable production of high-purity water by high-purity mixed-bed ion exchange resins should be available. The next most important group of reagents are mineral acids. Contamination of the sample by residual concentrations of metallic trace elements in the acids used for dissolution or decomposition represents a major problem. Purification of the acids is essential to ensure acceptable blanks.
Isothermal (isopiestic) distillation can produce volatile acids (and ammonia) of medium concentration in high-purity form. For example, pure hydrochloric acid (and ammonia) can be generated by placing an open container of concentrated reagent-grade acid adjacent to a

container of high-purity water, within a closed system (such as a desiccator) at room temperature. Acid vapours are continuously transferred into the water until equilibrium is obtained. Purification by sub-boiling distillation is based on motionless evaporation of the liquid by infrared heating at the surface to prevent violent boiling. Different purification systems are described in detail by Matthinson (1972), Kuehner et al. (1972), Dabeka et al. (1976), Tschopel et al. (1980), Mitchell (1982b), Moody and Beary (1982), Moody et al. (1989), and Paulsen et al. (1989). Acids of extremely high purity are produced by multiple batchwise distillation of reagent-grade acids in a silica apparatus, which is placed in a laminar-flow hood.

4. SAMPLE PRETREATMENT

If the determinands are heterogeneously distributed in the sample material, it may be preferable to homogenize prior to taking subsamples for analysis. However, this procedural step is problematic, since uncontrollable contamination through the homogenizing tool may occur. Cryogenic homogenization at liquid nitrogen temperature and application of high-purity material such as quartz, PTFE, titanium or stainless steel for the construction of homogenizing devices may help to minimize contamination (Iyengar, 1976; Iyengar and Kasperek, 1977; Klussmann et al., 1985).

5. SAMPLE DECOMPOSITION

For accurate direct measurements of metallic trace element contents in biological matrices, appropriate calibration (reference) standards are lacking in most instances. Therefore, multi-stage, easy to calibrate methods are still necessary, which include decomposition procedures and transformation of biological material into solution.
As a general rule wet sample is to be subject to decomposition procedures to avoid contamination or loss of determinands. A general sample decomposition procedure cannot be recommended due to the diverse composition of materials to be analysed, as well as to the different elements to be determined, and also because of the variety of possible analytical methods applied. However, the following minimum requirements should be met:

- complete destruction of all organic material of the sample,
- avoidance of determinand losses,
- avoidance of contamination.

Complete decomposition of the organic matrix is a prerequisite for a variety of the subsequently used instrumental determination techniques. Residual dissolved organic carbon from biological materials incompletely disintegrated after decomposition with nitric acid causes problems particularly in voltammetric and polarographic determinations. Both are sensitive to interference from chelating and electroactive organic components coexisting in incompletely decomposed samples during analysis (Pratt et al., 1988; Wurfels

et al., 1987, 1989). Residual dissolved organic carbon compounds even of low molecular weight can change the equilibria in the spray chambers for sample introduction in atomic emission spectrometry (AES), optical emission spectrometry (OES), and atomic absorption spectrophotometry (AAS) by changing the viscosity of the sample solution. In such cases, comparison with pure aquatic calibration standard solutions can lead to erroneous results. In graphite furnace atomic absorption spectrophotometry (GFAAS), residual organic carbon may undergo complicated secondary reactions with the analyte prior to or during the atomization process. Such 'matrix interferences' alter the rate at which atoms enter the optical path relative to that obtained for an undisturbed element standard (Harms, 1985; and other references cited there).

The comparatively simple dry ashing method using a muffle furnace is problematic, since both uncontrollable losses of the determinands and contamination through contact with the furnace material may occur.

Both, application of a carefully developed and controlled temperature programme and modifying the matrix prior to the ashing procedure (addition of ashing aids agents) may be suitable to prevent losses of volatile elements (special analytical problems concerning mercury determination are described in Attachment 1). The use of special materials (quartz, titanium, stainless steel) for the construction of sample containers may be helpful to minimise contamination.

In the widely applied wet ashing procedure in open systems, the sample is treated with acids, mainly nitric, sulphuric and perchloric acids, in different ratios and under different conditions. Usually large quantities of reagents and voluminous apparatus with large surfaces are needed for complete destruction of the organic material. Serious contamination problems (too high blank values) may arise, if insufficiently purified acids are used.

The rate of reaction and efficiency of acid decomposition increase substantially with elevated temperatures. Accordingly, closed-vessel techniques, using conventional heating or microwave energy, have an advantage over open systems. As a result of the closed systems with vessels manufactured of dense and very pure material (PTFE, PFA, quartz), loss of elements through volatilisation and contamination by desorption of impurities from the vessel surface are significantly reduced. In addition, since only small quantities of high-purity acid (usually nitric acid) need to be used, extremely low analytical blanks can be obtained. Kingston and Jassie (1986, 1988) comprehensively considered the fundamental parameters governing closed vessel acid decomposition at elevated temperatures using a microwave radiation field. Microwave systems enable a very fast energy transfer to the sample and a very rapid build up of high internal vessel temperature and pressure, with the advantage of an enormous reduction in digestion time occurs. Furthermore, a reduction of acid volume

(McCarthy and Ellis, 1991) and contamination reduction during the decomposition process were found (Dunemann, 1994; Sheppard et al., 1994).

The application of microwave energy must be carefully controlled to avoid explosions; a pressure-relief system is recommended for safe operation (Gilman and Grooms, 1988). At this stage of development, it can be concluded that advances in pressure and temperature feedback control features have contributed to the acceptance of microwave sample decomposition in analytical chemistry.

6. CALIBRATION

For calibration purposes, single element standard stock solutions at a concentration of 1000 mg/l, purchased from a qualified manufacturer, should be available. The actual concentration of the named element should be stated on the label together with the date of the preparation of the standard solution.

Fresh stock standard solutions should be compared with the old standard solutions. Traceability can be ensured by the use of CRM(s) or participation in intercomparison exercises (EURACHEM, 2003).

Single or mixed element working standard solutions for calibration purposes are prepared by dilution of the standard stock solutions using dilute acid, as required.
Both stock standard and working standard solutions are stored in polyethylene, borosilicate or quartz volumetric flasks. Working standard solutions at concentrations less than 100 ƒÊg/l should be freshly prepared for every batch of samples and kept no longer than two weeks.
The calibration procedure must meet some basic criteria in order to give the best estimate of the true (but unknown) element concentration of the sample analysed. These criteria are as follows:

- The amounts or concentrations of standards for the establishment of the calibration function must cover the range as related to practical conditions. The mean of the range should be roughly equal to the expected analyte concentration in the sample.
- The required analytical precision must be achievable and known throughout the entire range.
- The measured value (response) at the lower end of the range must be significantly different from the procedural analytical blank.
- The chemical and physical properties of the calibration standards must closely resemble those of the sample under investigation.
- The calibration standards must be processed through the entire analytical procedure in the same manner as the sample.

• The standard addition technique should be used only under very special circumstances (Cardone, 1986a, 1986b).

7. DETERMINATION

In an analytical series, especially with the number of samples >10, the control of calibration settings should be carried out with 2-3 calibration solution between environmental 10 samples. The analytical series should contain also a control sample of LRM or CRM.

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Annex III:

Recommended method for the determination of selected trace element in samples of marine origin by flame atomic absorption spectrometry

Recommended method for the determination of selected trace element in samples of marine origin by flame atomic absorption spectrometry

Marine Environmental Studies Laboratory in co-operation with MED POL

November 2011

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<u>NOTE:</u> This recommended method is not intended to be an analytical training manual. Therefore the method is written with the assumption that it will be performed by formally trained analytical chemists.

In addition the IAEA recommended methods are intended to be guidance methods that can be used by laboratories as a starting point for generating their own standard operating procedure. If performance data are included in the method they must not be used as absolute QC acceptance criteria,

The recommended protocol is mainly based on EPA 7000B method and ISO 11047 users are encouraged to consult this documents (US EPA, 2007; ISO 1998).

1. SCOPE:

This recommended method describes a protocol for measurement of Al, Ca, Co, Cr, Cu, Fe, Mg, Mn, Ni, Sr and Zn by flame (direct aspiration) atomic absorption spectrometry. The method is simple, rapid and applicable to a large number of environmental samples. This method is applicable when the element content in the digested solution is above the method limit. This limit will vary with the matrices and instrument model, indicative quantification limits are reported in table 1.

Table 1: Example of lower quantification limit for analyte in reagent water

Element	Lower quantification limit (mg l^{-1})
Al	0.5
Ca	0.02
Co	0.06
Cr	0.1
Cu	0.04
Fe	0.05
Mg	0.003
Mn	0.03
Ni	0.07
Sr	0.06
Zn	0.01

2. PRINCIPLE:

The method is based on the atomic absorption spectrometric measurement of the element in the mineralised solutions. In direct-aspiration atomic absorption spectrophotometry, the solution is aspirated and atomized in a flame. A light beam from a hollow cathode lamp or an electrodeless discharge lamp is directed through the flame into a monochromator, and onto a detector that measures the amount of absorbed light. Absorption depends upon the presence of free unexcited ground-state atoms in the flame. Because the wavelength of the light beam is characteristic of only the metal being determined, the light energy absorbed by the flame is a measure of the concentration of that metal in the sample. This principle is the basis of atomic absorption spectrophotometry.

3. SAMPLE PRE-TREATMENT:

Samples are prepared following the recommended method for microwave digestion of marine samples for determination of trace element content. (IAEA recommended method, 2011).

4. REAGENT:

All reagent used should be free of contamination of analyte of interest

4.1. Water: Reagent water (referenced also as water in the text) should be free of contamination

4.2. Caesium chloride solution, 4g l^{-1}: Dissolve 4g of CsCl of at least 99.999% purity in reagent water to 1 liter.

4.3. Caesium-Lanthanum solution: weigh 5.865g of La_2O_3 and 12.67g of CsCl in 100ml container, add 50ml of reagent water and 25ml of HCl and dilute to 100ml. Commercial solution specially produced for AAS may be used.

4.4. Commercial standard solution 1000µg ml^{-1}: Use a certified reference material solution; this solution should be accompanied by a certificate that should include at the minimum the traceability of the certified concentration as well as the expiration date. The density of the solution or the certified content in mg kg^{-1} should also be defined to allow preparation of the calibration solution by weighing.

5. MATERIAL:

This section does not list common laboratory glassware

5.1. Atomic absorption spectrophotometer: This shall be equipped with: a hollow cathode lamp or an electrode-less discharge lamp appropriate to the element of interest (operated at the current recommended for the lamp by the instrument manufacturer), a background correction system, a burner suitable for an air/acetylene or nitrous oxide/acetylene flame (operated following the manufacturer's instructions). Deuterium background correction is the minimum technical specification acceptable for background correction for the measurement wavelengths below 350 nm.

5.2. Glassware: All glassware, polypropylene, or fluorocarbon (PFA or TFM) containers, including sample bottles, flasks and pipets tips, should be washed in the following sequence -- 24h soaking in laboratory soap (or 10% alcohol) followed by 24h soaking in 10% nitric acid, followed by 10% soaking in water, final rinse in water, drying under laminar flow hood. Cleaned items should be kept in double sealed plastic bags.

If it can be documented through an active analytical quality control program using spiked samples and method blanks that certain steps in the cleaning procedure are not needed for routine samples, those steps may be eliminated from the procedure (i.e. For the levels measured by flame AAS some sterile plastic containers are sufficiently free of contamination in certain analytes.)

5.3. Pipettes: microliter pipettes size ranging from 50 to 10000µl as needed. The accuracy and precision of the pipettes used should be checked as a routine every 6 months and obtained results should be compared with the individual certificates.

5.4. Volumetric containers preferably in polypropylene of a suitable precision and accuracy

6. INTERFERENCES:

6.1. The most troublesome type of interference in atomic absorption spectrometry is usually termed “chemical” and is cause by lack of absorption of atoms bound in molecular combination in the flame. This phenomenon can occur when the flame is not sufficiently hot to dissociate the molecule. The addition of chemical buffer (i.e. Lanthanum or calcium) or the use of nitrous oxide/acetylene gas mixture will help to prevent this interference.

6.2. The presence of high dissolved solids in the sample may result in interference from non-atomic absorbance such as light scattering. In the absence of background correction, this can result in false positive, signal contribution from uncorrected background which cannot be compensated by the method of standard addition.

6.3. Ionisation interference occurs when the flame temperature is sufficiently high to generate the removal of an electron from a neutral atom, giving a positively charged ion. This type of interference can generally be controlled by the addition of a large excess (~1mg l-1) of an easily ionized element such as K or Cs.

6.4. Spectral interference can occur when an absorbing wavelength of an element present in the sample, but not being determined, falls within the width of the absorption line of the element of interest. This type of interference may sometimes be reduced by narrowing the slid width.

Specific conditions applied to individual anaytes in case of known interferences are displayed in table 2.

Table 2: Instrument parameter

Element	Wavelength (nm)	Flame type	Chemical buffer*	Background	Typical calibration range (mg l^{-1})
Al	324.7	acetylene/NO2	Caesium chloride	Deuterium	1.5-40
Ca	422.7	acetylene/NO2	Caesium Lanthanum		0.02-1.2
Co	240.7	acetylene/air		Deuterium	0.06-4
Cr	357.9	acetylene/NO2			0.3-6
Cr	357.9	acetylene/air	Caesium chloride		0.1-6
Cu	324.7	acetylene/air		Deuterium	0.04-3
Fe	248.3	acetylene/air	Caesium chloride	Deuterium	0.05-3
Mg	285.2	acetylene/air	Caesium Lanthanum	Deuterium	0.003-0.3
Mn	279.5	acetylene/air	Caesium Lanthanum	Deuterium	0.03-3
Ni	232.0	acetylene/air		Deuterium	0.07-4
Sr		acetylene/NO2	Caesium chloride		0.06-5
Zn	213.9	acetylene/air		Deuterium	0.01-1.5

* see 4.2, 4.3and 7.4 for use of chemical buffer

7. PROCEDURE:

7.1.Sample solution: Use sample prepared following the recommended method for digestion of marine samples for the determination of trace metal (IAEA, 2011)

7.2.Blank solution: Prepare at least two blank solutions with each batch of sample using same procedure than for samples

7.3.Preparation of calibration solutions:

7.3.1. Before each batch of determination prepare by appropriate dilution of 1000µg ml^{-1} stock standard solution (4.4) at least 4 standard solutions and one calibration blank solution covering the appropriate range of the linear part of the curve. The calibration standards and calibration blank should be prepared using the same type of acid or combination of acids and at the same concentration as will result in the test portion.

7.3.2. Calibration solutions should be prepared fresh each day.

7.3.3. If necessary intermediate stock standard solutions can be prepared in 10% nitric acid, these solutions should be prepared monthly.

7.3.4. All volumetric material (pipettes and containers) should be of appropriate precision and accuracy, if not available standard solution can be prepared by weighing.

7.3.5. Example of calibration curve are given in table 2.

7.4. Special case: Use of chemical buffer. If a chemical buffer is added, it should be at the same concentration as in the sample solution (7.1), the blank (7.2), calibration blank and standard solutions (7.3) following the recommendation of table 2.

For CsCl add 5ml of 4g l^{-1} for 50 ml of solution (4.2)

For CsLa solution add 0.5ml for 50ml of solution (4.3)

The chemical buffer will be added to a separate portion of sample and blank solutions that will need to be diluted to a known volume.

7.5. Calibration

7.5.1. Set up the atomic absorption spectrometer according to the manufacturer's instructions at the appropriate wavelength using appropriate conditions (see table 2), and with the suitable background correction system in operation.

7.5.2. Aspirate a calibration solution (7.3) and optimize the aspiration conditions, burner height and flame conditions to get the maximum signal.

7.5.3. Adjust the response of the instrument to zero absorbance whilst aspirating water

7.5.4. Aspirate the set of calibration solutions in ascending order and, as a zero member, the blank calibration solution (7.3).

NOTE: Care should be taken to ensure that, when using the more concentrated standards, the absorbance is < 1, and preferably not more than 0,6.
The calibration curve is automatically plot from instrument software. The obtained curve should be linear with $r<0.995$.
To correct for the instrumental drift the calibration should be performed every 20 samples or if the calibration verification has failed (7.8.1).

7.6. Aspirate blank (7.2) and sample solutions (7.1) and record their concentrations calculated by software using the calibration curve.

7.7. If the concentration of the test portion exceeds the calibration range dilute the test portion with the blank solution accordingly.

As an option to avoid too big dilution factors and/or to avoid a diluting large number of solutions, if all solutions are exceeding the calibration range, the burner can be turned from 0 to 90° to decrease the instrument's sensitivity. New calibration standard solutions should be prepared to match the sample range and the procedure should be repeated from (7.3).

7.8. Quality control solutions: Quality control solutions as describe below should be measured during the run.

7.8.1. **Initial Calibration Verification ICV**:

After initial calibration, the calibration curve must be verified by the use of initial calibration verification (ICV) standard.

The ICV standard is a standard solution made from an independent (second source) material at or near midrange. This solution as a calibration standard is prepared using the same type of acid or combination of acids and at the same concentration as will result in the test portion. If a chemical buffer is necessary it should be added in the ICV.

The acceptance criteria for the ICV standard must be ±10% of its true value

If the calibration curve cannot be verified within the specified limits, the cause must be determined and the instrument recalibrated before samples are analyzed. The analysis data for the ICV must be kept on file with the sample analysis

The calibration curve must also be verified at the end of each analysis batch and/or after every 10 samples. If the calibration cannot be verified within the specified limits, the sample analysis must be discontinued, the cause determined and the instrument recalibrated. All samples following the last acceptable test must be reanalyzed.

7.8.2. **Blank solution** (7.2): Maximum allowed blank concentration should be well documented and if blank solution exceeds this value all samples prepared along the contaminated blank should be prepared again and reanalysed.

7.8.3. **Post digestion spike**

Each unknown type of sample should be spike to check for potential matrix effect.

This spike is consider as a single point standard addition, and should be performed with a minimum dilution factor. The recovery of spike calculated as equation 1 should be 85-115%. If this test fails it is recommended to run analyses with standard addition method.

Spike solution: mix a fixe volume (V1) of sample solution, and a known volume (V2) of a standard solution of a known concentration (Cstandard)

Unspike solution: mix same fixe volume (V1) of sample solution, and same volume (V2) of reagent water

Measure concentration C (mg l^{-1}) in both solutions on the calibration curve (7.6), and calculate recovery as:

Equation 1 $$Cspike = \frac{Cstandard \times V2}{(V1+V2)}$$

Equation 2 $$R = \frac{C\ Spike\ Solution - C\ Unspike\ solution}{Cspike} \times 100$$

To be valid concentrations of spike and unspike solutions should be in the linearity range of the calibration curve and the Spike concentration (equation 1) should be in the range of 50-150% of the concentration of unspike solution.

7.8.4. **Dilution test**:

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the lower limit of quantitation after dilution), an analysis of a 1:5

dilution should agree within ±10% of the original determination. If not, then a chemical or physical interference effect should be suspected, and method of standard addition is recommended.

7.8.5. **Certified Reference Material**:

At least one certified reference material of a representative matrix should be prepared with each batch of sample, the calculated result should fall in the value of the certificate within the coverage uncertainty.(Linsinger, 2010), to show evidence of unbiased result.

Results of CRM should be recorded for quality control purpose and plot in control chart (UNEP/IOC/IAEA 1994).

An example of sequence order with recommended criteria and actions is given in table 3.

8. CALCULATION OF RESULTS:

Results are calculated with equation 3

Equation 3: $w(m) = \frac{(\rho 1 - \rho 0)}{m} \times f \times V \times R$

w(m) mass fraction of element m in the sample in mg kg^{-1}
ρ1: concentration of element m in milligrams per liter as measured in the sample solution
ρ0: concentration of element m in milligrams per liter as measured in the blank solution
f: is the dilution factor calculated as

$$f = \frac{final\ volume}{initial\ volume}$$

or equal to 1 if ρ1 is determined in undiluted solution
R: recovery calculated using CRM (see 7.8.5) or pre digestion spike

9. EXPRESSION OF RESULTS:

The rounding of values will depend of the uncertainty reported with the result; in general for this method no more than two significant figures will be reported.

Uncertainty component should be reported with all results. (ISO 2005, Nordtest 2004)

Example : w(Zn) = 8.5 ± 1.2 mg kg^{1}

Table 3: Example of an analytical sequence:

Solutions Description	Performance	Action
Calibration blank	< maximum allowed calibration blank value	Stop until resolve
Standard solution 1 -4	r>0.995	recalibrate in the linearity range
ICV	±10% of true value	Stop until resolve
Sample blank	< maximum allowed blank value	
CRM	Fall in certificate value within coverage uncertainty, or fall within acceptable criteria of the QC chart	Stop until resolve, check Matrix spike and run again with standard addition method if necessary
Matrix Spike	recovery 100% ± 15%	switch to standard addition, keep record for future analyses of the same matrix
Dilution Test	sample 1 = 5x sample 1 diluted 5x within 10%	switch to standard addition, keep record for future analyses of the same matrix
Unknown Sample 1-10	should ≥ standard 1 and ≤ standard 4	report as <minimum quantification limit or dilute
ICV	±10% of true value	Stop until resolve
Unknown Sample 11-20	should ≥ standard 1 and ≤ standard 4	report as <minimum quantification limit or dilute
Calibration blank	< maximum allowed calibration blank value	Stop until resolve
Standard solution 1 -4	r>0.995	recalibrate in the linearity range
ICV	±10% of true value	Stop until resolve
Ect….		

10. **REFERENCES:**

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Nordtest (2004) Handbook For Calculation Of Measurement Uncertainty In Environmental Laboratories Edition 2 http://www.nordicinnovation.net/nordtestfiler/tec537.pdf)

UNEP/IOC/IAEA (1994) reference method 57: Quality assurance and good laboratory practice, UNEP, 1994

Annex IV:

Recommended method for the determination of selected trace element in samples of marine origin by atomic absorption spectrometry using graphite furnace

Recommended method for the determination of selected trace element in samples of marine origin by atomic absorption spectrometry using graphite furnace

Marine Environmental Studies Laboratory in co-operation with MED POL

November 2011

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NOTE: This recommended method is not intended to be an analytical training manual. Therefore the method is written with the assumption that it will be performed by formally trained analytical chemist.
In addition, the IAEA recommended methods are intended to be guidance methods that can be used by laboratories as a starting point for generating their own standard operating procedure. If performance data are included in the method they must not be used as absolute QC acceptance criteria.

The recommended protocol is mainly based on EPA 7010 method and ISO 15586 users are encouraged to consult this documents (US EPA, 2007; ISO 2003)

1. SCOPE:

This International Standard includes principles and procedures for the determination of trace levels of: As, Cd, Co, Cr, Cu, Ni, Pb, and V in samples from marine origin, using atomic absorption spectrometry with electro thermal atomization in a graphite furnace. The method is applicable to the determination of low concentrations of elements. The detection limit of the method for each element depends on the sample matrix as well as the instrument, the type of atomizer and the use of chemical modifiers. Table 1 gives approximate working range and characteristic masses.

Table 1 Approximate characteristic masses and typical working range using 20µl sample volume

Element	Characteristic mass M_0^* pg	Working range ng ml^{-1}
As	15	5-50
Cd	0.8	0.2-2
Co	10	3-30
Cr	3	2-20
Cu	10	3-30
Ni	13	5-50
Pb	15	5-50
V	35	10-100

The characteristic mass (m_0) of an element is the mass in pg corresponding to a signal of 0.00044 unity using peak area as integration

2. PRINCIPLE:

An aliquot of sample solution (5-50 µL) is introduced into a graphite tube of the GF AAS and atomized by rapid heating at high temperature. A light beam is directed through the graphite tube, into a monochromator, and onto a detector that measures the amount of light absorbed by the atomized element in the tube. Each metal has its own characteristic wavelength therefore a source hollow cathode lamp composed of that element is used. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.

3. SAMPLE PRE-TREATMENT:

Samples are prepared following the recommended method for microwave digestion of marine samples for determination of trace element content. (IAEA recommended method, 2011)

4. REAGENTS:

4.1. Water: Reagent water (referenced also as water in the text) should be free of contamination

4.2. Concentrated acid solution as used for sample preparation (section 3)

4.3. Commercial standard solution 1000µg ml^{-1}: Use certified reference material solution; this solution should be accompanied by a certificate that should include at least the traceability of the certified concentration as well as the expiration date. The density of the solution or the certified content in mg kg-1 should also be defined to allow preparation of calibration solution by weighing.

4.4. Calibration solutions: Prepare calibration solutions from the standard solutions (4.3) by appropriate dilution. Intermediate standard solutions should be prepared in 2% (v/v) nitric acid. For calibration solution use the same amount of acid as that of the samples solutions.

Calibration solutions below 1 mg/l should not be used for more than one month, and those below 100 µg/l should not be used for more than one day.

4.5. Blank calibration solution: Prepare a blank calibration solution in the same way as the calibration solution but without adding standard. The final amount of acid will be the same as that of the sample solutions.

4.6. Palladium nitrate/magnesium nitrate modifier

$Pd(NO_3)_2$ solution is commercially available (10 g/l). Dissolve 0,259 g of $Mg(NO_3)_2 \cdot 6H_2O$ in 100 ml of water. Mix the palladium nitrate solution with twice as much magnesium nitrate solution. 10 µl of the mixed solution is equal to 15 µg Pd and 10 µg $Mg(NO_3)_2$. The mixture is also commercially available.

Prepare a fresh solution monthly.

4.7. Magnesium nitrate modifier

Dissolve 0,865 g of $Mg(NO_3)_2 \cdot 6H_2O$ in 100 ml of water. 10 µl of this solution is equal to 50 µg $Mg(NO_3)_2$.

4.8. Ammonium dihydrogen phosphate modifier

Dissolve 2,0 g of $NH_4H_2PO_4$ in 100 ml of water. 10 µl of this solution is equal to 200 µg $NH_4H_2PO_4$.

4.9. Ammonium dihydrogen phosphate/magnesium nitrate modifier

Dissolve 2,0 g of $NH_4H_2PO_4$ and 0,173 g of $Mg(NO_3)_2 \cdot 6H_2O$ in 100 ml of water. 10 µl of this solution is equal to 200 µg $NH_4H_2PO_4$ and 10 µg $Mg(NO_3)_2$.

4.10. Palladium/Ammonium dihydrogen phosphate/magnesium nitrate modifier

Mix 2ml of $Pd(NO_3)_2$ solution is commercially available (10 g/l), 2ml of $Mg(NO_3)_2$ solution prepared as (4.7), 0.5ml of $NH_4H_2PO_4$ prepared as (4.8) and dilute with water to 10ml. 4µl of this solution is equal to 8µg of Pd, 4µg of $Mg(NO_3)_2$ and 4µg of $NH_4H_2PO_4$.

4.11. Nickel modifier

Dissolve 0,200 g of nickel powder in 1 ml concentrated nitric acid and dilute to 100 ml with water. 10 µl of this solution is equal to 20 µg Ni. Solutions of $Ni(NO_3)_2$ are also commercially available.

4.12. Iridium solution 1000µg ml^{-1}

Use commercial solution (standard)

4.13. Argon

5. MATERIALS:

5.1. Glassware: All glassware, polypropylene, or fluorocarbon (PFA or TFM) containers, including sample bottles, flasks and pipettes tips, should be washed in the following sequence -- 24h soaking in laboratory soap (or 10% alcohol) followed by 24h soaking in 10% nitric acid, followed by 10% soaking in water, final rinsing in water, drying under laminar flow hood. Cleaned items should be kept in double sealed plastic bags

5.2. Pipettes: microliter pipettes size ranging from 50 to 10000µl as needed. The accuracy and precision of the pipettes used should be checked as a routine every 6 months and the obtained results should be compared with the individual certificates.

5.3. Volumetric containers preferably in polypropylene of suitable precision and accuracy

5.4. Atomic Absorption Spectrometer equipped with graphite furnace, background correction system and necessary hallow cathode lamp.

5.5. Auto sampler

5.6. Polypropylene cups for automatic sampler cleaned as explained in (5.1)

5.7. Graphite tubes: pyrolytically-coated with platforms, preferably for highly and medium volatile elements, whereas elements of low volatility should be atomized from the wall. Provided satisfactory results are achieved, manufacturer's recommendations regarding the use of graphite tubes and platforms should be followed.

6. INTERFERENCES:

Some sample solutions, may contain large amounts of substances that may affect the results. High concentrations of chloride may cause low results, because the volatility of many elements is increased and analyte loss may occur during the pyrolysis step. Matrix effects may be overcome, partially or completely, by the optimization of the temperature program, the use of pyrolytically-coated tubes and platforms, the use of chemical modifiers, the standard addition technique and the use of background correction.

7. CHEMICAL MODIFICATION:

Chemical modifiers are used to overcome spectral and/or non-spectral interferences in a sample (matrix effects). In general, the aim of chemical modification is to allow a pyrolysis temperature that is high enough to remove the bulk of concomitants before the atomization step. In order to ascertain that the modification works, the spike procedures is performed with and without the addition of a chosen chemical modifier and recovery are compared

Spike experiment:

Spike solution: mix a fixe volume (V1) of sample solution, and a known volume (V2) of a standard solution of a known concentration (Cstandard)

Unspike solution: mix same fixe volume (V1) of sample solution, and same volume (V2) of reagent water

Measure concentration C (mg l^{-1}) in both solutions on the calibration curve, and calculate recovery as:

Equation 1 $$Cspike = \frac{Cstandard \times V2}{(V1+V2)}$$

Equation 2 $$\mathrm{R} = \frac{C\ Spike\ Solution - C\ Unspike\ solution}{Cspike} \times 100$$

To be valid concentrations of spike and unspike solutions should be in the linearity range of the calibration curve and Spike concentration (equation 1) should be in the range of 50-150% of the concentration of unspike solution. The recovery should be 100 ± 15%

In Table 2 some recommendations of chemical modifiers are given.

Other chemical modifiers may be used if they show consistent results. Graphite tube can also be pretreated with Iridium (Vasileva 2001) as following:

Inject 50µl of the solution and run the temperature program below

Step	Temperature (°C)	Ramp Time (s)	Hold Time (s)
1	100	5	30
2	1200	20	5
3	100	5	2
4	2500	2	10

Repeat this 3 times, the coating is stable for about 200 injections and can be repeated

If chemical modifiers are used, add them to test samples, sample blank solutions, calibration solutions, and blank calibration solutions. Preferably inject the modifier solution with the auto sampler directly into the atomizer after the sample is delivered.

Table 2 Recommended chemical modifiers

Element	Chemical modifier	Amount* µg
As	Pd + $Mg(NO_3)_2$ or $NH_4H_2PO_4$	15+10 200
Cd	Pd + $Mg(NO_3)_2$+$NH_4H_2PO_4$ or Ir coating	8+4+4
Co	Pd + $Mg(NO_3)_2$	15+10
Cr	$Mg(NO_3)_2$	50
Cu	None	
Ni	$Mg(NO_3)_2$	50
Pb	Pd + $Mg(NO_3)_2$+$NH_4H_2PO_4$ or Ir coating	8+4+4
V	None	

**These amounts are only recommendation, significantly lower amounts may be required in some atomizers, see also recommendations from instrument manufacturers.*

8. PROCEDURE

8.1. Switch on the instrument and perform the optimization according to the manufacturer's instructions. Install an appropriate graphite tube, and set up the auto sampler.

8.2. Program the graphite furnace and the auto sampler. Examples of temperature program are given in table 3.

Note: Method for specific element and matrix should be developed and all necessary information should be stored with at least:

- *Temperature program*
- *Matrix modifier*
- *Type of graphite tube*
- *Matrix effect*
- *Type of calibration curve*
- *Typical m_0 obtained with the program*
- *Linearity*

Table 3 Example of temperature program

Element	Cu	Cu	Cd	Cd	Pb	Pb	As	As	Cr	Cr
Sample type	Sediment	Biota	Sediment	Biota	Sediment	Biota	Sediment	Biota	Sediment	Biota
Wavelengt(nm)	327.4	327.4	228.8	228.8	283.3	283.3	193.7	193.7	357.9	357.9
Graphite tube	Partition Tube	Partition Tube	platform	platform	platform	platform	platform	platform	Partition Tube	Partition Tube
Matrix Modifier	none	none	none	Pd,Mg, Amonium Phosphate	none	Pd,Mg ,Amonium Phosphate	Pd,Mg	Pd,Mg	none	none
Peak Measurement	area	area	area	area	area	area	area	area	area	area
M0(pg/0.0044 UA) on standard	13	13	1	1	16	16	15	15	2.5	2.5
Ashing T° (C°)	700	700	300	700	400	925	1400	1400	1100	1100
Atomisation T° (C°)	2300	2300	1800	1900	2100	2200	2600	2600	2600	2600
Remark							Number of Fire is critical	Standard Addition often required. Number of fire is critical	Use peak Height for lower concentration (peak shape)	Standard Addition often required. Use peak Height for lower concentration (peak shape)

8.3. Generality for measurements:

All measurements should be performed with at least duplicate injections of solutions; the relative standard deviation should be less than 5% for a signal above 0.01 unit of absorbance.

It is recommended to work in peak area.

Check the number of firing and change the graphite tube when appropriate, if graphite tube is changed during a run, the instrument needs to be recalibrated.

8.4. Run the calibration:

8.4.1. **Standard calibration technique**: Perform the calibration with a blank calibration solution (4.5) and 3 to 5 equidistant calibration solutions (4.4) for an appropriate concentration range.

To correct for the instrumental drift calibration should be performed every 10 samples (if possible the option of reslope using the middle standard point should be applied every 5 samples)

Calibration solutions can be prepared by the auto sampler from the highest standard solution, the minimum volume uptake should not be less than 4µl.

The blank calibration solution should be free of analyte, or below a well-documented maximum allowed calibration blank value (i.e. validation, control charts..).

It should be stressed that the linearity of the calibration curve is often limited. The calibration curve is automatically plot by instrument software, if linear regression is set checked that $r \leq 0.995$ or switch to second order equation.

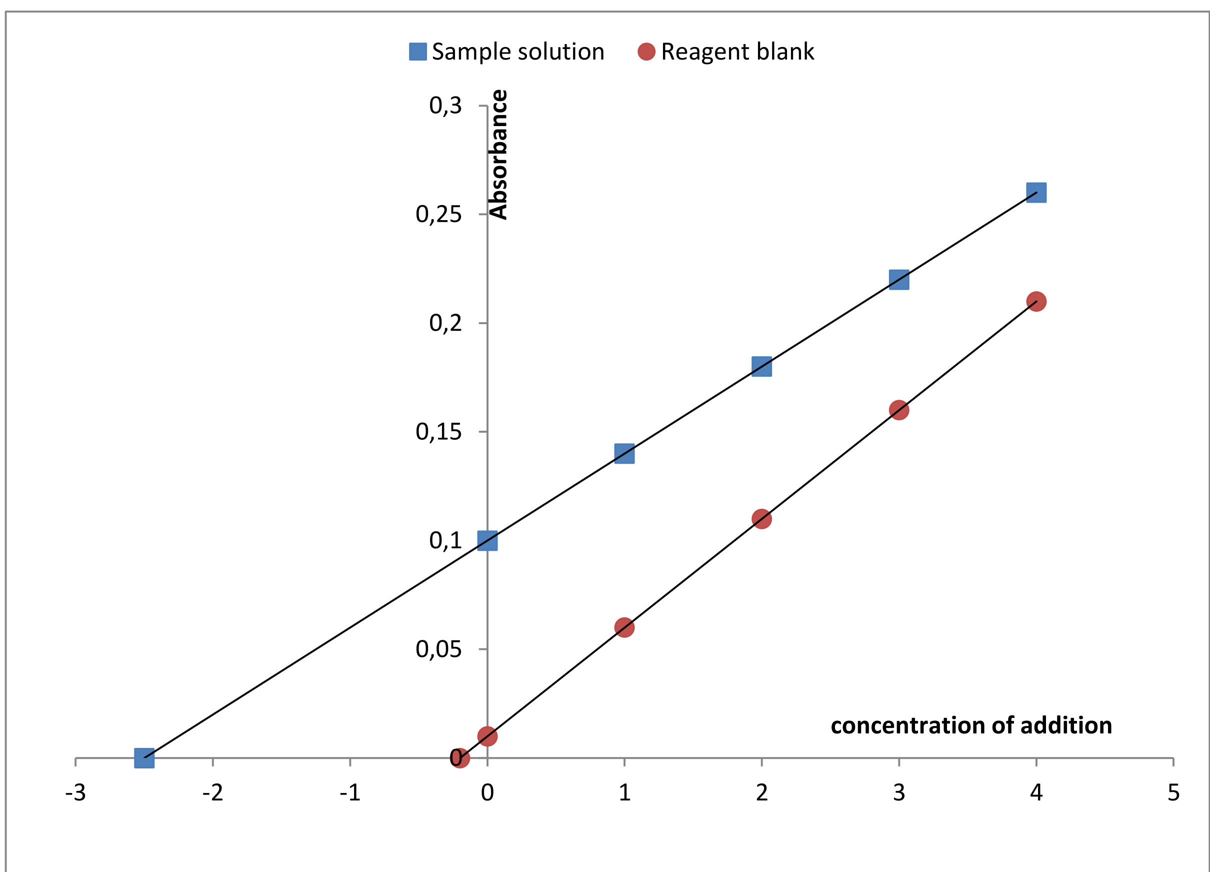
8.4.2. **Standard addition method**: This technique involves preparing same aliquots of sample solution with increasing amount of analyte. As describe in section 7 for the spike experiment using an increasing concentration of standard (V1 and V2 should stay the same). The auto sampler can be programed to perform standard addition. Determine the analyte concentration in the reagent blank solution the same way. Example of standard addition is given in figure 1. The concentration is obtained by dividing the absorbance of zero addition by the slope.

The standard addition should be performed for each type of matrix (i.e. a sediment sample solution cannot be measured with a standard addition curve done on a fish sample solution). For similar sample matrices (i.e. same fish species) the slope obtained with one sample can be used for other measurements respecting recalibration every 10samples.

For standard addition to be valid the following limitation should be taken into consideration:

- The resulting calibration should be linear (r≤0.995), software calibration equation is a linear regression
- The additions should represent ideally 50, 100, 150 and 200% of the sample concentration
- The standard addition technic cannot be used to correct for spectral interferences, such as unspecific background absorption, and should not be used if interferences change the signal by a factor of more than three.

Figure 1 Standard addition example



8.5. Measure sample blank and sample solutions (prepared following section 3) record the concentration as calculated by the software and calculate results following equation 3 (section 9), if samples exceed the highest point of calibration dilute appropriately. As an option a smaller volume of solution can be injected to stay under linear range of the instrument.

8.6. Quality control solutions: Quality control solutions as described below should be measured during the run. An example of a sequence order with recommended criteria and action is given in table 4.

Table 4 Example of analytical sequence:

Solutions Description	Performance	Action
Calibration blank	< maximum allowed calibration blank value	Stop until resolve
Standard solution 1 -4	r>0.995	recalibrate in the linearity range
ICV	±10% of true value	Stop until resolve
Sample blank	< maximum allowed blank value	
CRM	Fall in certificate value within coverage uncertainty, or fall within acceptable criteria of the QC chart	Stop until resolve, check Matrix spike and run again with standard addition method if necessary
Matrix Spike	recovery 100% ± 15%	switch to standard addition, keep record for future analyses of the same matrix
Dilution Test	sample 1 = 5x sample 1 diluted 5x within 10%	switch to standard addition, keep record for future analyses of the same matrix
Unknown Sample 1-10	should ≥ standard 1 and ≤ standard 4	report as <minimum quantification limit or dilute

ETC…(restart sequence from calibration blank)

8.6.1. **Initial Calibration Verification ICV**:

After the initial calibration, the calibration curve must be verified using the initial calibration verification (ICV) standard.

The ICV standard is a standard solution made from an independent (second source) material at or near midrange. This solution as calibration standard is prepared using the same type of acid or combination of acids and at the same concentration as will result in the test portion.

The acceptance criteria for the ICV standard must be ±10% of its true value

If the calibration curve cannot be verified within the specified limits, the cause must be determined and the instrument recalibrated before samples are analyzed. The analysis data for the ICV must be kept on file with the sample analysis

The calibration curve must also be verified at the end of each analysis batch and/or after every 10 samples. If the calibration cannot be verified within the specified limits, the sample analysis must be discontinued, the cause determined and the instrument recalibrated. All samples following the last acceptable test must be reanalyzed.

8.6.2. **Blank solution** (4.5): Maximum allowed blank concentration should be well documented and if blank solution exceeds this value all samples prepared along the contaminated blank should be prepared again and re analyzed.

8.6.3. **Post digestion spike**

Each unknown type of sample should be spike to check for potential matrix effect.

This spike is consider as a single point standard addition, and should be performed with a minimum dilution factor. Recovery of spike calculated as equation 1 should be 85-115%. If this test failed it is recommended to run analyses with standard addition method. (see section 7 for detail)

8.6.4. **Dilution test**:

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the lower limit of quantitation after dilution), an analysis of a 1:5 dilution should agree within ±10% of the original determination. If not, then a chemical or physical interference effect should be suspected, and method of standard addition is recommended.

8.6.5. **Certified reference material**:

At least one certified reference material of a representative matrix will be prepared with each batch of sample, the calculated result should be comparable with the value of the certificate within the coverage uncertainty.(Linsinger, 2010), to show evidence of unbias result.

Results of CRM should be record for quality control purpose and plot in control chart (UNEP/IOC/IAEA 1994)

9. CALCULATION OF RESULTS:

Results are calculated with equation 3

Equation 3: $w(m) = \frac{(\rho 1 - \rho 0)}{m} \times f \times V \times R$

w(m) mass fraction of element m in the sample in mg kg^{-1}
ρ1: concentration of element m in milligrams per liter as measured in the sample solution
ρ0: concentration of element m in milligrams per liter as measured in the blank solution
f: is the dilution factor calculated as

$$f = \frac{final\ volume}{initial\ volume}$$

or equal to 1 if ρ1 is determined in undiluted solution
R: recovery calculated using CRM (see 8.6.5) or pre digestion spike

10. EXPRESSION OF RESULTS:

The rounding of values will depend of the uncertainty reported with the result. Uncertainty component should be reported with all results. (ISO 1995, Nordtest 2004)

Example : w(Pb) = 8.5 ± 1.2 mg kg^{1}

11. REFERENCES:

EPA (2007) U.S. Environmental Protection Agency, EPA method 7010: Graphite furnace Atomic Absorption Spectrophotometry, Rev 0, Febuary 2007, (http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/7010.pdf)

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Vasileva. E, Baeten. H, Hoenig. M (2001), Advantages of the iridium permanent modifier in fast programs applied to trace-element analysis of plant samples by electrothermal atomic absorption spectrometry, Fresenius J Anal Chem (2001) 369 :491–495

Annex V:

METHOD 200.8

DETERMINATION OF TRACE ELEMENTS IN WATERS AND WASTES

BY INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY

Revision 5.4

EMMC Version

METHOD 200.8

DETERMINATION OF TRACE ELEMENTS IN WATERS AND WASTES BY INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY

Revision 5.4
EMMC Version

S.E. Long (Technology Applications Inc.), T.D. Martin, and E.R. Martin - Method 200.8, Revisions 4.2 and 4.3 (1990)

S.E. Long (Technology Applications Inc.) and T.D. Martin - Method 200.8, Revision 4.4 (1991)

J.T. Creed, C.A. Brockhoff, and T.D. Martin - Method 200.8, Revision 5.4 (1994)

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
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DETERMINATION OF TRACE ELEMENTS IN WATERS AND WASTES BY INDUCTIVELY COUPLED PLASMA - MASS SPECTROMETRY

1.0 SCOPE AND APPLICATION

1.1 This method provides procedures for determination of dissolved elements in ground waters, surface waters and drinking water. It may also be used for determination of total recoverable element concentrations in these waters as well as wastewaters, sludges and soils samples. This method is applicable to the following elements:

Analyte		Chemical Abstract Services Registry Number (CASRN)
Aluminum	(Al)	7429-90-5
Antimony	(Sb)	7440-36-0
Arsenic	(As)	7440-38-2
Barium	(Ba)	7440-39-3
Beryllium	(Be)	7440-41-7
Cadmium	(Cd)	7440-43-9
Chromium	(Cr)	7440-47-3
Cobalt	(Co)	7440-48-4
Copper	(Cu)	7440-50-8
Lead	(Pb)	7439-92-1
Manganese	(Mn)	7439-96-5
Mercury	(Hg)	7439-97-6
Molybdenum	(Mo)	7439-98-7
Nickel	(Ni)	7440-02-0
Selenium	(Se)	7782-49-2
Silver	(Ag)	7440-22-4
Thallium	(Tl)	7440-28-0
Thorium	(Th)	7440-29-1
Uranium	(U)	7440-61-1
Vanadium	(V)	7440-62-2
Zinc	(Zn)	7440-66-6

Estimated instrument detection limits (IDLs) for these elements are listed in Table 1. These are intended as a guide to instrumental limits typical of a system optimized for multielement determinations and employing commercial instrumentation and pneumatic nebulization sample introduction. However, actual method detection limits (MDLs) and linear working ranges will be dependent on the sample matrix, instrumentation and selected operating conditions. Given in Table 7 are typical MDLs for both total recoverable determinations by "direct analysis" and where sample digestion is employed.

1.2 For reference where this method is approved for use in compliance monitoring programs [e.g., Clean Water Act (NPDES) or Safe Drinking Water Act (SDWA)] consult both the appropriate sections of the Code of Federal Regulation (40 CFR Part 136 Table 1B for NPDES, and Part 141 § 141.23 for drinking water), and the latest Federal Register announcements.

1.3 Dissolved elements are determined after suitable filtration and acid preservation. In order to reduce potential interferences, dissolved solids should not exceed 0.2% (w/v) (Section 4.1.4).

1.4 With the exception of silver, where this method is approved for the determination of certain metal and metalloid contaminants in drinking water, samples may be analyzed directly by pneumatic nebulization without acid digestion if the samples have been properly preserved with acid and have turbidity of <1 NTU at the time of analysis. This total recoverable determination procedure is referred to as "direct analysis".

1.5 For the determination of total recoverable analytes in aqueous and solid samples a digestion/extraction is required prior to analysis when the elements are not in solution (e.g., soils, sludges, sediments and aqueous samples that may contain particulate and suspended solids). Aqueous samples containing suspended or particulate material ≥1% (w/v) should be extracted as a solid type sample (Section 11.2.2).

1.6 The total recoverable sample digestion procedure given in this method is not suitable for the determination of volatile organo-mercury compounds. However, for "direct analysis" of drinking water (turbidity <1 NTU), the combined concentrations of inorganic and organo-mercury in solution can be determined by "direct analysis" pneumatic nebulization provided gold is added to both samples and standards alike to eliminate memory interference effects.

1.7 Silver is only slightly soluble in the presence of chloride unless there is a sufficient chloride concentration to form the soluble chloride complex. Therefore, low recoveries of silver may occur in samples, fortified sample matrices and even fortified blanks if determined as a dissolved analyte or by "direct analysis" where the sample has not been processed using the total recoverable mixed acid digestion. For this reason it is recommended that samples be digested prior to the determination of silver. The total recoverable sample digestion procedure given in this method is suitable for the determination of silver in aqueous samples containing concentrations up to 0.1 mg/L. For the analysis of wastewater samples containing higher concentrations of silver, succeeding smaller volume, well mixed sample aliquots must be prepared until the analysis solution contains <0.1 mg/L silver. The extraction of solid samples containing concentrations of silver >50 mg/kg should be treated in a similar manner.

1.8 The total recoverable sample digestion procedure given in this method will solubilize and hold in solution only minimal concentrations of barium in the presence of free sulfate. For the analysis of barium in samples having varying

and unknown concentrations of sulfate, analysis should be completed as soon as possible after sample preparation.

1.9 This method should be used by analysts experienced in the use of inductively coupled plasma mass spectrometry (ICP-MS), the interpretation of spectral and matrix interferences and procedures for their correction. A minimum of six months experience with commercial instrumentation is recommended.

1.10 Users of the method data should state the data-quality objectives prior to analysis. Users of the method must document and have on file the required initial demonstration performance data described in Section 9.2 prior to using the method for analysis.

2.0 SUMMARY OF METHOD

2.1 An aliquot of a well mixed, homogeneous aqueous or solid sample is accurately weighed or measured for sample processing. For total recoverable analysis of a solid or an aqueous sample containing undissolved material, analytes are first solubilized by gentle refluxing with nitric and hydrochloric acids. After cooling, the sample is made up to volume, is mixed and centrifuged or allowed to settle overnight prior to analysis. For the determination of dissolved analytes in a filtered aqueous sample aliquot, or for the "direct analysis" total recoverable determination of analytes in drinking water where sample turbidity is <1 NTU, the sample is made ready for analysis by the appropriate addition of nitric acid, and then diluted to a predetermined volume and mixed before analysis.

2.2 The method describes the multi-element determination of trace elements by ICP-MS.[1-3] Sample material in solution is introduced by pneumatic nebulization into a radiofrequency plasma where energy transfer processes cause desolvation, atomization and ionization. The ions are extracted from the plasma through a differentially pumped vacuum interface and separated on the basis of their mass-to-charge ratio by a quadrupole mass spectrometer having a minimum resolution capability of 1 amu peak width at 5% peak height. The ions transmitted through the quadrupole are detected by an electron multiplier or Faraday detector and the ion information processed by a data handling system. Interferences relating to the technique (Section 4.0) must be recognized and corrected for. Such corrections must include compensation for isobaric elemental interferences and interferences from polyatomic ions derived from the plasma gas, reagents or sample matrix. Instrumental drift as well as suppressions or enhancements of instrument response caused by the sample matrix must be corrected for by the use of internal standards.

3.0 DEFINITIONS

3.1 **Calibration Blank** - A volume of reagent water acidified with the same acid matrix as in the calibration standards. The calibration blank is a zero standard and is used to calibrate the ICP instrument (Section 7.6.1).

3.2 **Calibration Standard (CAL)** - A solution prepared from the dilution of stock standard solutions. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration (Section 7.4).

3.3 **Dissolved Analyte** - The concentration of analyte in an aqueous sample that will pass through a 0.45 µm membrane filter assembly prior to sample acidification (Section 11.1).

3.4 **Field Reagent Blank (FRB)** - An aliquot of reagent water or other blank matrix that is placed in a sample container in the laboratory and treated as a sample in all respects, including shipment to the sampling site, exposure to the sampling site conditions, storage, preservation, and all analytical procedures. The purpose of the FRB is to determine if method analytes or other interferences are present in the field environment (Section 8.5).

3.5 **Instrument Detection Limit (IDL)** - The concentration equivalent to the analyte signal which is equal to three times the standard deviation of a series of 10 replicate measurements of the calibration blank signal at the selected analytical mass(es). (Table 1).

3.6 **Internal Standard** - Pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes that are components of the same sample or solution. The internal standard must be an analyte that is not a sample component (Sections 7.5 and 9.4.5).

3.7 **Laboratory Duplicates (LD1 and LD2)** - Two aliquots of the same sample taken in the laboratory and analyzed separately with identical procedures. Analyses of LD1 and LD2 indicates precision associated with laboratory procedures, but not with sample collection, preservation, or storage procedures.

3.8 **Laboratory Fortified Blank (LFB)** - An aliquot of LRB to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements (Sections 7.9 and 9.3.2).

3.9 **Laboratory Fortified Sample Matrix (LFM)** - An aliquot of an environmental sample to which known quantities of the method analytes are added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations (Section 9.4).

3.10 **Laboratory Reagent Blank (LRB)** - An aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, and internal standards that are used with other samples. The LRB is used to determine if method analytes or other interferences

are present in the laboratory environment, reagents, or apparatus (Sections 7.6.2 and 9.3.1).

3.11 **Linear Dynamic Range (LDR)** - The concentration range over which the instrument response to an analyte is linear (Section 9.2.2).

3.12 **Method Detection Limit (MDL)** - The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero (Section 9.2.4 and Table 7).

3.13 **Quality Control Sample (QCS)** - A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check either laboratory or instrument performance (Sections 7.8 and 9.2.3).

3.14 **Solid Sample** - For the purpose of this method, a sample taken from material classified as either soil, sediment or sludge.

3.15 **Stock Standard Solution** - A concentrated solution containing one or more method analytes prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source (Section 7.3).

3.16 **Total Recoverable Analyte** - The concentration of analyte determined either by "direct analysis" of an unfiltered acid preserved drinking water sample with turbidity of <1 NTU (Section 11.2.1), or by analysis of the solution extract of a solid sample or an unfiltered aqueous sample following digestion by refluxing with hot dilute mineral acid(s) as specified in the method (Sections 11.2 and 11.3).

3.17 **Tuning Solution** - A solution which is used to determine acceptable instrument performance prior to calibration and sample analyses (Section 7.7).

3.18 **Water Sample** - For the purpose of this method, a sample taken from one of the following sources: drinking, surface, ground, storm runoff, industrial or domestic wastewater.

4.0 INTERFERENCES

4.1 Several interference sources may cause inaccuracies in the determination of trace elements by ICP-MS. These are:

4.1.1 Isobaric elemental interferences - Are caused by isotopes of different elements which form singly or doubly charged ions of the same nominal mass-to-charge ratio and which cannot be resolved by the mass spectrometer in use. All elements determined by this method have, at a minimum, one isotope free of isobaric elemental interference. Of the analytical isotopes recommended for use with this method (Table 4), only molybdenum-98 (ruthenium) and selenium-82 (krypton) have isobaric elemental interferences. If alternative analytical isotopes having higher

natural abundance are selected in order to achieve greater sensitivity, an isobaric interference may occur. All data obtained under such conditions must be corrected by measuring the signal from another isotope of the interfering element and subtracting the appropriate signal ratio from the isotope of interest. A record of this correction process should be included with the report of the data. It should be noted that such corrections will only be as accurate as the accuracy of the isotope ratio used in the elemental equation for data calculations. Relevant isotope ratios should be established prior to the application of any corrections.

4.1.2 Abundance sensitivity - Is a property defining the degree to which the wings of a mass peak contribute to adjacent masses. The abundance sensitivity is affected by ion energy and quadrupole operating pressure. Wing overlap interferences may result when a small ion peak is being measured adjacent to a large one. The potential for these interferences should be recognized and the spectrometer resolution adjusted to minimize them.

4.1.3 Isobaric polyatomic ion interferences - Are caused by ions consisting of more than one atom which have the same nominal mass-to-charge ratio as the isotope of interest, and which cannot be resolved by the mass spectrometer in use. These ions are commonly formed in the plasma or interface system from support gases or sample components. Most of the common interferences have been identified[3], and these are listed in Table2 together with the method elements affected. Such interferences must be recognized, and when they cannot be avoided by the selection of alternative analytical isotopes, appropriate corrections must be made to the data. Equations for the correction of data should be established at the time of the analytical run sequence as the polyatomic ion interferences will be highly dependent on the sample matrix and chosen instrument conditions. In particular, the common ^{82}Kr interference that affects the determination of both arsenic and selenium, can be greatly reduced with the use of high purity krypton free argon.

4.1.4 Physical interferences - Are associated with the physical processes which govern the transport of sample into the plasma, sample conversion processes in the plasma, and the transmission of ions through the plasma-mass spectrometer interface. These interferences may result in differences between instrument responses for the sample and the calibration standards. Physical interferences may occur in the transfer of solution to the nebulizer (e.g., viscosity effects), at the point of aerosol formation and transport to the plasma (e.g., surface tension), or during excitation and ionization processes within the plasma itself. High levels of dissolved solids in the sample may contribute deposits of material on the extraction and/or skimmer cones reducing the effective diameter of the orifices and therefore ion transmission. Dissolved solids levels not exceeding 0.2% (w/v) have been recommended[3] to reduce such effects. Internal standardization may be effectively used to compensate for many physical interference effects.[4] Internal standards ideally should have similar

analytical behavior to the elements being determined.

4.1.5 Memory interferences - Result when isotopes of elements in a previous sample contribute to the signals measured in a new sample. Memory effects can result from sample deposition on the sampler and skimmer cones, and from the buildup of sample material in the plasma torch and spray chamber. The site where these effects occur is dependent on the element and can be minimized by flushing the system with a rinse blank between samples (Section 7.6.3). The possibility of memory interferences should be recognized within an analytical run and suitable rinse times should be used to reduce them. The rinse times necessary for a particular element should be estimated prior to analysis. This may be achieved by aspirating a standard containing elements corresponding to 10 times the upper end of the linear range for a normal sample analysis period, followed by analysis of the rinse blank at designated intervals. The length of time required to reduce analyte signals to within a factor of 10 of the method detection limit, should be noted. Memory interferences may also be assessed within an analytical run by using a minimum of three replicate integrations for data acquisition. If the integrated signal values drop consecutively, the analyst should be alerted to the possibility of a memory effect, and should examine the analyte concentration in the previous sample to identify if this was high. If a memory interference is suspected, the sample should be reanalyzed after a long rinse period. In the determination of mercury, which suffers from severe memory effects, the addition of 100 µg/L gold will effectively rinse 5 µg/L mercury in approximately two minutes. Higher concentrations will require a longer rinse time.

5.0 SAFETY

5.1 The toxicity or carcinogenicity of reagents used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.[5,8] A reference file of material data handling sheets should also be available to all personnel involved in the chemical analysis. Specifically, concentrated nitric and hydrochloric acids present various hazards and are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection, protective clothing and observe proper mixing when working with these reagents.

5.2 The acidification of samples containing reactive materials may result in the release of toxic gases, such as cyanides or sulfides. Acidification of samples should be done in a fume hood.

5.3 All personnel handling environmental samples known to contain or to have been

in contact with human waste should be immunized against known disease causative agents.

5.4 Analytical plasma sources emit radiofrequency radiation in addition to intense UV radiation. Suitable precautions should be taken to protect personnel from such hazards. The inductively coupled plasma should only be viewed with proper eye protection from UV emissions.

5.5 It is the responsibility of the user of this method to comply with relevant disposal and waste regulations. For guidance see Sections 14.0 and 15.0.

6.0 EQUIPMENT AND SUPPLIES

6.1 Inductively coupled plasma mass spectrometer:

6.1.1 Instrument capable of scanning the mass range 5-250 amu with a minimum resolution capability of 1 amu peak width at 5% peak height. Instrument may be fitted with a conventional or extended dynamic range detection system.

Note: If an electron multiplier detector is being used, precautions should be taken, where necessary, to prevent exposure to high ion flux. Otherwise changes in instrument response or damage to the multiplier may result.

6.1.2 Radio-frequency generator compliant with FCC regulations.

6.1.3 Argon gas supply - High purity grade (99.99%). When analyses are conducted frequently, liquid argon is more economical and requires less frequent replacement of tanks than compressed argon in conventional cylinders (Section 4.1.3).

6.1.4 A variable-speed peristaltic pump is required for solution delivery to the nebulizer.

6.1.5 A mass-flow controller on the nebulizer gas supply is required. A water-cooled spray chamber may be of benefit in reducing some types of interferences (e.g., from polyatomic oxide species).

6.1.6 If an electron multiplier detector is being used, precautions should be taken, where necessary, to prevent exposure to high ion flux. Otherwise changes in instrument response or damage to the multiplier may result. Samples having high concentrations of elements beyond the linear range of the instrument and with isotopes falling within scanning windows should be diluted prior to analysis.

6.2 Analytical balance, with capability to measure to 0.1 mg, for use in weighing solids, for preparing standards, and for determining dissolved solids in digests or extracts.

6.3 A temperature adjustable hot plate capable of maintaining a temperature of 95°C.

6.4 (Optional) A temperature adjustable block digester capable of maintaining a temperature of 95°C and equipped with 250 mL constricted digestion tubes.

6.5 (Optional) A steel cabinet centrifuge with guard bowl, electric timer and brake.

6.6 A gravity convection drying oven with thermostatic control capable of maintaining 105°C ± 5°C.

6.7 (Optional) An air displacement pipetter capable of delivering volumes ranging from 0.1-2500 µL with an assortment of high quality disposable pipet tips.

6.8 Mortar and pestle, ceramic or nonmetallic material.

6.9 Polypropylene sieve, 5-mesh (4 mm opening).

6.10 Labware - For determination of trace levels of elements, contamination and loss are of prime consideration. Potential contamination sources include improperly cleaned laboratory apparatus and general contamination within the laboratory environment from dust, etc. A clean laboratory work area designated for trace element sample handling must be used. Sample containers can introduce positive and negative errors in the determination of trace elements by (1) contributing contaminants through surface desorption or leaching, (2) depleting element concentrations through adsorption processes. All reusable labware (glass, quartz, polyethylene, PTFE, FEP, etc.) should be sufficiently clean for the task objectives. Several procedures found to provide clean labware include soaking overnight and thoroughly washing with laboratory-grade detergent and water, rinsing with tap water, and soaking for four hours or more in 20% (V/V) nitric acid or a mixture of dilute nitric and hydrochloric acid (1+2+9), followed by rinsing with reagent grade water and storing clean.

Note: Chromic acid must not be used for cleaning glassware.

6.10.1 Glassware - Volumetric flasks, graduated cylinders, funnels and centrifuge tubes (glass and/or metal free plastic).

6.10.2 Assorted calibrated pipettes.

6.10.3 Conical Phillips beakers (Corning 1080-250 or equivalent), 250 mL with 50 mm watch glasses.

6.10.4 Griffin beakers, 250 mL with 75 mm watch glasses and (optional) 75 mm ribbed watch glasses.

6.10.5 (Optional) PTFE and/or quartz beakers, 250 mL with PTFE covers.

6.10.6 Evaporating dishes or high-form crucibles, porcelain, 100 mL capacity.

6.10.7 Narrow-mouth storage bottles, FEP (fluorinated ethylene propylene) with ETFE (ethylene tetrafluorethylene) screw closure, 125-250 mL capacities.

6.10.8 One-piece stem FEP wash bottle with screw closure, 125 mL capacity.

7.0 REAGENTS AND STANDARDS

7.1 Reagents may contain elemental impurities that might affect the integrity of analytical data. Owing to the high sensitivity of ICP-MS, high-purity reagents should be used whenever possible. All acids used for this method must be of ultra high-purity grade. Suitable acids are available from a number of manufacturers or may be prepared by sub-boiling distillation. Nitric acid is preferred for ICP-MS in order to minimize polyatomic ion interferences. Several polyatomic ion interferences result when hydrochloric acid is used (Table 2), however, it should be noted that hydrochloric acid is required to maintain stability in solutions containing antimony and silver. When hydrochloric acid is used, corrections for the chloride polyatomic ion interferences must be applied to all data.

7.1.1 Nitric acid, concentrated (sp.gr. 1.41).

7.1.2 Nitric acid (1+1) - Add 500 mL conc. nitric acid to 400 mL of regent grade water and dilute to 1 L.

7.1.3 Nitric acid (1+9) - Add 100 mL conc. nitric acid to 400 mL of reagent grade water and dilute to 1 L.

7.1.4 Hydrochloric acid, concentrated (sp.gr. 1.19).

7.1.5 Hydrochloric acid (1+1) - Add 500 mL conc. hydrochloric acid to 400 mL of reagent grade water and dilute to 1 L.

7.1.6 Hydrochloric acid (1+4) - Add 200 mL conc. hydrochloric acid to 400 mL of reagent grade water and dilute to 1 L.

7.1.7 Ammonium hydroxide, concentrated (sp.gr. 0.902).

7.1.8 Tartaric acid (CASRN 87-69-4).

7.2 Reagent water - All references to reagent grade water in this method refer to ASTM Type I water (ASTM D1193).[9] Suitable water may be prepared by passing distilled water through a mixed bed of anion and cation exchange resins.

7.3 Standard Stock Solutions - Stock standards may be purchased from a reputable commercial source or prepared from ultra high-purity grade chemicals or metals (99.99-99.999% pure). All salts should be dried for one hour at 105°C, unless otherwise specified. Stock solutions should be stored in FEP bottles. Replace stock standards when succeeding dilutions for preparation of the multielement stock standards can not be verified.

CAUTION: Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.

The following procedures may be used for preparing standard stock solutions:

Note: Some metals, particularly those which form surface oxides require cleaning prior to being weighed. This may be achieved by pickling the surface of the metal in acid. An amount in excess of the desired weight should be pickled repeatedly, rinsed with water, dried and weighed until the desired weight is achieved.

7.3.1 Aluminum solution, stock 1 mL = 1000 µg Al: Pickle aluminum metal in warm (1+1) HCl to an exact weight of 0.100 g. Dissolve in 10 mL conc. HCl and 2 mL conc. nitric acid, heating to effect solution. Continue heating until volume is reduced to 4 mL. Cool and add 4 mL reagent grade water. Heat until the volume is reduced to 2 mL. Cool and dilute to 100 mL with reagent grade water.

7.3.2 Antimony solution, stock 1 mL = 1000 µg Sb: Dissolve 0.100 g antimony powder in 2 mL (1+1) nitric acid and 0.5 mL conc. hydrochloric acid, heating to effect solution. Cool, add 20 mL reagent grade water and 0.15 g tartaric acid. Warm the solution to dissolve the white precipitate. Cool and dilute to 100 mL with reagent grade water.

7.3.3 Arsenic solution, stock 1 mL = 1000 µg As: Dissolve 0.1320 g As_2O_3 in a mixture of 50 mL reagent grade water and 1 mL conc. ammonium hydroxide. Heat gently to dissolve. Cool and acidify the solution with 2 mL conc. nitric acid. Dilute to 100 mL with reagent grade water.

7.3.4 Barium solution, stock 1 mL = 1000 µg Ba: Dissolve 0.1437 g $BaCO_3$ in a solution mixture of 10 mL reagent grade water and 2 mL conc. nitric acid. Heat and stir to effect solution and degassing. Dilute to 100 mL with reagent grade water.

7.3.5 Beryllium solution, stock 1 mL = 1000 µg Be: Dissolve 1.965 g $BeSO_4{\bullet}4H_2O$ (DO NOT DRY) in 50 mL reagent grade water. Add 1 mL conc. nitric acid. Dilute to 100 mL with reagent grade water.

7.3.6 Bismuth solution, stock 1 mL = 1000 µg Bi: Dissolve 0.1115 g Bi_2O_3 in 5 mL conc. nitric acid. Heat to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.7 Cadmium solution, stock 1 mL = 1000 µg Cd: Pickle cadmium metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.8 Chromium solution, stock 1 mL = 1000 µg Cr: Dissolve 0.1923 g CrO_3 in a solution mixture of 10 mL reagent grade water and 1 mL conc. nitric

acid. Dilute to 100 mL with reagent grade water.

7.3.9 Cobalt solution, stock 1 mL = 1000 µg Co: Pickle cobalt metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.10 Copper solution, stock 1 mL = 1000 µg Cu: Pickle copper metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.11 Gold solution, stock 1 mL = 1000 µg Au: Dissolve 0.100 g high purity (99.9999%) Au shot in 10 mL of hot conc. nitric acid by dropwise addition of 5 mL conc. HCl and then reflux to expel oxides of nitrogen and chlorine. Cool and dilute to 100 mL with reagent grade water.

7.3.12 Indium solution, stock 1 mL = 1000 µg In: Pickle indium metal in (1+1) nitric acid to an exact weight of 0.100 g. Dissolve in 10 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.13 Lead solution, stock 1 mL = 1000 µg Pb: Dissolve 0.1599 g $PbNO_3$ in 5 mL (1+1) nitric acid. Dilute to 100 mL with reagent grade water.

7.3.14 Magnesium solution, stock 1 mL = 1000 µg Mg: Dissolve 0.1658 g MgO in 10 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.15 Manganese solution, stock 1 mL = 1000 µg Mn: Pickle manganese flake in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.16 Mercury solution, stock, 1 mL = 1000 µg Hg: <u>DO NOT DRY</u>. **CAUTION:** highly toxic element. Dissolve 0.1354 g $HgCl_2$ in reagent water. Add 5.0 mL concentrated HNO_3 and dilute to 100 mL with reagent water.

7.3.17 Molybdenum solution, stock 1 mL = 1000 µg Mo: Dissolve 0.1500 g MoO_3 in a solution mixture of 10 mL reagent grade water and 1 mL conc. ammonium hydroxide., heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.18 Nickel solution, stock 1 mL = 1000 µg Ni: Dissolve 0.100 g nickel powder in 5 mL conc. nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.19 Scandium solution, stock 1 mL = 1000 µg Sc: Dissolve 0.1534 g Sc_2O_3 in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to

100 mL with reagent grade water.

7.3.20 Selenium solution, stock 1 mL = 1000 µg Se: Dissolve 0.1405 g SeO_2 in 20 mL ASTM Type I water. Dilute to 100 mL with reagent grade water.

7.3.21 Silver solution, stock 1 mL = 1000 µg Ag: Dissolve 0.100 g silver metal in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water. Store in dark container.

7.3.22 Terbium solution, stock 1 mL = 1000 µg Tb: Dissolve 0.1176 g Tb_4O_7 in 5 mL conc. nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.23 Thallium solution, stock 1 mL = 1000 µg Tl: Dissolve 0.1303 g $TlNO_3$ in a solution mixture of 10 mL reagent grade water and 1 mL conc. nitric acid. Dilute to 100 mL with reagent grade water.

7.3.24 Thorium solution, stock 1 mL = 1000 µg Th: Dissolve 0.2380 g $Th(NO_3)_4 \bullet 4H_2O$ (DO NOT DRY) in 20 mL reagent grade water. Dilute to 100 mL with reagent grade water.

7.3.25 Uranium solution, stock 1 mL = 1000 µg U: Dissolve 0.2110 g $UO_2(NO_3)_2 \bullet 6H_2O$ (DO NOT DRY) in 20 mL reagent grade water and dilute to 100 mL with reagent grade water.

7.3.26 Vanadium solution, stock 1 mL = 1000 µg V: Pickle vanadium metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.27 Yttrium solution, stock 1 mL = 1000 µg Y: Dissolve 0.1270 g Y_2O_3 in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.3.28 Zinc solution, stock 1 mL = 1000 µg Zn: Pickle zinc metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with reagent grade water.

7.4 Multielement Stock Standard Solutions - Care must be taken in the preparation of multielement stock standards that the elements are compatible and stable. Originating element stocks should be checked for the presence of impurities which might influence the accuracy of the standard. Freshly prepared standards should be transferred to acid cleaned, not previously used FEP fluorocarbon bottles for storage and monitored periodically for stability. The following combinations of elements are suggested:

Standard Solution A		Standard Solution B
Aluminum	Mercury	Barium
Antimony	Molybdenum	Silver
Arsenic	Nickel	
Beryllium	Selenium	
Cadmium	Thallium	
Chromium	Thorium	
Cobalt	Uranium	
Copper	Vanadium	
Lead	Zinc	
Manganese		

Except for selenium and mercury, multielement stock standard solutions A and B (1 mL = 10 µg) may be prepared by diluting 1.0 mL of each single element stock standard in the combination list to 100 mL with reagent water containing 1% (v/v) nitric acid. For mercury and selenium in solution A, aliquots of 0.05 mL and 5.0 mL of the respective stock standards should be diluted to the specified 100 mL (1 ml = 0.5 µg Hg and 50 µg Se). Replace the multielement stock standards when succeeding dilutions for preparation of the calibration standards cannot be verified with the quality control sample.

7.4.1 Preparation of calibration standards - fresh multielement calibration standards should be prepared every two weeks or as needed. Dilute each of the stock multielement standard solutions A and B to levels appropriate to the operating range of the instrument using reagent water containing 1% (v/v) nitric acid. The element concentrations in the standards should be sufficiently high to produce good measurement precision and to accurately define the slope of the response curve. Depending on the sensitivity of the instrument, concentrations ranging from 10-200 µg/L are suggested, except mercury, which should be limited to ≤5 µg/L. It should be noted the selenium concentration is always a factor of 5 greater than the other analytes. If the direct addition procedure is being used (Method A, Section 10.3), add internal standards (Section 7.5) to the calibration standards and store in FEP bottles. Calibration standards should be verified initially using a quality control sample (Section 7.8).

7.5 Internal Standards Stock Solution - 1 mL = 100 µg. Dilute 10 mL of scandium, yttrium, indium, terbium and bismuth stock standards (Section 7.3) to 100 mL with reagent water, and store in a FEP bottle. Use this solution concentrate for addition to blanks, calibration standards and samples, or dilute by an appropriate amount using 1% (v/v) nitric acid, if the internal standards are being added by peristaltic pump (Method B, Section 10.3).

Note: If mercury is to be determined by the "direct analysis" procedure, add an aliquot of the gold stock standard (Section 7.3.11) to the internal standard solution sufficient to provide a concentration of 100 µg/L in final the dilution of all blanks, calibration standards, and samples.

7.6 Blanks - Three types of blanks are required for this method. A calibration blank is used to establish the analytical calibration curve, the laboratory reagent blank is used to assess possible contamination from the sample preparation procedure and to assess spectral background and the rinse blank is used to flush the instrument between samples in order to reduce memory interferences.

7.6.1 Calibration blank - Consists of 1% (v/v) nitric acid in reagent grade water. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards.

7.6.2 Laboratory reagent blank (LRB) - Must contain all the reagents in the same volumes as used in processing the samples. The LRB must be carried through the same entire preparation scheme as the samples including digestion, when applicable. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards to the solution after preparation is complete.

7.6.3 Rinse blank - Consists of 2% (v/v) nitric acid in reagent grade water.

Note: If mercury is to be determined by the "direct analysis" procedure, add gold (Section 7.3.11) to the rinse blank to a concentration of 100 µg/L.

7.7 Tuning Solution - This solution is used for instrument tuning and mass calibration prior to analysis. The solution is prepared by mixing beryllium, magnesium, cobalt, indium and lead stock solutions (Section 7.3) in 1% (v/v) nitric acid to produce a concentration of 100 µg/L of each element. Internal standards are not added to this solution. (Depending on the sensitivity of the instrument, this solution may need to be diluted 10-fold.)

7.8 Quality Control Sample (QCS) - The QCS should be obtained from a source outside the laboratory. The concentration of the QCS solution analyzed will depend on the sensitivity of the instrument. To prepare the QCS dilute an appropriate aliquot of analytes to a concentration ≤100 µg/L in 1% (v/v) nitric acid. Because of lower sensitivity, selenium may be diluted to a concentration of <500 µg/L, however, in all cases, mercury should be limited to a concentration of ≤5 µg/L. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards after dilution, mix and store in a FEP bottle. The QCS should be analyzed as needed to meet data-quality needs and a fresh solution should be prepared quarterly or more frequently as needed.

7.9 Laboratory Fortified Blank (LFB) - To an aliquot of LRB, add aliquots from multielement stock standards A and B (Section 7.4) to prepared the LFB. Depending on the sensitivity of the instrument, the fortified concentration used should range from 40-100 µg/L for each analyte, except selenium and mercury. For selenium the concentration should range from 200-500 µg/L, while the concentration range mercury should be limited to 2-5 µg/L. The LFB must be carried through the same entire preparation scheme as the samples including sample digestion, when applicable. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards to this solution after

preparation has been completed.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

8.1 Prior to the collection of an aqueous sample, consideration should be given to the type of data required, (i.e., dissolved or total recoverable), so that appropriate preservation and pretreatment steps can be taken. The pH of all aqueous samples **must** be tested immediately prior to aliquoting for processing or "direct analysis" to ensure the sample has been properly preserved. If properly acid preserved, the sample can be held up to 6 months before analysis.

8.2 For the determination of dissolved elements, the sample must be filtered through a 0.45 µm pore diameter membrane filter at the time of collection or as soon thereafter as practically possible. Use a portion of the sample to rinse the filter flask, discard this portion and collect the required volume of filtrate. Acidify the filtrate with (1+1) nitric acid immediately following filtration to pH <2.

8.3 For the determination of total recoverable elements in aqueous samples, samples are **not** filtered, but acidified with (1+1) nitric acid to pH <2 (normally, 3 mL of (1+1) acid per liter of sample is sufficient for most ambient and drinking water samples). Preservation may be done at the time of collection, however, to avoid the hazards of strong acids in the field, transport restrictions, and possible contamination it is recommended that the samples be returned to the laboratory within two weeks of collection and acid preserved upon receipt in the laboratory. Following acidification, the sample should be mixed, held for 16 hours, and then verified to be pH <2 just prior withdrawing an aliquot for processing or "direct analysis". If for some reason such as high alkalinity the sample pH is verified to be >2, more acid must be added and the sample held for 16 hours until verified to be pH <2. See Section 8.1.

Note: When the nature of the sample is either unknown or known to be hazardous, acidification should be done in a fume hood. See Section 5.2.

8.4 Solid samples require no preservation prior to analysis other than storage at 4°C. There is no established holding time limitation for solid samples.

8.5 For aqueous samples, a field blank should be prepared and analyzed as required by the data user. Use the same container and acid as used in sample collection.

9.0 QUALITY CONTROL

9.1 Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an initial demonstration of laboratory capability, and the periodic analysis of laboratory reagent blanks, fortified blanks and calibration solutions as a continuing check on performance. The laboratory is required to maintain performance records that define the quality of the data thus generated.

9.2 Initial Demonstration of Performance (mandatory)

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of linear calibration ranges and analysis of quality control samples) and laboratory performance (determination of method detection limits) prior to analyses conducted by this method.

9.2.2 Linear calibration ranges - Linear calibration ranges are primarily detector limited. The upper limit of the linear calibration range should be established for each analyte by determining the signal responses from a minimum of three different concentration standards, one of which is close to the upper limit of the linear range. Care should be taken to avoid potential damage to the detector during this process. The linear calibration range which may be used for the analysis of samples should be judged by the analyst from the resulting data. The upper LDR limit should be an observed signal no more than 10% below the level extrapolated from lower standards. Determined sample analyte concentrations that are greater than 90% of the determined upper LDR limit must be diluted and reanalyzed. The LDRs should be verified whenever, in the judgement of the analyst, a change in analytical performance caused by either a change in instrument hardware or operating conditions would dictate they be redetermined.

9.2.3 Quality control sample (QCS) - When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS (Section 7.8). To verify the calibration standards the determined mean concentration from three analyses of the QCS must be within ±10% of the stated QCS value. If the QCS is used for determining acceptable on-going instrument performance, analysis of the QCS prepared to a concentration of 100 µg/L must be within ±10% of the stated value or within the acceptance limits listed in Table 8, whichever is the greater. (If the QCS is not within the required limits, an immediate second analysis of the QCS is recommended to confirm unacceptable performance.) If the calibration standards and/or acceptable instrument performance cannot be verified, the source of the problem must be identified and corrected before either proceeding on with the initial determination of method detection limits or continuing with on-going analyses.

9.2.4 Method detection limits (MDL) should be established for all analytes, using reagent water (blank) fortified at a concentration of two to five times the estimated detection limit.[7] To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = (t) \times (S)$$

where:

t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom [t = 3.14 for seven replicates]

S = standard deviation of the replicate analyses

Note: If additional confirmation is desired, reanalyze the seven replicate aliquots on two more nonconsecutive days and again calculate the MDL values for each day. An average of the three MDL values for each analyte may provide for a more appropriate MDL estimate. If the relative standard deviation (RSD) from the analyses of the seven aliquots is <10%, the concentration used to determine the analyte MDL may have been inappropriately high for the determination. If so, this could result in the calculation of an unrealistically low MDL. Concurrently, determination of MDL in reagent water represents a best case situation and does not reflect possible matrix effects of real world samples. However, successful analyses of LFMs (Section 9.4) can give confidence to the MDL value determined in reagent water. Typical single laboratory MDL values using this method are given in Table 7.

The MDLs must be sufficient to detect analytes at the required levels according to compliance monitoring regulation (Section 1.2). MDLs should be determined annually, when a new operator begins work or whenever, in the judgement of the analyst, a change in analytical performance caused by either a change in instrument hardware or operating conditions would dictate they be redetermined.

9.3 Assessing Laboratory Performance (mandatory)

9.3.1 Laboratory reagent blank (LRB) - The laboratory must analyze at least one LRB (Section 7.6.2) with each batch of 20 or fewer of samples of the same matrix. LRB data are used to assess contamination from the laboratory environment and to characterize spectral background from the reagents used in sample processing. LRB values that exceed the MDL indicate laboratory or reagent contamination should be suspected. When LRB values constitute 10% or more of the analyte level determined for a sample or is 2.2 times the analyte MDL whichever is greater, fresh aliquots of the samples must be prepared and analyzed again for the affected analytes after the source of contamination has been corrected and acceptable LRB values have been obtained.

9.3.2 Laboratory fortified blank (LFB) - The laboratory must analyze at least one LFB (Section 7.9) with each batch of samples. Calculate accuracy as percent recovery using the following equation:

$$R = \frac{LFB - LRB}{s} \times 100$$

where:

R = percent recovery
LFB = laboratory fortified blank
LRB = laboratory reagent blank
s = concentration equivalent of analyte added to fortify the LBR solution

If the recovery of any analyte falls outside the required control limits of 85-115%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

9.3.3 The laboratory must use LFB analyses data to assess laboratory performance against the required control limits of 85-115% (Section 9.3.2). When sufficient internal performance data become available (usually a minimum of 20-30 analyses), optional control limits can be developed from the mean percent recovery (x) and the standard deviation (S) of the mean percent recovery. These data can be used to establish the upper and lower control limits as follows:

UPPER CONTROL LIMIT = x + 3S
LOWER CONTROL LIMIT = x - 3S

The optional control limits must be equal to or better than the required control limits of 85-115%. After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.3.4 Instrument performance - For all determinations the laboratory must check instrument performance and verify that the instrument is properly calibrated on a continuing basis. To verify calibration run the calibration blank and calibration standards as surrogate samples immediately following each calibration routine, after every ten analyses and at the end of the sample run. The results of the analyses of the standards will indicate whether the calibration remains valid. The analysis of all analytes within the standard solutions must be within ±10% of calibration. If the calibration cannot be verified within the specified limits, the instrument must be recalibrated. (The instrument responses from the calibration check may be used for recalibration purposes, however, it must be verified before continuing sample analysis.) If the continuing calibration check is not confirmed within ±15%, the previous 10 samples must be reanalyzed after recalibration. If the sample matrix is responsible for the calibration drift, it is recommended that the previous 10 samples are reanalyzed in groups of five between calibration checks to prevent a similar drift situation from occurring.

9.4 Assessing Analyte Recovery and Data Quality

9.4.1 Sample homogeneity and the chemical nature of the sample matrix can affect analyte recovery and the quality of the data. Taking separate aliquots from the sample for replicate and fortified analyses can in some cases assess the effect. Unless otherwise specified by the data user, laboratory or program, the following laboratory fortified matrix (LFM) procedure (Section 9.4.2) is required.

9.4.2 The laboratory must add a known amount of analyte to a minimum of 10% of the routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis and for total recoverable determinations added prior to sample preparation. For water samples, the added analyte concentration must be the same as that used in the laboratory fortified blank (Section 7.9). For solid samples, the concentration added should be 100 mg/kg equivalent (200 µg/L in the analysis solution) except silver which should be limited to 50 mg/kg (Section 1.8). Over time, samples from all routine sample sources should be fortified.

9.4.3 Calculate the percent recovery for each analyte, corrected for background concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range of 70-130%. Recovery calculations are not required if the concentration of the analyte added is less than 30% of the sample background concentration. Percent recovery may be calculated in units appropriate to the matrix, using the following equation:

$$R = \frac{C_s - C}{s} \times 100$$

where:

- R = percent recovery
- C_s = fortified sample concentration
- C = sample background concentration
- s = concentration equivalent of analyte added to fortify the sample

9.4.4 If recovery of any analyte falls outside the designated range and laboratory performance for that analyte is shown to be in control (Section 9.3), the recovery problem encountered with the fortified sample is judged to be matrix related, not system related. The data user should be informed that the result for that analyte in the unfortified sample is suspect due to either the heterogeneous nature of the sample or an uncorrected matrix effect.

9.4.5 Internal standards responses - The analyst is expected to monitor the responses from the internal standards throughout the sample set being

analyzed. Ratios of the internal standards responses against each other should also be monitored routinely. This information may be used to detect potential problems caused by mass dependent drift, errors incurred in adding the internal standards or increases in the concentrations of individual internal standards caused by background contributions from the sample. The absolute response of any one internal standard must not deviate more than 60-125% of the original response in the calibration blank. If deviations greater than these are observed, flush the instrument with the rinse blank and monitor the responses in the calibration blank. If the responses of the internal standards are now within the limit, take a fresh aliquot of the sample, dilute by a further factor of two, add the internal standards and reanalyze. If after flushing the response of the internal standards in the calibration blank are out of limits, terminate the analysis and determine the cause of the drift. Possible causes of drift may be a partially blocked sampling cone or a change in the tuning condition of the instrument.

10.0 CALIBRATION AND STANDARDIZATION

10.1 Operating conditions - Because of the diversity of instrument hardware, no detailed instrument operating conditions are provided. The analyst is advised to follow the recommended operating conditions provided by the manufacturer. It is the responsibility of the analyst to verify that the instrument configuration and operating conditions satisfy the analytical requirements and to maintain quality control data verifying instrument performance and analytical results. Instrument operating conditions which were used to generate precision and recovery data for this method (Section 13.0) are included in Table 6.

10.2 Precalibration routine - The following precalibration routine must be completed prior to calibrating the instrument until such time it can be documented with periodic performance data that the instrument meets the criteria listed below without daily tuning.

10.2.1 Initiate proper operating configuration of instrument and data system. Allow a period of not less than 30 minutes for the instrument to warm up. During this process conduct mass calibration and resolution checks using the tuning solution. Resolution at low mass is indicated by magnesium isotopes 24, 25, and 26. Resolution at high mass is indicated by lead isotopes 206, 207, and 208. For good performance adjust spectrometer resolution to produce a peak width of approximately 0.75 amu at 5% peak height. Adjust mass calibration if it has shifted by more than 0.1 amu from unit mass.

10.2.2 Instrument stability must be demonstrated by running the tuning solution (Section 7.7) a minimum of five times with resulting relative standard deviations of absolute signals for all analytes of less than 5%.

10.3 Internal Standardization - Internal standardization must be used in all analyses to correct for instrument drift and physical interferences. A list of acceptable

internal standards is provided in Table 3. For full mass range scans, a minimum of three internal standards must be used. Procedures described in this method for general application, detail the use of five internal standards; scandium, yttrium, indium, terbium and bismuth. These were used to generate the precision and recovery data attached to this method. Internal standards must be present in all samples, standards and blanks at identical levels. This may be achieved by directly adding an aliquot of the internal standards to the CAL standard, blank or sample solution (Method A, Section 10.3), or alternatively by mixing with the solution prior to nebulization using a second channel of the peristaltic pump and a mixing coil (Method B, Section 10.3). The concentration of the internal standard should be sufficiently high that good precision is obtained in the measurement of the isotope used for data correction and to minimize the possibility of correction errors if the internal standard is naturally present in the sample. Depending on the sensitivity of the instrument, a concentration range of 20-200 µg/L of each internal standard is recommended. Internal standards should be added to blanks, samples and standards in a like manner, so that dilution effects resulting from the addition may be disregarded.

10.4 Calibration - Prior to initial calibration, set up proper instrument software routines for quantitative analysis. The instrument must be calibrated using one of the internal standard routines (Method A or B) described in Section 10.3. The instrument must be calibrated for the analytes to be determined using the calibration blank (Section 7.6.1) and calibration standards A and B (Section 7.4.1) prepared at one or more concentration levels. A minimum of three replicate integrations are required for data acquisition. Use the average of the integrations for instrument calibration and data reporting.

10.5 The rinse blank should be used to flush the system between solution changes for blanks, standards and samples. Allow sufficient rinse time to remove traces of the previous sample (Section 4.1.5). Solutions should be aspirated for 30 seconds prior to the acquisition of data to allow equilibrium to be established.

11.0 PROCEDURE

11.1 Aqueous Sample Preparation - Dissolved Analytes

11.1.1 For the determination of dissolved analytes in ground and surface waters, pipet an aliquot (≥20 mL) of the filtered, acid preserved sample into a 50 mL polypropylene centrifuge tube. Add an appropriate volume of (1+1) nitric acid to adjust the acid concentration of the aliquot to approximate a 1% (v/v) nitric acid solution (e.g., add 0.4 mL (1+1) HNO_3 to a 20 mL aliquot of sample). If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards, cap the tube and mix. The sample is now ready for analysis (Section 1.2). Allowance for sample dilution should be made in the calculations.

Note: If a precipitate is formed during acidification, transport, or storage, the sample aliquot must be treated using the procedure in Section 11.2 prior to analysis.

11.2 Aqueous Sample Preparation - Total Recoverable Analytes

11.2.1 For the "direct analysis" of total recoverable analytes in drinking water samples containing turbidity <1 NTU, treat an unfiltered acid preserved sample aliquot using the sample preparation procedure described in Section 11.1.1 while making allowance for sample dilution in the data calculation. For the determination of total recoverable analytes in all other aqueous samples or for preconcentrating drinking water samples prior to analysis follow the procedure given in Sections 11.2.2 through 11.2.8.

11.2.2 For the determination of total recoverable analytes in aqueous samples (other than drinking water with <1 NTU turbidity), transfer a 100 mL (±1 mL) aliquot from a well mixed, acid preserved sample to a 250 mL Griffin beaker (Sections 1.2, 1.3, 1.7, and 1.8). (When necessary, smaller sample aliquot volumes may be used.)

Note: If the sample contains undissolved solids >1%, a well mixed, acid preserved aliquot containing no more than 1 g particulate material should be cautiously evaporated to near 10 mL and extracted using the acid-mixture procedure described in Sections 11.3.3 through 11.3.7.

11.2.3 Add 2 mL (1+1) nitric acid and 1.0 mL of (1+1) hydrochloric acid to the beaker containing the measured volume of sample. Place the beaker on the hot plate for solution evaporation. The hot plate should be located in a fume hood and previously adjusted to provide evaporation at a temperature of approximately but no higher than 85°C. (See the following note.) The beaker should be covered with an elevated watch glass or other necessary steps should be taken to prevent sample contamination from the fume hood environment.

Note: For proper heating adjust the temperature control of the hot plate such that an uncovered Griffin beaker containing 50 mL of water placed in the center of the hot plate can be maintained at a temperature approximately but no higher than 85°C. (Once the beaker is covered with a watch glass the temperature of the water will rise to approximately 95°C.)

11.2.4 Reduce the volume of the sample aliquot to about 20 mL by gentle heating at 85°C. DO NOT BOIL. This step takes about two hours for a 100 mL aliquot with the rate of evaporation rapidly increasing as the sample volume approaches 20 mL. (A spare beaker containing 20 mL of water can be used as a gauge.)

11.2.5 Cover the lip of the beaker with a watch glass to reduce additional evaporation and gently reflux the sample for 30 minutes. (Slight boiling may occur, but vigorous boiling must be avoided to prevent loss of the $HCl-H_2O$ azeotrope.)

11.2.6 Allow the beaker to cool. Quantitatively transfer the sample solution to

a 50 mL volumetric flask or 50 mL class A stoppered graduated cylinder, make to volume with reagent water, stopper and mix.

11.2.7 Allow any undissolved material to settle overnight, or centrifuge a portion of the prepared sample until clear. (If after centrifuging or standing overnight the sample contains suspended solids that would clog the nebulizer, a portion of the sample may be filtered for their removal prior to analysis. However, care should be exercised to avoid potential contamination from filtration.)

11.2.8 Prior to analysis, adjust the chloride concentration by pipetting 20 mL of the prepared solution into a 50 mL volumetric flask, dilute to volume with reagent water and mix. (If the dissolved solids in this solution are >0.2%, additional dilution may be required to prevent clogging of the extraction and/or skimmer cones. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards and mix. The sample is now ready for analysis. Because the effects of various matrices on the stability of diluted samples cannot be characterized, all analyses should be performed as soon as possible after the completed preparation.

11.3 Solid Sample Preparation - Total Recoverable Analytes

11.3.1 For the determination of total recoverable analytes in solid samples, mix the sample thoroughly and transfer a portion (>20 g) to tared weighing dish, weigh the sample and record the wet weight (WW). (For samples with <35% moisture a 20 g portion is sufficient. For samples with moisture >35% a larger aliquot 50-100 g is required.) Dry the sample to a constant weight at 60°C and record the dry weight (DW) for calculation of percent solids (Section 12.6). (The sample is dried at 60°C to prevent the loss of mercury and other possible volatile metallic compounds, to facilitate sieving, and to ready the sample for grinding.)

11.3.2 To achieve homogeneity, sieve the dried sample using a 5-mesh polypropylene sieve and grind in a mortar and pestle. (The sieve, mortar and pestle should be cleaned between samples.) From the dried, ground material weigh accurately a representative 1.0 ± 0.01 g aliquot (W) of the sample and transfer to a 250 mL Phillips beaker for acid extraction.

11.3.3 To the beaker add 4 mL of (1+1) HNO_3 and 10 mL of (1+4) HCl. Cover the lip of the beaker with a watch glass. Place the beaker on a hot plate for reflux extraction of the analytes. The hot plate should be located in a fume hood and previously adjusted to provide a reflux temperature of approximately 95°C. (See the following note.)

Note: For proper heating adjust the temperature control of the hot plate such that an uncovered Griffin beaker containing 50 mL of water placed in the center of the hot plate can be maintained at a temperature approximately but no higher than 85°C. (Once the beaker is covered with a watch glass the temperature of the water will rise to approximately

95°C.) Also, a block digester capable of maintaining a temperature of 95°C and equipped with 250 mL constricted volumetric digestion tubes may be substituted for the hot plate and conical beakers in the extraction step.

11.3.4 Heat the sample and gently reflux for 30 minutes. Very slight boiling may occur, however vigorous boiling must be avoided to prevent loss of the HCl-H_2O azeotrope. Some solution evaporation will occur (3-4 mL).

11.3.5 Allow the sample to cool and quantitatively transfer the extract to a 100 mL volumetric flask. Dilute to volume with reagent water, stopper and mix.

11.3.6 Allow the sample extract solution to stand overnight to separate insoluble material or centrifuge a portion of the sample solution until clear. (If after centrifuging or standing overnight the extract solution contains suspended solids that would clog the nebulizer, a portion of the extract solution may be filtered for their removal prior to analysis. However, care should be exercised to avoid potential contamination from filtration.)

11.3.7 Prior to analysis, adjust the chloride concentration by pipetting 20 mL of the prepared solution into a 100 mL volumetric flask, dilute to volume with reagent water and mix. (If the dissolved solids in this solution are >0.2%, additional dilution may be required to prevent clogging of the extraction and/or skimmer cones. If the direct addition procedure (Method A, Section 10.3) is being used, add internal standards and mix. The sample extract is now ready for analysis. Because the effects of various matrices on the stability of diluted samples cannot be characterized, all analyses should be performed as soon as possible after the completed preparation.

Note: Determine the percent solids in the sample for use in calculations and for reporting data on a dry weight basis.

11.4 Sample Analysis

11.4.1 For every new or unusual matrix, it is highly recommended that a semi-quantitative analysis be carried out to screen the sample for elements at high concentration. Information gained from this may be used to prevent potential damage to the detector during sample analysis and to identify elements which may be higher than the linear range. Matrix screening may be carried out by using intelligent software, if available, or by diluting the sample by a factor of 500 and analyzing in a semi-quantitative mode. The sample should also be screened for background levels of all elements chosen for use as internal standards in order to prevent bias in the calculation of the analytical data.

11.4.2 Initiate instrument operating configuration. Tune and calibrate the instrument for the analytes of interest (Section 10.0).

11.4.3 Establish instrument software run procedures for quantitative analysis. For all sample analyses, a minimum of three replicate integrations are required for data acquisition. Use the average of the integrations for data reporting.

11.4.4 All masses which might affect data quality must be monitored during the analytical run. As a minimum, those masses prescribed in Table 4 must be monitored in the same scan as is used for the collection of the data. This information should be used to correct the data for identified interferences.

11.4.5 During the analysis of samples, the laboratory must comply with the required quality control described in Sections 9.3 and 9.4. Only for the determination of dissolved analytes or the "direct analysis" of drinking water with turbidity of <1 NTU is the sample digestion step of the LRB, LFB, and LFM not required.

11.4.6 The rinse blank should be used to flush the system between samples. Allow sufficient time to remove traces of the previous sample or a minimum of one minute (Section 4.1.5). Samples should be aspirated for 30 seconds prior to the collection of data.

11.4.7 Samples having concentrations higher than the established linear dynamic range should be diluted into range and reanalyzed. The sample should first be analyzed for the trace elements in the sample, protecting the detector from the high concentration elements, if necessary, by the selection of appropriate scanning windows. The sample should then be diluted for the determination of the remaining elements. Alternatively, the dynamic range may be adjusted by selecting an alternative isotope of lower natural abundance, provided quality control data for that isotope have been established. The dynamic range must not be adjusted by altering instrument conditions to an uncharacterized state.

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 Elemental equations recommended for sample data calculations are listed in Table 5. Sample data should be reported in units of µg/L for aqueous samples or mg/kg dry weight for solid samples. Do not report element concentrations below the determined MDL.

12.2 For data values less than 10, two significant figures should be used for reporting element concentrations. For data values greater than or equal to 10, three significant figures should be used.

12.3 For aqueous samples prepared by total recoverable procedure (Section 11.2), multiply solution concentrations by the dilution factor 1.25. If additional dilutions were made to any samples or an aqueous sample was prepared using the acid-mixture procedure described in Section 11.3, the appropriate factor should be applied to the calculated sample concentrations.

12.4 For total recoverable analytes in solid samples (Section 11.3), round the solution analyte concentrations (µg/L in the analysis solution) as instructed in Section 12.2. Multiply the µ/L concentrations in the analysis solution by the factor 0.005 to calculate the mg/L analyte concentration in the 100 mL extract solution. (If additional dilutions were made to any samples, the appropriate factor should be applied to calculate analyte concentrations in the extract solution.) Report the data up to three significant figures as mg/kg dry-weight basis unless specified otherwise by the program or data user. Calculate the concentration using the equation below:

$$\text{Sample Conc. (mg/kg) dry-weight basis} = \frac{C \times V}{W}$$

where:

C = Concentration in the extract (mg/L)
V = Volume of extract (L, 100 mL = 0.1L)
W = Weight of sample aliquot extracted (g x 0.001 = kg)

Do not report analyte data below the estimated solids MDL or an adjusted MDL because of additional dilutions required to complete the analysis.

12.5 To report percent solids in solid samples (Sect. 11.3) calculate as follows:

$$\%\ \text{solids (S)} = \frac{DW}{WW} \times 100$$

where:

DW = Sample weight (g) dried at 60°C
WW = Sample weight (g) before drying

Note: If the data user, program or laboratory requires that the reported percent solids be determined by drying at 105°C, repeat the procedure given in Section 11.3 using a separate portion (>20 g) of the sample and dry to constant weight at 103-105°C.

12.6 Data values should be corrected for instrument drift or sample matrix induced interferences by the application of internal standardization. Corrections for characterized spectral interferences should be applied to the data. Chloride interference corrections should be made on all samples, regardless of the addition of hydrochloric acid, as the chloride ion is a common constituent of environmental samples.

12.7 If an element has more than one monitored isotope, examination of the concentration calculated for each isotope, or the isotope ratios, will provide useful information for the analyst in detecting a possible spectral interference.

Consideration should therefore be given to both primary and secondary isotopes in the evaluation of the element concentration. In some cases, secondary isotopes may be less sensitive or more prone to interferences than the primary recommended isotopes, therefore differences between the results do not necessarily indicate a problem with data calculated for the primary isotopes.

12.8 The QC data obtained during the analyses provide an indication of the quality of the sample data and should be provided with the sample results.

13.0 METHOD PERFORMANCE

13.1 Instrument operating conditions used for single laboratory testing of the method are summarized in Table 6. Total recoverable digestion and "direct analysis" MDLs determined using the procedure described in Section 9.2.4, are listed in Table 7.

13.2 Data obtained from single laboratory testing of the method are summarized in Table 9 for five water samples representing drinking water, surface water, ground water and waste effluent. Samples were prepared using the procedure described in Section 11.2. For each matrix, five replicates were analyzed and the average of the replicates used for determining the sample background concentration for each element. Two further pairs of duplicates were fortified at different concentration levels. For each method element, the sample background concentration, mean percent recovery, the standard deviation of the percent recovery and the relative percent difference between the duplicate fortified samples are listed in Table 8.

13.3 Data obtained from single laboratory testing of the method are summarized in Table 10 for three solid samples consisting of SRM 1645 River Sediment, EPA Hazardous Soil and EPA Electroplating Sludge. Samples were prepared using the procedure described in Section 11.3. For each method element, the sample background concentration, mean percent recovery, the standard deviation of the percent recovery and the relative percent difference between the duplicate fortified samples were determined as for Section 13.2.

13.4 Data obtained from single laboratory testing of the method for drinking water analysis using the "direct analysis" procedure (Section 11.2.1) are given in Table 11. Three drinking water samples of varying hardness collected from Regions 4, 6, and 10 were fortified to contain 1 µg/L of all metal primary contaminants, except selenium, which was added to a concentration of 20 µg/L. For each matrix, four replicate aliquots were analyzed to determine the sample background concentration of each analyte and four fortified aliquots were analyzed to determine mean percent recovery in each matrix. Listed in the Table 11 are the average mean percent recovery of each analyte in the three matrices and the standard deviation of the mean percent recoveries.

13.5 Listed in Table 12 are the regression equations for precision and bias developed from the joint USEPA/Association of Official Analytical Chemists (AOAC) multilaboratory validation study conducted on this method. These equations

were developed from data received from 13 laboratories on reagent water, drinking water and ground water. Listed in Tables 13 and 14, respectively, are the precision and recovery data from a wastewater digestate supplied to all laboratories and from a wastewater of the participant's choice. For a complete review of the study see Reference 11, Section 16.0 of this method.

14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction", available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street N.W., Washington D.C. 20036, (202)872-4477.

15.0 WASTE MANAGEMENT

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult "The Waste Management Manual for Laboratory Personnel", available from the American Chemical Society at the address listed in the Section 14.2.

16.0 REFERENCES

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17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

TABLE 1: ESTIMATED INSTRUMENT DETECTION LIMITS

Element	Recommended Analytical Mass	Scanning Mode[1]	Selection Ion Monitoring Mode[2,3]
Aluminum	27	0.05	0.02
Antimony	123	0.08	0.008
Arsenic(3)	75	0.9	0.02
Barium	137	0.5	0.03
Beryllium	9	0.1	0.02
Cadmium	111	0.1	0.02
Chromium	52	0.07	0.04
Cobalt	59	0.03	0.002
Copper	63	0.03	0.004
Lead	206, 207, 208	0.08	0.015
Manganese	55	0.1	0.007
Mercury	202	n.a	0.2
Molybdenum	98	0.1	0.005
Nickel	60	0.2	0.07
Selenium(3)	82	5	1.3
Silver	107	0.05	0.004
Thallium	205	0.09	0.014
Thorium	232	0.03	0.005
Uranium	238	0.02	0.005
Vanadium	51	0.02	0.006
Zinc	66	0.2	0.07

Instrument detection limits (3σ) estimated from seven replicate integrations of the blank (1% v/v nitric acid) following calibration of the instrument with three replicate integrations of a multi-element standard.

[1]Instrument operating conditions and data acquisition mode are given in Table 6.

[2]IDLs determined using state-of-the-art instrumentation (1994). Data for ^{75}As, ^{77}Se, and ^{82}Se were acquired using a dwell time of 4.096 seconds with 1500 area count per sec ^{83}Kr present in argon supply. All other data were acquired using a dwell time of 1.024 seconds per AMU monitored.

TABLE 2: COMMON MOLECULAR ION INTERFERENCES IN ICP-MS

BACKGROUND MOLECULAR IONS

Molecular Ion	Mass	Element Interference[a]
NH^+	15	
OH^+	17	
OH_2^+	18	
C_2^+	24	
CN^+	26	
CO^+	28	
N_2^+	28	
N_2H^+	29	
NO^+	30	
NOH^+	31	
O_2^+	32	
O_2H^+	33	
$^{36}ArH^+$	37	
$^{38}ArH^+$	39	
$^{40}ArH^+$	41	
CO_2^+	44	
CO_2H^+	45	Sc
ArC^+, ArO^+	52	Cr
ArN^+	54	Cr
$ArNH^+$	55	Mn
ArO^+	56	
$ArOH^+$	57	
$^{40}Ar^{36}Ar^+$	76	Se
$^{40}Ar^{38}Ar^+$	78	Se
$^{40}Ar^+$	80	Se

[a]method elements or internal standards affected by the molecular ions.

TABLE 2: COMMON MOLECULAR ION INTERFERENCES IN ICP-MS (Cont'd)

MATRIX MOLECULAR IONS

Molecular Ion		Mass	Element Interference[a]
Bromide[12]			
	$^{81}BrH^+$	82	Se
	$^{79}BrO^+$	95	Mo
	$^{81}BrO^+$	97	Mo
	$^{81}BrOH^+$	98	Mo
	$Ar^{81}Br^+$	121	Sb
Chloride			
	$^{35}ClO^+$	51	V
	$^{35}ClOH^+$	52	Cr
	$^{37}ClO^+$	53	Cr
	$^{37}ClOH^+$	54	Cr
	$Ar^{35}Cl^+$	75	As
	$Ar^{37}Cl^+$	77	Se
Sulphate			
	$^{32}SO^+$	48	
	$^{32}SOH^+$	49	
	$^{34}SO^+$	50	V, Cr
	$^{34}SOH^+$	51	V
	SO_2^+, S_2^+	64	Zn
	$Ar^{32}S^+$	72	
	$Ar^{34}S^+$	74	
Phosphate			
	PO^+	47	
	POH^+	48	
	PO_2^+	63	Cu
	ArP^+	71	
Group I, II Metals			
	$ArNa^+$	63	Cu
	ArK^+	79	
	$ArCa^+$	80	

TABLE 2: COMMON MOLECULAR ION INTERFERENCES IN ICP-MS (Cont'd)

MATRIX MOLECULAR IONS

Molecular Ion	Mass	Element Interference[a]
Matrix Oxides[*]		
TiO	62-66	Ni, Cu, Zn
ZrO	106-112	Ag, Cd
MoO	108-116	Cd

[*]Oxide interferences will normally be very small and will only impact the method elements when present at relatively high concentrations. Some examples of matrix oxides are listed of which the analyst should be aware. It is recommended that Ti and Zr isotopes are monitored in solid waste samples, which are likely to contain high levels of these elements. Mo is monitored as a method analyte.

TABLE 3: INTERNAL STANDARDS AND LIMITATIONS OF USE

Internal Standard	Mass	Possible Limitation
^{6}Lithium	6	a
Scandium	45	polyatomic ion interference
Yttrium	89	a,b
Rhodium	103	
Indium	115	isobaric interference by Sn
Terbium	159	
Holmium	165	
Lutetium	175	
Bismuth	209	a

a May be present in environmental samples.

b In some instruments Yttrium may form measurable amounts of YO^+ (105 amu)and YOH^+ (106 amu). If this is the case, care should be taken in the use of the cadmium elemental correction equation.

Internal standards recommended for use with this method are shown in bold face. Preparation procedures for these are included in Section 7.3.

TABLE 4: RECOMMENDED ANALYTICAL ISOTOPES AND ADDITIONAL MASSES WHICH MUST BE MONITORED

Isotope	Element of Interest
27	Aluminum
121, 123	Antimony
75	Arsenic
135, 137	Barium
9	Beryllium
106, 108, 111, 114	Cadmium
52, 53	Chromium
59	Cobalt
63, 65	Copper
206, 207, 208	Lead
55	Manganese
95, 97, 98	Molybdenum
60, 62	Nickel
77, 82	Selenium
107, 109	Silver
203, 205	Thallium
232	Thorium
238	Uranium
51	Vanadium
66, 67, 68	Zinc
83	Krypton
99	Ruthenium
105	Palladium
118	Tin

NOTE: Isotopes recommended for analytical determination are underlined.

TABLE 5: RECOMMENDED ELEMENTAL EQUATIONS FOR DATA CALCULATIONS

Element	Elemental Equation	Note
Al	$(1.000)\ (^{27}C)$	
Sb	$(1.000)\ (^{123}C)$	
As	$(1.000)\ (^{75}C)-(3.127)\ [(^{77}C)-(0.815)\ (^{82}C)]$	(1)
Ba	$(1.000)\ (^{137}C)$	
Be	$(1.000)\ (^{9}C)$	
Cd	$(1.000)\ (^{111}C)-(1.073)\ [(^{108}C)-(0.712)\ (^{106}C)]$	(2)
Cr	$(1.000)\ (^{52}C)$	(3)
Co	$(1.000)\ (^{59}C)$	
Cu	$(1.000)\ (^{63}C)$	
Pb	$(1.000)\ (^{206}C)+(1.000)\ [(^{207}C)+(1.000)\ (^{208}C)]$	(4)
Mn	$(1.000)\ (^{55}C)$	
Mo	$(1.000)\ (^{98}C)-(0.146)\ (^{99}C)$	(5)
Ni	$(1.000)\ (^{60}C)$	
Se	$(1.000)\ (^{82}C)$	(6)
Ag	$(1.000)\ (^{107}C)$	
Tl	$(1.000)\ (^{205}C)$	
Th	$(1.000)\ (^{232}C)$	
U	$(1.000)\ (^{238}C)$	
V	$(1.000)\ (^{51}C)-(3.127)\ [(^{53}C)-(0.113)\ (^{52}C)]$	(7)
Zn	$(1.000)\ (^{66}C)$	

TABLE 5: RECOMMENDED ELEMENTAL EQUATIONS FOR DATA CALCULATIONS

Element	Elemental Equation	Note
Bi	(1.000) (^{209}C)	
In	(1.000) (^{209}C)-(0.016) (^{118}C)	(8)
Sc	(1.000) (^{45}C)	
Tb	(1.000) (^{159}C)	
Y	(1.000) (^{89}C)	

C - Calibration blank subtracted counts at specified mass.

(1) - Correction for chloride interference with adjustment for ^{77}Se. ArCl 75/77 ratio may be determined from the reagent blank. Isobaric mass 82 must be from Se only and not BrH^+.

(2) - Correction for MoO interference. Isobaric mass 106 must be from Cd only not ZrO^+. An additional isobaric elemental correction should be made if palladium is present.

(3) - In 0.4% v/v HCl, the background from ClOH will normally be small. However the contribution may be estimated from the reagent blank. Isobaric mass must be from Cr only not ArC^+.

(4) - Allowance for isotopic variability of lead isotopes.

(5) - Isobaric elemental correction for ruthenium.

(6) - Some argon supplies contain krypton as an impurity. Selenium is corrected for ^{82}Kr by background subtraction.

(7) - Correction for chloride interference with adjustment for ^{53}Cr. ClO 51/53 ratio may be determined from the reagent blank. Isobaric mass 52 must be from Cr only not ArC^+.

(8) - Isobaric elemental correction for tin.

TABLE 6: INSTRUMENT OPERATING CONDITIONS FOR PRECISION AND RECOVERY DATA[1]

Instrument	VG PlasmaQuad Type I
Plasma foward power	1.35 kW
Coolant flow rate	13.5 L/min.
Auxillary flow rate	0.6 L/min.
Nebulizer flow rate	0.78 L/min.
Solution uptake rate	0.6 mL/min.
Spray chamber temperature	15°C
Data Acquistion	
Detector mode	Pulse counting
Replicate integrations	3
Mass range	8-240 amu
Dwell time	320 µs
Number of MCA channels	2048
Number of scan sweeps	85
Total acquisition time	3 minutes per sample

[1]The described instrument and operating conditions were used to determine the scanning mode MDL data listed in Table 7 and the precision and recovery data given in Tables 9 and 10.

TABLE 7: METHOD DETECTION LIMITS

	Scanning Mode[1] Total Recoverable		Selection Ion Monitoring Mode[2] Total Recoverable	Selection Ion Monitoring Mode[2] Direct Analysis[3]
^{AMU}Element	Aqueous µg/L	Solids mg/kg	Aqueous µg/L	Aqueous µg/L
^{27}Al	1.0	0.4	1.7	0.04
^{123}Sb	0.4	0.2	0.04	0.02
^{75}As	1.4	0.6	0.4	0.1
^{137}Ba	0.8	0.4	0.04	0.04
^{9}Be	0.3	0.1	0.02	0.03
^{111}Cd	0.5	0.2	0.03	0.03
^{52}Cr	0.9	0.4	0.08	0.08
^{59}Co	0.09	0.04	0.004	0.003
^{63}Cu	0.5	0.2	0.02	0.01
^{206,207,208}Pb	0.6	0.3	0.05	0.02
^{55}Mn	0.1	0.05	0.02	0.04
^{202}Hg	n.a.	n.a.	n.a	0.2
^{98}Mo	0.3	0.1	0.01	0.01
^{60}Ni	0.5	0.2	0.06	0.03
^{82}Se	7.9	3.2	2.1	0.5
^{107}Ag	0.1	0.05	0.005	0.005
^{205}Tl	0.3	0.1	0.02	0.01
^{232}Th	0.1	0.05	0.02	0.01
^{238}U	0.1	0.05	0.01	0.01
^{51}V	2.5	1.0	0.9	0.05
^{66}Zn	1.8	0.7	0.1	0.2

[1]Data acquisition mode given in Table 6. Total recoverable MDL concentrations are computed for original matrix with allowance for sample dilution during preparation. Listed MDLs for solids calculated from determined aqueous MDLs.

[2]MDLs determined using state-of-the-art instrumentation (1994). Data for ^{75}As, ^{77}Se, and ^{82}Se were acquired using a dwell time of 4.096 seconds with 1500 area count per seconds ^{83}Kr present in argon supply. All other data were acquired using a dwell time of 1.024 seconds per AMU monitored.

[3]MDLs were determined from analysis of seven undigested aqueous sample aliquots.

n.a. - Not applicable. Total recoverable digestion not suitable for organo-mercury compounds.

TABLE 8: ACCEPTANCE LIMITS FOR QC CHECK SAMPLE

METHOD PERFORMANCE (μg/L)[1]

Element	QC Check Sample Conc.	Average Recovery	Standard Deviation[2] (S_r)	Acceptance Limits[3] μg/L
Aluminum	100	100.4	5.49	84-117
Antimony	100	99.9	2.40	93-107
Arsenic	100	101.6	3.66	91-113
Barium	100	99.7	2.64	92-108
Beryllium	100	105.9	4.13	88-112[4]
Cadmium	100	100.8	2.32	94-108
Chromium	100	102.3	3.91	91-114
Cobalt	100	97.7	2.66	90-106
Copper	100	100.3	2.11	94-107
Lead	100	104.0	3.42	94-114
Manganese	100	98.3	2.71	90-106
Molybdenum	100	101.0	2.21	94-108
Nickel	100	100.1	2.10	94-106
Selenium	100	103.5	5.67	86-121
Silver	100	101.1	3.29	91-111[5]
Thallium	100	98.5	2.79	90-107
Thorium	100	101.4	2.60	94-109
Uranium	100	102.6	2.82	94-111
Vanadium	100	100.3	3.26	90-110
Zinc	100	105.1	4.57	91-119

[1]Method performance characteristics calculated using regression equations from collaborative study, Reference 11.

[2]Single-analyst standard deviation, S_r.

[3]Acceptance limits calculated as average recovery ± three standard deviations.

[4]Acceptance limits centered at 100% recovery.

[5]Statistics estimated from summary statistics at 48 and 64 μg/L.

TABLE 9: PRECISION AND RECOVERY DATA IN AQUEOUS MATRICES

DRINKING WATER

Element	Sample Conc. µg/L	Low Spike µg/L	Average Recovery R (%)	S (R)	RPD	High Spike µg/L	Average Recovery R (%)	S (R)	RPD
Al	175	50	115.8	5.9	0.4	200	102.7	1.6	1.1
Sb	<0.4	10	99.1	0.7	2.0	100	100.8	0.7	2.0
As	<1.4	50	99.7	0.8	2.2	200	102.5	1.1	2.9
Ba	43.8	50	94.8	3.9	5.8	200	95.6	0.8	1.7
Be	<0.3	10	113.5	0.4	0.9	100	111.0	0.7	1.8
Cd	<0.5	10	97.0	2.8	8.3	100	101.5	0.4	1.0
Cr	<0.9	10	111.0	3.5	9.0	100	99.5	0.1	0.2
Co	0.11	10	94.4	0.4	1.1	100	93.6	0.5	1.4
Cu	3.6	10	101.8	8.8	17.4	100	91.6	0.3	0.3
Pb	0.87	10	97.8	2.0	2.8	100	99.0	0.8	2.2
Mn	0.96	10	96.9	1.8	4.7	100	95.8	0.6	1.8
Mo	1.9	10	99.4	1.6	3.4	100	98.6	0.4	1.0
Ni	1.9	10	100.2	5.7	13.5	100	95.2	0.5	1.3
Se	<7.9	50	99.0	1.8	5.3	200	93.5	3.5	10.7
Ag	<0.1	50	100.7	1.5	4.2	200	99.0	0.4	1.0
Tl	<0.3	10	97.5	0.4	1.0	100	98.5	1.7	4.9
Th	<0.1	10	109.0	0.7	1.8	100	106.0	1.4	3.8
U	0.23	10	110.7	1.4	3.5	100	107.8	0.7	1.9
V	<2.5	50	101.4	0.1	0.4	200	97.5	0.7	2.1
Zn	5.2	50	103.4	3.3	7.7	200	96.4	0.5	1.0

S (R) Standard deviation of percent recovery.
RPD Relative percent difference between duplicate spike determinations.
< Sample concentration below established method detection limit.

TABLE 9: PRECISION AND RECOVERY DATA IN AQUEOUS MATRICES (Cont'd)

WELL WATER

Element	Sample Conc. µg/L	Low Spike µg/L	Average Recovery R (%)	S (R)	RPD	High Spike µg/L	Average Recovery R (%)	S (R)	RPD
Al	34.3	50	100.1	3.9	0.8	200	102.6	1.1	1.3
Sb	0.46	10	98.4	0.9	1.9	100	102.5	0.7	1.9
As	<1.4	50	110.0	6.4	16.4	200	101.3	0.2	0.5
Ba	106	50	95.4	3.9	3.3	200	104.9	1.0	1.6
Be	<0.3	10	104.5	0.4	1.0	100	101.4	1.2	3.3
Cd	106	10	88.6	1.7	3.8	100	98.6	0.6	1.6
Cr	<0.9	10	111.0	0.0	0.0	100	103.5	0.4	1.0
Co	2.4	10	100.6	1.0	1.6	100	104.1	0.4	0.9
Cu	37.4	10	104.3	5.1	1.5	100	100.6	0.8	1.5
Pb	3.5	10	95.2	2.5	1.5	100	99.5	1.4	3.9
Mn	2770	10	*	*	1.8	100	*	*	0.7
Mo	2.1	10	103.8	1.1	1.6	100	102.9	0.7	1.9
Ni	11.4	10	116.5	6.3	6.5	100	99.6	0.3	0.0
Se	<7.9	50	127.3	8.4	18.7	200	101.3	0.2	0.5
Ag	<0.1	50	99.2	0.4	1.0	200	101.5	1.4	3.9
Tl	<0.3	10	93.9	0.1	0.0	100	100.4	1.8	5.0
Th	<0.1	10	103.0	0.7	1.9	100	104.5	1.8	4.8
U	1.8	10	106.0	1.1	1.6	100	109.7	2.5	6.3
V	<2.5	50	105.3	0.8	2.1	200	105.8	0.2	0.5
Zn	554	50	*	*	1.2	200	102.1	5.5	3.2

S (R) Standard deviation of percent recovery.
RPD Relative percent difference between duplicate spike determinations.
< Sample concentration below established method detection limit.
* Spike concentration <10% of sample background concentration.

TABLE 9: PRECISION AND RECOVERY DATA IN AQUEOUS MATRICES (Cont'd)

POND WATER

Element	Sample Conc. µg/L	Low Spike µg/L	Average Recovery R (%)	S (R)	RPD	High Spike µg/L	Average Recovery R (%)	S (R)	RPD
Al	610	50	*	*	1.7	200	78.2	9.2	5.5
Sb	<0.4	10	101.1	1.1	2.9	100	101.5	3.0	8.4
As	<1.4	50	100.8	2.0	5.6	200	96.8	0.9	2.6
Ba	28.7	50	102.1	1.8	2.4	200	102.9	3.7	9.0
Be	<0.3	10	109.1	0.4	0.9	100	114.4	3.9	9.6
Cd	<0.5	10	106.6	3.2	8.3	100	105.8	2.8	7.6
Cr	2.0	10	107.0	1.0	1.6	100	100.0	1.4	3.9
Co	0.79	10	101.6	1.1	2.7	100	101.7	1.8	4.9
Cu	5.4	10	107.5	1.4	1.9	100	98.1	2.5	6.8
Pb	1.9	10	108.4	1.5	3.2	100	106.1	0.0	0.0
Mn	617	10	*	*	1.1	100	139.0	11.1	4.0
Mo	0.98	10	104.2	1.4	3.5	100	104.0	2.1	5.7
Ni	2.5	10	102.0	2.3	4.7	100	102.5	2.1	5.7
Se	<7.9	50	102.7	5.6	15.4	200	105.5	1.4	3.8
Ag	0.12	50	102.5	0.8	2.1	200	105.2	2.7	7.1
Tl	<0.3	10	108.5	3.2	8.3	100	105.0	2.8	7.6
Th	0.19	10	93.1	3.5	10.5	100	93.9	1.6	4.8
U	0.30	10	107.0	2.8	7.3	100	107.2	1.8	4.7
V	3.5	50	96.1	5.2	14.2	200	101.5	0.2	0.5
Zn	6.8	50	99.8	1.7	3.7	200	100.1	2.8	7.7

S (R) Standard deviation of percent recovery.
RPD Relative percent difference between duplicate spike determinations.
< Sample concentration below established method detection limit.
* Spike concentration <10% of sample background concentration.

TABLE 9: PRECISION AND RECOVERY DATA IN AQUEOUS MATRICES (Cont'd)

SEWAGE TREATMENT PRIMARY EFFLUENT

Element	Sample Conc. µg/L	Low Spike µg/L	Average Recovery R (%)	S (R)	RPD	High Spike µg/L	Average Recovery R (%)	S (R)	RPD
Al	1150	50	*	*	3.5	200	100.0	13.8	1.5
Sb	1.5	10	95.7	0.4	0.9	100	104.5	0.7	1.9
As	<1.4	50	104.2	4.5	12.3	200	101.5	0.7	2.0
Ba	202	50	79.2	9.9	2.5	200	108.6	4.6	5.5
Be	<0.3	10	110.5	1.8	4.5	100	106.4	0.4	0.9
Cd	9.2	10	101.2	1.3	0.0	100	102.3	0.4	0.9
Cr	128	10	*	*	1.5	100	102.1	1.7	0.4
Co	13.4	10	95.1	2.7	2.2	100	99.1	1.1	2.7
Cu	171	10	*	*	2.4	100	105.2	7.1	0.7
Pb	17.8	10	95.7	3.8	1.1	100	102.7	1.1	2.5
Mn	199	10	*	*	1.5	100	103.4	2.1	0.7
Mo	136	10	*	*	1.4	100	105.7	2.4	2.1
Ni	84.0	10	88.4	16.3	4.1	100	98.0	0.9	0.0
Se	<7.9	50	112.0	10.9	27.5	200	108.8	3.0	7.8
Ag	10.9	50	97.1	0.7	1.5	200	102.6	1.4	3.7
Tl	<0.3	10	97.5	0.4	1.0	100	102.0	0.0	0.0
Th	0.11	10	15.4	1.8	30.3	100	29.3	0.8	8.2
U	0.71	10	109.4	1.8	4.3	100	109.3	0.7	1.8
V	<2.5	50	90.9	0.9	0.6	200	99.4	2.1	6.0
Zn	163	50	85.8	3.3	0.5	200	102.0	1.5	1.9

S (R) Standard deviation of percent recovery.
RPD Relative percent difference between duplicate spike determinations.
< Sample concentration below established method detection limit.
* Spike concentration <10% of sample background concentration.

TABLE 9: PRECISION AND RECOVERY DATA IN AQUEOUS MATRICES (Cont'd)

INDUSTRIAL EFFLUENT

Element	Sample Conc. µg/L	Low Spike µg/L	Average Recovery R (%)	S (R)	RPD	High Spike µg/L	Average Recovery R (%)	S (R)	RPD
Al	44.7	50	98.8	8.7	5.7	200	90.4	2.1	2.2
Sb	2990	10	*	*	0.3	100	*	*	0.0
As	<1.4	50	75.1	1.8	6.7	200	75.0	0.0	0.0
Ba	100	50	96.7	5.5	3.4	200	102.9	1.1	0.7
Be	<0.3	10	103.5	1.8	4.8	100	100.0	0.0	0.0
Cd	10.1	10	106.5	4.4	2.4	100	97.4	1.1	2.8
Cr	171	10	*	*	0.0	100	127.7	2.4	1.7
Co	1.3	10	90.5	3.2	8.7	100	90.5	0.4	1.3
Cu	101	10	*	*	0.9	100	92.5	2.0	1.6
Pb	294	10	*	*	2.6	100	108.4	2.1	0.0
Mn	154	10	*	*	2.8	100	103.6	3.7	1.6
Mo	1370	10	*	*	1.4	100	*	*	0.7
Ni	17.3	10	107.4	7.4	5.0	100	88.2	0.7	1.0
Se	15.0	50	129.5	9.3	15.1	200	118.3	1.9	3.6
Ag	<0.1	50	91.8	0.6	1.7	200	87.0	4.9	16.1
Tl	<0.3	10	90.5	1.8	5.5	100	98.3	1.0	2.8
Th	0.29	10	109.6	1.2	2.7	100	108.7	0.0	0.0
U	0.17	10	104.8	2.5	6.6	100	109.3	0.4	0.9
V	<2.5	50	74.9	0.1	0.3	200	72.0	0.0	0.0
Zn	43.4	50	85.0	4.0	0.6	200	97.6	1.0	0.4

S (R) Standard deviation of percent recovery.
RPD Relative percent difference between duplicate spike determinations.
< Sample concentration below established method detection limit.
* Spike concentration <10% of sample background concentration.

TABLE 10: PRECISION AND RECOVERY DATA IN SOLID MATRICES

EPA HAZARDOUS SOIL #884

Element	Sample Conc. (mg/kg)	Low+ Spike (mg/kg)	Average Recovery R (%)	S (R)	RPD	High+ Spike (mg/kg)	Average Recovery R (%)	S (R)	RPD
Al	5170	20	*	*	–	100	*	*	–
Sb	5.4	20	69.8	2.5	4.7	100	70.4	1.8	6.5
As	8.8	20	104.7	5.4	9.1	100	102.2	2.2	5.4
Ba	113	20	54.9	63.6	18.6	100	91.0	9.8	0.5
Be	0.6	20	100.1	0.6	1.5	100	102.9	0.4	1.0
Cd	1.8	20	97.3	1.0	1.4	100	101.7	0.4	1.0
Cr	83.5	20	86.7	16.1	8.3	100	105.5	1.3	0.0
Co	7.1	20	98.8	1.2	1.9	100	102.9	0.7	1.8
Cu	115	20	86.3	13.8	3.4	100	151.7	4.2	4.6
Pb	152	20	85.0	45.0	13.9	100	85.2	25.7	23.7
Mn	370	20	*	*	12.7	100	95.2	10.4	2.2
Mo	4.8	20	95.4	1.5	2.9	100	102.3	0.7	2.0
Ni	19.2	20	101.7	3.8	1.0	100	100.7	0.8	0.8
Se	<3.2	20	79.5	7.4	26.4	100	94.8	9.4	26.5
Ag	1.1	20	96.1	0.6	0.5	100	97.9	0.8	2.3
Tl	0.24	20	94.3	1.1	3.1	100	76.0	1.0	2.9
Th	1.0	20	69.8	0.6	1.3	100	102.9	2.2	7.9
U	1.1	20	100.1	0.2	0.0	100	106.7	0.0	0.0
V	17.8	20	109.2	4.2	2.3	100	113.4	1.3	2.4
Zn	128	20	87.0	27.7	5.5	100		12.9	14.1

S (R) Standard deviation of percent recovery.
RPD Relative percent difference between duplicate spike determinations.
< Sample concentration below established method detection limit.
* Spike concentration <10% of sample background concentration.
– Not determined.
+ Equivalent.

TABLE 10: PRECISION AND RECOVERY DATA IN SOLID MATRICES

NBS 1645 RIVER SEDIMENT

Element	Sample Conc. (mg/kg)	Low+ Spike (mg/kg)	Average Recovery R (%)	S (R)	RPD	High+ Spike (mg/kg)	Average Recovery R (%)	S (R)	RPD
Al	5060	20	*	*	–	100	*	*	–
Sb	21.8	20	73.9	6.5	9.3	100	81.2	1.5	3.9
As	67.2	20	104.3	13.0	7.6	100	107.3	2.1	2.9
Ba	54.4	20	105.6	4.9	2.8	100	98.6	2.2	3.9
Be	0.59	20	88.8	0.2	0.5	100	87.9	0.1	0.2
Cd	8.3	20	92.9	0.4	0.0	100	95.7	1.4	3.9
Cr	29100	20	*	*	–	100	*	*	–
Co	7.9	20	97.6	1.3	2.6	100	103.1	0.0	0.0
Cu	112	20	121.0	9.1	1.5	100	105.2	2.2	1.8
Pb	742	20	*	*	–	100	–	–	–
Mn	717	20	*	*	–	100	–	–	–
Mo	17.1	20	89.8	8.1	12.0	100	98.4	0.7	0.9
Ni	41.8	20	103.7	6.5	4.8	100	102.2	0.8	0.0
Se	<3.2	20	108.3	14.3	37.4	100	93.9	5.0	15.1
Ag	1.8	20	94.8	1.6	4.3	100	96.2	0.7	1.9
Tl	1.2	20	91.2	1.3	3.6	100	94.4	0.4	1.3
Th	0.90	20	91.3	0.9	2.6	100	92.3	0.9	2.8
U	0.79	20	95.6	1.8	5.0	100	98.5	1.2	3.5
V	21.8	20	91.8	4.6	5.7	100	100.7	0.6	0.8
Zn	1780	20	*	*	–	100	*	*	–

S (R) Standard deviation of percent recovery.
RPD Relative percent difference between duplicate spike determinations.
< Sample concentration below established method detection limit.
* Spike concentration <10% of sample background concentration.
– Not determined.
+ Equivalent.

TABLE 10: PRECISION AND RECOVERY DATA IN SOLID MATRICES

EPA ELECTROPLATING SLUDGE #286

Element	Sample Conc. (mg/kg)	Low+ Spike (mg/kg)	Average Recovery R (%)	S (R)	RPD	High+ Spike (mg/kg)	Average Recovery R (%)	S (R)	RPD
Al	5110	20	*	*	–	100	*	*	–
Sb	8.4	20	55.4	1.5	4.1	100	61.0	0.2	0.9
As	41.8	20	91.0	2.3	1.7	100	94.2	0.8	1.5
Ba	27.3	20	1.8	7.1	8.3	100	0	1.5	10.0
Be	0.25	20	92.0	0.9	2.7	100	93.4	0.3	0.9
Cd	112	20	85.0	5.2	1.6	100	88.5	0.8	0.5
Cr	7980	20	*	*	–	100	*	*	–
Co	4.1	20	89.2	1.8	4.6	100	88.7	1.5	4.6
Cu	740	20	*	*	6.0	100	61.7	20.4	5.4
Pb	1480	20	*	*	–	100	*	*	–
Mn	295	20	*	*	–	100	–	–	–
Mo	13.3	20	82.9	1.2	1.3	100	89.2	0.4	1.0
Ni	450	20	*	*	6.8	100	83.0	10.0	4.5
Se	3.5	20	89.7	3.7	4.2	100	91.0	6.0	18.0
Ag	5.9	20	89.8	2.1	4.6	100	85.1	0.4	1.1
Tl	1.9	20	96.9	0.9	2.4	100	98.9	0.9	2.4
Th	3.6	20	91.5	1.3	3.2	100	97.4	0.7	2.0
U	2.4	20	107.7	2.0	4.6	100	109.6	0.7	1.8
V	21.1	20	105.6	1.8	2.1	100	97.4	1.1	2.5
Zn	13300	20	*	*	–	100	*	*	–

S (R) Standard deviation of percent recovery.
RPD Relative percent difference between duplicate spike determinations.
< Sample concentration below established method detection limit.
* Spike concentration <10% of sample background concentration.
– Not determined.
+ Equivalent.

TABLE 11: PRIMARY DRINKING WATER CONTAMINANTS PRECISION AND RECOVERY DATA

Analyte	Regional Sample Background Concentration, µg/L (IV)	(VI)	(X)	Average Mean[1] % Recovery	S (R)
Antimony	0.16	0.07	0.03	114%	1.9
Arsenic	< MDL	2.4	1.0	93	8.5
Barium	4.6	280	14.3	(*)	–
Beryllium	< MDL	< MDL	< MDL	100%	8.2
Cadmium	0.05	0.05	0.03	81	4.0
Chromium	0.71	5.1	0.10	94	2.5
Copper	208	130	14.3	(*)	–
Lead	1.2	1.2	2.5	91	2.6
Mercury	< MDL	0.23	< MDL	86	11.4
Nickel	1.7	3.6	0.52	101%	11.5
Selenium	< MDL	4.3	< MDL	98	8.4
Thallium	< MDL	0.01	< MDL	100	1.4

[1]The three regional waters were fortified with 1.0 µg/L of all analytes listed, except selenium, which was fortified to 20 µg/L.

(*) Recovery of barium and copper was not calculated because the analyte addition was <20% the sample background concentration in all waters. (Recovery calculations are not required if the concentration of the analyte added is less than 30% of the sample background concentration. Section 9.4.3).

S (R) Standard deviation of the mean percent recoveries.

TABLE 12: SUMMARY STATISTICS AND DESCRIPTIVE EQUATIONS FOR THE 20 ANALYTES TESTED IN THE COLLABORATIVE STUDY

		Reagent Water				Finished Drinking Water				Ground Water			
Analyte	C[a]	$\bar{X}$[b]	S_R	S_r	Regr. Equations	$\bar{X}$	S_R	S_r	Regr. Equations	$\bar{X}$	S_R	S_r	Regr. Equations
Aluminum	8.00	10.01	2.33	1.74	$\bar{X} = 0.992C + 1.19$	11.18	9.02	6.34	$\bar{X} = 0.954C + 2.38$	9.86	7.10	2.70	$\bar{X} = 0.946C + 2.20$
	12.00	10.98	5.16		$S_R = 0.056\bar{X} + 2.59^c$	11.02	3.02		$S_R = 7.70^d$	13.40	10.27		$S_R = 0.169\bar{X} + 6.22^c$
	56.00	59.13	5.55	4.19	$S_r = 0.042\bar{X} + 1.27$	56.97	7.14	6.18	$S_r = 0.013\bar{X} + 6.17$	51.75	10.78	16.92	$S_r = 0.172\bar{X} + 0.75^c$
	80.00	82.59	4.92			82.73	8.01			82.83	33.37		
	160.00	158.95	11.82	8.90		159.89	11.94	10.59		155.40	15.39	19.27	
	200.00	200.89	8.61			189.98	12.97			189.64	31.46		
Antimony	2.80	2.75	0.27	0.27	$\bar{X} = 0.999C + 0.04$	2.73	0.29	0.17	$\bar{X} = 0.983C + 0.03$	2.82	0.19	0.22	$\bar{X} = 1.003C + 0.01$
	4.00	4.22	0.46		$S_R = 0.013\bar{X} + 0.61^e$	4.10	0.47		$S_R = 0.049\bar{X} + 0.19$	4.02	0.35		$S_R = 0.059\bar{X} + 0.04$
	20.00	19.76	1.09	0.85	$S_r = 0.022\bar{X} + 0.20$	19.17	1.37	0.66	$S_r = 0.026\bar{X} + 0.08$	20.12	0.82	0.97	$S_r = 0.058\bar{X} + 0.02$
	28.00	27.48	1.38			26.48	1.72			27.77	1.38		
	80.00	82.52	2.24	1.76		83.43	2.05	2.46		80.34	9.14	6.80	
	100.00	98.06	1.34			97.19	5.31			101.09	2.89		
Arsenic	8.00	8.64	3.01	3.02	$\bar{X} = 1.013C + 0.50$	9.00	3.13	1.96	$\bar{X} = 0.993C + 0.57$	10.40	5.17	4.90	$\bar{X} = 0.949C + 0.91$
	12.00	12.58	3.18		$S_R = 0.031\bar{X} + 2.74$	11.37	1.77		$S_R = 0.018\bar{X} + 2.55$	7.85	4.62		$S_R = 0.048\bar{X} + 4.52$
	56.00	55.44	4.64	3.51	$S_r = 0.007\bar{X} + 2.95$	53.77	4.12	4.07	$S_r = 0.031\bar{X} + 1.65$	53.25	3.49	7.88	$S_r = 0.059\bar{X} + 4.29$
	80.00	85.15	2.54			87.72	4.14			83.60	12.46		
	160.00	161.80	11.15	3.96		157.56	4.83	6.30		159.86	11.67	14.94	
	200.00	201.52	10.81			197.99	10.66			194.41	18.24		
Barium	8.01	7.58	0.50	0.48	$\bar{X} = 1.001C - 0.36$	8.21	1.21	1.11	$\bar{X} = 0.995C + 0.37$	8.04	2.60	2.24	$\bar{X} = 1.055C - 0.21$
	12.00	11.81	1.05		$S_R = 0.039\bar{X} + 0.31$	12.56	1.79		$S_R = 0.045\bar{X} + 0.97^c$	12.85	1.45		$S_R = 0.020\bar{X} + 2.05$
	48.00	47.32	1.60	1.82	$S_r = 0.024\bar{X} + 0.25$	49.13	3.72	3.77	$S_r = 0.040\bar{X} + 0.72^c$	50.12	2.98	2.19	$S_r = 0.014\bar{X} + 2.08$
	64.00	65.52	2.90			65.30	4.16			69.53	2.66		
	160.00	157.09	6.53	4.07		155.25	7.82	5.67		164.44	8.81	6.61	
	200.00	198.53	8.28			196.52	5.70			208.32	9.22		
Beryllium	2.80	3.31	0.81	0.26	$\bar{X} = 1.056C + 0.32$	3.15	0.47	0.31	$\bar{X} = 1.055C + 0.20$	3.02	0.46	0.22	$\bar{X} = 1.049C + 0.08$
	4.00	4.45	0.73		$S_R = 0.067\bar{X} + 0.55$	4.45	0.51		$S_R = 0.057\bar{X} + 0.28$	4.27	0.44		$S_R = 0.084\bar{X} + 0.16$
	20.00	22.38	2.76	1.00	$S_r = 0.038\bar{X} + 0.11$	21.27	1.23	0.63	$S_r = 0.016\bar{X} + 0.25$	21.55	1.72	1.10	$S_r = 0.043\bar{X} + 0.06$
	28.00	30.02	2.86			29.57	1.67			29.24	2.09		
	80.00	84.18	4.79	4.02		87.59	6.89	1.88		84.23	9.05	4.32	
	100.00	102.88	5.90			102.64	6.27			103.39	10.17		
Cadmium	4.00	4.01	0.34	0.20	$\bar{X} = 1.007C + 0.07$	4.11	0.88	0.71	$\bar{X} = 0.985C + 0.10$	3.98	0.48	0.14	$\bar{X} = 0.944C + 0.11$
	6.00	6.32	0.49		$S_R = 0.041\bar{X} + 0.19$	5.87	0.58		$S_R = 0.031\bar{X} + 0.65$	5.62	0.73		$S_R = 0.017\bar{X} + 1.09^e$
	20.00	19.81	1.12	0.86	$S_r = 0.022\bar{X} + 0.10^f$	19.57	1.45	1.26	$S_r = 0.021\bar{X} + 0.61$	18.15	1.73	0.88	$S_r = 0.029\bar{X} + 0.01$
	28.00	28.33	0.94			27.68	1.27			26.86	2.59		
	80.00	81.28	4.91	1.33		80.62	4.45	2.02		77.83	3.05	1.88	
	100.00	100.11	3.24			98.15	3.60			95.31	2.04		

TABLE 12: SUMMARY STATISTICS AND DESCRIPTIVE EQUATIONS FOR THE 20 ANALYTES TESTED IN THE COLLABORATIVE STUDY

		Reagent Water				Finished Drinking Water				Ground Water			
Analyte	C[a]	$\bar{X}$[b]	S_R	S_r	Regr. Equations	$\bar{X}$	S_R	S_r	Regr. Equations	$\bar{X}$	S_R	S_r	Regr. Equations
Chromium	8.00	8.27	0.32	1.54	$\bar{X} = 1.017C + 0.62$	9.46	2.34	2.08	$\bar{X} = 0.990C + 1.45$	8.98	1.47	0.37	$\bar{X} = 1.026C + 0.89$
	12.00	13.88	3.10		$S_R = 0.066\bar{X} + 0.48$	13.10	2.39		$S_R = 0.015\bar{X} + 2.19$	13.42	1.13		$S_R = 0.067\bar{X}\ 0.68$
	56.00	57.86	4.03	2.68	$S_r = 0.026\bar{X} + 1.25$	56.04	2.24	1.29	$S_r = 2.18$[d]	59.35	5.99	5.42	$S_r = 0.068\bar{X} - 0.37$
	80.00	84.73	2.65			84.38	3.18			83.90	5.70		
	160.00	157.66	13.62	6.97		158.24	5.12	3.16		164.58	14.11	9.80	
	200.00	197.43	9.47			196.72	7.47			199.88	11.19		
Cobalt	0.80	0.88	0.10	0.05	$\bar{X} = 0.977C + 0.01$	0.92	0.45	0.31	$\bar{X} = 0.964C + 0.06$	0.85	0.13	0.09	$\bar{X} = 0.989C - 0.01$
	1.21	0.98	0.04		$S_R = 0.028\bar{X} + 0.06$	1.02	0.10		$S_R = 0.019\bar{X} + 0.32$	1.04	0.18		$S_R = 0.057\bar{X} + 0.09$
	20.10	20.77	0.74	0.67	$S_r = 0.027\bar{X} + 0.02$	20.45	0.91	0.53	$S_r = 0.014\bar{X} + 0.30$	20.81	1.11	1.12	$S_r = 0.012\bar{X} + 0.40$[e]
	28.20	27.75	0.96			27.29	1.22			28.07	2.16		
	80.50	78.59	2.29	2.31		78.04	3.72	1.84		79.26	4.66	1.34	
	101.00	98.79	2.94			97.62	4.62			99.41	4.22		
Copper	4.00	3.88	0.73	0.59	$\bar{X} = 1.003C - 0.05$	3.33	0.85	0.99	$\bar{X} = 0.976C - 0.38$	3.86	1.40	0.71	$\bar{X} = 0.977C - 0.01$
	6.00	6.14	1.00		$S_R = 0.037\bar{X} + 0.64$	5.95	1.78		$S_R = 0.063\bar{X} + 0.86$	5.96	0.95		$S_R = 0.073\bar{X} + 0.92$
	20.00	20.07	1.08	0.92	$S_r = 0.016\bar{X} + 0.51$	18.90	1.64	1.51	$S_r = 0.029\bar{X} + 0.86$	18.97	1.68	2.32	$S_r = 0.077\bar{X} + 0.35$
	28.00	27.97	1.94			27.21	2.76			27.44	2.58		
	80.00	79.80	3.22	1.91		76.64	5.30	3.42		79.30	9.05	6.54	
	100.00	99.57	4.42			96.17	5.64			97.54	11.16		
Lead	4.00	4.00	1.57	1.62	$\bar{X} = 1.043C - 0.31$	3.44	1.15	1.18	$\bar{X} = 1.032C - 0.30$	4.20	1.13	1.76	$\bar{X} = 1.012C + 0.15$
	6.00	5.56	2.00		$S_R = 0.064\bar{X} + 1.43$[c]	6.84	1.10		$S_R = 0.015\bar{X} + 1.06$	6.27	2.38		$S_R = 0.048\bar{X} + 1.27$
	20.00	20.54	2.91	4.36	$S_r = 3.42$[d]	20.18	1.20	1.44	$S_r = 0.011\bar{X} + 1.13$	19.57	2.72	0.88	$S_r = 1.78$[d]
	28.00	30.90	4.58			28.08	1.57			28.55	1.73		
	80.00	80.57	3.13	4.29		80.92	2.30	2.07		82.47	4.38	2.69	
	100.00	102.93	6.62			101.60	3.23			102.47	3.58		
Manganese	0.80	0.86	0.15	0.09	$\bar{X} = 0.983C + 0.02$	0.96	0.32	0.42	$\bar{X} = 0.989C + 0.10$	0.64	0.22	0.17	$\bar{X} = 0.954C - 0.16$
	1.20	1.09	0.12		$S_R = 0.026\bar{X} + 0.11$	1.13	0.38		$S_R = 0.047\bar{X} + 0.29$	0.90	0.21		$S_R = 0.103\bar{X} + 0.14$
	20.00	20.43	0.89	0.72	$S_r = 0.027\bar{X} + 0.06$	21.06	1.32	0.96	$S_r = 0.021\bar{X} + 0.40$	19.61	2.60	2.62	$S_r = 0.025\bar{X} + 0.09$[e]
	28.00	27.53	0.41			27.60	1.47			25.65	4.10		
	80.00	79.00	3.16	2.38		79.57	4.18	2.01		77.38	6.13	2.90	
	100.00	97.60	2.51			97.97	4.10			95.86	6.74		

TABLE 12: SUMMARY STATISTICS AND DESCRIPTIVE EQUATIONS FOR THE 20 ANALYTES TESTED IN THE COLLABORATIVE STUDY

		Reagent Water				Finished Drinking Water				Ground Water			
Analyte	C[a]	$\bar{X}$[b]	S_R	S_r	Regr. Equations	$\bar{X}$	S_R	S_r	Regr. Equations	$\bar{X}$	S_R	S_r	Regr. Equations
Molybdenum	2.80	2.63	0.32	0.16	$\bar{X} = 1.012C - 0.20$	2.80	0.20	0.32	$\bar{X} = 1.013C - 0.07$	3.00	0.47	0.42	$\bar{X} = 1.032C - 0.09$
	4.00	3.85	0.31		$S_R = 0.032\bar{X} + 0.22$	3.95	0.47		$S_R = 0.037\bar{X} + 0.17$	3.60	0.90		$S_R = 0.55\bar{X} + 0.43$
	20.00	19.75	0.64	0.64	$S_r = 0.021X + 0.09$	19.78	0.60	1.16	$S_r = 0.035X + 0.20$	20.69	1.37	1.11	$S_r = 0.042X + 0.27$
	28.00	27.87	1.07			27.87	1.51			28.80	2.01		
	80.00	83.07	3.07	1.78		85.65	3.50	3.07		84.26	4.13	4.81	
	100.00	100.08	4.32			99.06	2.89			103.57	6.10		
Nickel	4.00	4.02	0.41	0.50	$\bar{X} = 1.000C + 0.12$	3.66	0.53	1.03	$\bar{X} = 0.953C - 0.19$	4.81	2.06	2.82	$\bar{X} = 1.022C + 0.66$
	6.00	6.36	0.91		$S_R = 0.051\bar{X} + 0.31$	5.44	1.32		$S_R = 0.046\bar{X} + 0.56$	6.67	3.66		$S_R = 0.091\bar{X} + 2.03$
	20.00	19.93	1.30	0.63	$S_r = 0.017X + 0.40$	18.42	0.87	1.11	$S_r = 0.023X + 0.91$	20.58	3.71	2.37	$S_r = 0.008X + 2.75$[c]
	28.00	28.02	1.25			27.09	1.68			30.73	3.75		
	80.00	79.29	2.95	2.55		75.84	4.40	3.94		82.71	9.49	5.42	
	100.00	100.87	7.20			95.83	4.41			101.00	9.89		
Selenium	32.00	33.54	4.63	1.57	$\bar{X} = 1.036C - 0.06$	32.57	4.37	3.65	$\bar{X} = 1.022C + 0.14$	32.46	4.95	3.24	$\bar{X} = 1.045C - 0.83$
	40.00	41.03	6.04		$S_R = 0.051\bar{X} + 3.24$	42.18	3.71		$S_R = 0.056\bar{X} + 2.10$	41.46	3.30		$S_R = 0.037\bar{X} + 2.97$
	80.00	81.40	5.86	5.44	$S_r = 0.061X - 0.64$	79.97	6.66	5.28	$S_r = 0.040X + 2.15$	81.63	6.94	5.65	$S_r = 0.058X + 1.02$
	96.10	98.34	8.57			94.94	7.90			98.92	4.39		
	160.00	163.58	15.69	9.86		163.48	9.17	10.06		167.54	8.69	12.98	
	200.00	214.30	10.57			212.19	16.49			209.21	14.65		
Silver	0.80	0.93	0.09	0.14	$\bar{X} = 0.917C + 0.26$	0.70	0.34	0.34	$\bar{X} = 0.888C + 0.09$	0.70	0.26	0.10	$\bar{X} = 0.858C - 0.00$
	1.20	1.51	0.23		$S_R = 0.196\bar{X} - 0.09$	1.37	0.33		$S_R = 0.186\bar{X} + 0.17$	0.98	0.28		$S_R = 0.169\bar{X} + 0.14$
	48.00	49.39	3.25	1.81	$S_r = 0.053X + 0.08$	45.43	6.78	5.15	$S_r = 0.164X + 0.18$	45.59	4.27	2.70	$S_r = 0.120X - 0.01$
	64.00	63.54	2.75			60.35	2.22			59.71	6.58		
	160.00	136.42	48.31	12.19		119.06	55.28	36.34		121.43	42.55	28.19	
	200.00	153.74	57.34			172.15	31.92			160.69	27.15		
Thallium	2.80	2.89	0.23	0.22	$\bar{X} = 0.984X + 0.08$	2.88	0.40	0.16	$\bar{X} = 1.010C + 0.01$	2.88	0.14	0.12	$\bar{X} = 1.023C - 0.06$
	4.00	3.92	0.15		$S_R = 0.035\bar{X} + 0.09$	3.96	0.21		$S_R = 0.040\bar{X} + 0.21$	3.88	0.37		$S_R = 0.056\bar{X} + 0.04$
	20.00	19.27	0.99	0.67	$S_r = 0.027X + 0.13$	19.77	1.13	0.83	$S_r = 0.039X + 0.02$	20.22	1.05	0.65	$S_r = 0.049X - 0.06$
	28.00	28.08	0.83			27.61	1.24			28.65	1.50		
	80.00	81.29	3.65	2.86		85.32	4.08	4.05		83.97	6.10	6.05	
	100.00	96.69	2.86			100.07	4.33			101.09	4.15		
Thorium	0.80	0.93	0.16	0.09	$\bar{X} = 1.013C + 0.08$	0.78	0.13	0.07	$\bar{X} = 1.019C - 0.06$	0.87	0.17	0.07	$\bar{X} = 1.069C - 0.03$
	1.20	1.22	0.19		$S_R = 0.036\bar{X} + 0.13$	1.09	0.19		$S_R = 0.035\bar{X} + 0.12$	1.15	0.17		$S_R = 0.041\bar{X} + 0.13$
	20.00	20.88	0.90	0.71	$S_r = 0.025X + 0.07$	21.66	0.94	0.54	$S_r = 0.024X + 0.05$	21.78	0.90	0.94	$S_r = 0.027X + 0.04$
	28.00	27.97	1.11			28.09	0.83			29.86	1.65		
	80.10	81.14	2.99	2.14		79.99	2.03	2.60		86.00	3.43	1.95	
	100.00	102.64	3.39			100.50	4.56			107.35	4.72		

TABLE 12: SUMMARY STATISTICS AND DESCRIPTIVE EQUATIONS FOR THE 20 ANALYTES TESTED IN THE COLLABORATIVE STUDY

		Reagent Water				Finished Drinking Water				Ground Water			
Analyte	C[a]	$\bar{X}$[b]	S_R	S_r	Regr. Equations	$\bar{X}$	S_R	S_r	Regr. Equations	$\bar{X}$	S_R	S_r	Regr. Equations
Uranium	0.80	0.86	0.05	0.08	$\bar{X} = 1.026C - 0.02$	0.85	0.15	0.09	$\bar{X} = 1.026C - 0.04$	0.84	0.23	0.19	$\bar{X} = 1.058C - 0.06$
	1.20	1.10	0.11		$S_R = 0.048\bar{X} + 0.02$	1.05	0.13		$S_R = 0.044\bar{X} + 0.11$	1.10	0.14		$S_R = 0.039\bar{X} + 0.17$
	20.10	21.38	0.99	0.82	$S_r = 0.027\bar{X} + 0.05$	22.30	1.40	0.46	$S_r = 0.022\bar{X} + 0.07$	21.56	1.11	1.08	$S_r = 0.028\bar{X} + 0.16$
	28.10	28.36	1.10			28.89	1.47			29.86	1.83		
	80.30	82.47	4.03	2.16		80.31	2.00	2.71		85.01	3.76	2.00	
	100.00	103.49	5.24			100.70	5.30			106.47	3.74		
Vanadium	32.00	31.02	2.68	2.19	$\bar{X} = 1.025C - 2.21$	33.15	2.51	2.28	$\bar{X} = 1.022C - 0.30$	33.25	3.83	1.87	$\bar{X} = 1.076C - 1.87$
	40.00	38.54	2.94		$S_R = 3.79^d$	40.20	1.88		$S_R = 0.023\bar{X} + 1.45$	40.34	3.08		$S_R = 0.033\bar{X} + 2.25$
	80.00	79.14	4.94	4.29	$S_r = 3.26^d$	77.83	4.18	2.75	$S_r = 0.023\bar{X} + 1.38$	84.42	3.97	2.93	$S_r = 0.049\bar{X} - 0.09$
	96.00	93.47	3.85			96.32	1.34			98.70	5.03		
	160.00	162.43	5.67	3.30		161.89	7.63	6.56		170.94	9.09	11.55	
	200.00	208.20	2.65			214.91	5.89			217.90	11.36		
Zinc	8.00	8.33	2.56	1.78	$\bar{X} = 1.042C + 0.87$	11.60	6.18	5.72	$\bar{X} = 0.943C + 2.54$	7.29	1.12	2.20	$\bar{X} = 0.962C + 0.07$
	12.00	15.49	4.18		$S_R = 0.041\bar{X} + 2.60$	10.21	4.96		$S_R = 0.048\bar{X} + 5.27$	12.66	3.24		$S_R = 0.093\bar{X} + 0.92$
	56.00	56.07	2.91	2.47	$S_r = 0.030\bar{X} + 1.42$	56.83	7.66	4.56	$S_r = 0.004\bar{X} + 5.66^c$	54.86	5.12	7.24	$S_r = 0.069\bar{X} + 1.55$
	80.00	85.53	5.81			82.88	8.34			78.62	8.56		
	160.00	165.17	7.78	9.87		156.69	17.01	9.48		150.12	12.52	10.84	
	200.00	207.27	14.61			191.59	17.21			184.37	16.59		

[a] True Value for the concentration added (μg/L)
[b] Mean Recovery (μg/L)
[c] $COD_w < 0.5$ - Use of regression equation outside study concentration range not recommended.
[d] $COD_w < 0$ - Mean precision is reported.
[e] $COD_w < 0$ - Unweighted linear regression equation presented.

TABLE 13: BACKGROUND AND SPIKE MEASUREMENTS IN WASTEWATER DIGESTATE[a]

	Background		Concentrate 1					Concentrate 2					
	Conc. µg/L	Std Dev µg/L	Spike µg/L	Found µg/L	Std Dev µg/L	% Rec %	RSD %	Spike µg/L	Found µg/L	Std Dev µg/L	% Rec %	RSD %	RSD_r %
Be	0.0	0.0	100	94.5	11.8	94.5	12.5	125	118.1	14.7	94.5	12.4	3.5
Al	78.2	12.4	200	260.9	41.2	91.4	15.8	250	309.1	48.5	92.4	15.7	2.7
Cr	19.5	8.1	200	222.2	23.3	101.4	10.5	250	274.3	26.6	101.9	9.7	2.0
V	1.9	2.8	250	271.8	36.5	108.0	13.4	200	219.3	30.1	108.7	13.7	2.6
Mn	296.6	24.7	125	419.0	35.7	97.9	8.5	100	397.4	34.8	100.8	8.8	1.0
Co	2.5	0.4	125	124.7	12.3	97.8	9.9	101	100.7	9.4	97.2	9.3	2.8
Ni	47.3	5.0	125	161.7	4.9	91.5	3.0	100	142.7	5.6	95.4	3.9	2.1
cu	77.4	13.2	125	194.5	29.5	93.7	15.2	100	172.3	26.6	94.9	15.4	2.2
Zn	77.4	4.9	200	257.4	16.3	90.0	6.3	250	302.5	21.1	90.0	7.0	1.8
As	0.8	1.1	200	194.9	8.0	97.1	4.1	250	244.7	12.8	97.6	5.2	3.4
Se	4.5	6.2	250	236.8	14.2	92.9	6.0	200	194.3	9.3	94.9	4.8	3.8
Mo	166.1	9.4	100	269.8	19.0	103.7	7.0	125	302.0	18.0	108.7	6.0	1.5
Ag	0.6	0.7	200	176.0	14.6	87.7	8.3	250	214.6	17.8	85.6	8.3	2.3
Cd	2.7	1.1	125	117.0	4.8	91.4	4.1	100	96.6	3.2	93.9	3.3	2.9
Sb	3.3	0.2	100	100.2	4.8	96.9	4.8	125	125.9	4.3	98.1	3.4	1.8
Ba	68.6	3.3	250	321.0	19.4	101.0	6.0	200	279.3	17.2	105.4	6.2	2.5
Tl	0.1	0.1	100	103.3	8.0	103.2	7.7	125	129.2	8.9	103.3	6.9	2.1
Pb	6.9	0.5	125	135.1	7.8	102.6	5.8	100	110.3	6.3	103.4	5.7	1.8
Th	0.1	0.1	125	140.2	19.5	112.1	13.9	100	113.3	15.4	113.2	13.6	2.7
U	0.4	0.2	125	141.2	19.3	112.6	13.7	100	113.6	16.0	113.2	14.1	2.5

[a]Results from 10 participating laboratories. Wastewater digestate supplied with the study materials. Mean background concentrations determined by the participants.

TABLE 14: SPIKE MEASUREMENTS IN PARTICIPANTS WASTEWATER[a]

	Concentrate 1					Concentrate 2					
	Spike µg/L	Found µg/L	Std Dev µg/L	% Rec %	RSD %	Spike µg/L	Found µg/L	Std Dev µg/L	% Rec %	RSD %	RSD_r %
Be	101	103.4	12.0	103.4	11.6	125	128.2	13.6	102.6	10.6	2.4
Al	200	198.7	23.9	99.4	12.0	250	252.4	15.5	101.0	6.1	2.9
Cr	200	205.4	12.3	102.7	6.0	250	253.4	15.4	101.4	6.1	1.1
V	250	246.5	4.4	98.6	1.8	200	196.8	2.8	98.4	1.4	2.0
Mn	125	119.0	5.4	95.2	4.5	100	95.5	4.3	95.5	4.5	0.8
Co	125	125.8	7.0	100.6	5.6	101	99.5	5.3	98.5	5.3	1.8
Ni	125	127.4	9.7	101.9	7.6	100	101.0	7.5	101.0	7.4	1.7
cu	125	126.8	5.3	101.4	4.2	100	105.3	3.6	105.3	3.4	2.8
Zn	200	201.4	36.7	100.7	18.2	250	246.4	29.7	98.6	12.1	2.6
As	200	207.3	11.9	103.7	5.7	250	263.0	2.6	105.2	1.0	3.2
Se	250	256.8	26.4	102.7	10.3	200	214.0	18.7	107.3	8.7	3.6
Mo	100	98.6	4.6	98.6	4.7	125	123.2	6.7	98.6	5.4	2.2
Ag	200	200.7	48.9	100.4	24.4	250	231.2	63.5	92.5	27.5	8.2
Cd	125	123.2	11.5	98.6	9.3	100	95.8	2.9	95.8	3.0	5.8
Sb	100	92.2	4.4	92.2	4.8	125	119.0	1.0	95.2	0.8	2.8
Ba	250	245.2	12.8	98.1	5.2	200	204.7	12.1	102.4	5.9	2.1
Tl	100	100.0	0.9	100.0	0.9	125	128.0	6.0	102.4	4.7	3.5
Pb	125	125.8	5.1	100.6	4.1	100	100.8	2.7	100.8	2.7	2.2
Th	125	124.2	7.6	99.4	6.1	100	99.8	5.7	99.8	5.7	3.2
U	125	130.4	10.3	104.3	7.9	100	106.4	6.8	106.4	6.4	2.3

[a]Results from five participating laboratories. Mean concentrations before spiking are not listed because they varied considerably among the different wastewaters.

Annex VI:

Recommended Method for the

DETERMINATION OF TOTAL MERCURY IN MARINE SAMPLES BY THERMAL DECOMPOSITION AMALGAMATION AND ATOMIC ABSORPTION SPECTROPHOTOMETRY

REPORT

Recommended Method for the

DETERMINATION OF TOTAL MERCURY IN MARINE SAMPLES BY THERMAL DECOMPOSITION AMALGAMATION AND ATOMIC ABSORPTION SPECTROPHOTOMETRY

IAEA/NAEL

Marine Environmental Studies Laboratory in co-operation with

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Recommended Method for the
DETERMINATION OF TOTAL MERCURY IN MARINE SAMPLES BY
THERMAL DECOMPOSITION AMALGAMATION
AND ATOMIC ABSORPTION SPECTROPHOTOMETRY

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<u>NOTE:</u> This method is not intended to be an analytical training manual. Therefore, this method is written with the assumption that it will be performed by formally trained analytical chemist.

In addition, the IAEA recommended methods are intended to be guidance methods that can be used by laboratories as a starting point for generating their own standard operating procedure. If performance data are included in the method, they shall not be used as absolute QC acceptance criteria.

1. SCOPE

The method hereinafter describes the protocol for the determination of total mercury (inorganic and organic) in sediment and biological material.

By using this method, the total mercury in solid samples can be determined without sample chemical pre-treatment.

The recommended protocol is mainly based on the EPA 7473 method; users are encouraged to consult this document (EPA, 2007).

2. PRINCIPLE

The sample is dried and then chemically decomposed under oxygen in the decomposition furnace. The decomposition products are carried out to the catalytic section of the furnace, where oxidation is completed (halogens and nitrogen/sulfur oxides are trapped). The mercury present in the remaining decomposition products is selectively trapped on an amalgamator. After flushing the system with oxygen, the mercury vapour is released by rapid heating of the amalgamator, and carried through the absorbance cell in the light path of a single wavelength atomic absorption spectrophotometer. The absorbance is measured at 253.7 nm as a function of mercury quantity (ng).

The typical working range is 0.1–500 ng. The mercury vapour is carried through a long (first) and a short path length absorbance cell. The same quantity of mercury is measured twice with different sensitivity resulting in a dynamic range that spans four orders of magnitude.

The typical detection limit is 0.01 ng of mercury.

3. SAMPLE PRE-TREATMENT

The sediment samples are prepared following the recommendations of UNEP (2005);

The marine organisms are prepared following the recommendations of UNEP (1984, 1994).

4. REAGENTS

The reagents used shall meet the purity requirement of the subsequent analysis

4.1. ULTRAPUR WATER (type MilliQ)

4.2. NITRIC ACID 65%

4.3. POTASSIUM DICHROMATE OXIDIZING SOLUTION (10% w/v)

Weight 25 g of $K_2Cr_2O_7$ in 250 ml glass bottle, fill it up to 250 ml with water, and shake until total dissolution of solids. Keep the bottle tightly closed in a double plastic bag, and in an Hg free environment (i.e. laminar flow hood). This solution is stable indefinitely and rarely becomes contaminated.

4.4. COMMERCIAL STANDARD SOLUTION 1000 µg ml^{-1} MERCURY

Use a certified reference material solution; this solution should be accompanied by a certificate stipulating at minimum the traceability of the certified concentration, as well as the expiry date. The density of the solution, or the certified content in mg kg^{-1} should also be defined, to allow for the preparation of the calibration solution by weighing. Stock solutions should be kept at 5°C.

5. MATERIAL

5.1. SOLID MERCURY ANALYZER

Optionally equipped with an auto-sampler.

5.2. ANALYTICAL BALANCE

With a 0.001 g precision at least.

5.3. VOLUMETRIC CONTAINERS

Preferably in Teflon or glass.

5.4. PIPETTES

Some microliter pipettes sized ranging from 50 to 10000 µl are needed. The accuracy and precision of the pipettes used should be checked as a routine every 6 months, and the results obtained should be compared with the individual certificates.

5.5. METAL SPATULA (inox).

5.6. SAMPLE BOAT

Metal or metal alloy. Before measurement, sample boats are cleaned by heating over a flame until constant “red” to remove mercury.

5.7. OXYGEN

It should be of high purity and free of mercury. If there is a possible mercury contamination from oxygen, install a gold mesh filter between the cylinder and the instrument to prevent any mercury from entering the instrument.

6. CALIBRATION

6.1. PRIMARY CALIBRATION. This is the calibration of the instrument working range. This calibration is performed initially (usually done by the manufacturer and stored in the instrument), and/or when any significant instrumental parameters are changed (i.e. after maintenance).

6.2. PREPARE STANDARD SOLUTIONS of appropriate concentration by dilution of a commercial standard (see 4.4). It is recommended to prepare standard solution in Teflon or glass container, in 1 or 0.5% HNO_3 (see 4.2) and 0.1% (v/v) potassium dichromate (see 4.3). Fresh mercury standard should be prepared daily. Prepare a zero calibration solution using the same quantity of acid and potassium dichromate.

6.3. START THE INSTRUMENT according to the manufacturer recommendations.

6.4. CLEAN THE SYSTEM. Inject 100 µl of water and start the measurement with the recommended parameters (see 7.1). Repeat the cleaning until the absorbance is below 0.001ABS.

6.5. SET THE INSTRUMENT PARAMETERS (see 7.1) for selected volume (usually 100 µl) and inject the zero calibration, at least three measurements should be done. The zero solution serves to correct the amount of mercury in water and reagent used for preparing the calibration curve, hence the important of keeping the injected volume equal at all points of the calibration curve. If the amount of mercury in the zero calibration is high (i.e. more than 0.01 ng), it is recommended to check for contamination sources and to prepare new standard solution with clean acid.

6.6. STANDARDS ARE MEASURED from the lowest to the highest at least twice. The maximum relative standard deviation between readings should be 3% (except for zero calibration); if higher it is recommended to carry out more measurements.

6.7. EXAMPLE OF AMOUNTS used for recalibration (primary):

First Range:

Standard (ng ml^{-1})	1	3	10	30	100	300
Volume injected (µl)	100	100	100	100	100	100
Quantity of Hg (ng)	0.1	0.3	1	3	10	30

Second Range:

Standard (µg ml^{-1})	1	2	3	4	5	6
Volume injected (µl)	100	100	100	100	100	100
Quantity of Hg (ng)	100	200	300	400	500	600

Note: The calibration of the second range might induce problems for subsequent analysis, due to the relatively high quantity of mercury introduced (especially with memory effect). It should be performed only if there is a probability of using it (i.e. measuring samples with high mercury level > 1µg g^{-1}). After the reading of the last calibration point, clean the system (see 6.4).

6.8. ALTERNATIVE CALIBRATION CURVE can be performed using a solid certified reference material. In this case, weigh accurately a CRM onto a tare sample boat, set up the instrument according to the sample type (see 7.1) and measure the absorbance. The matrix of the CRM should be as similar as possible to the sample of interest. Repeat this procedure with different weights of the CRM and/or with different CRM, to get results in the desired working range.

6.9. CONSTRUCT A CALIBRATION CURVE by plotting the absorbance against Nano grams of mercury (this could be done automatically by the software). The type of equation will depend on the levels, as the response is not linear over the entire working range.

6.10. DAILY CALIBRATION: calibration performed every day with a minimum number of standards to ensure that the primary calibration is valid. It can be performed by using either liquid standard (see 6.2) or solid certified reference material (CRM) see 6.8. It should be performed in the range of interest, with at least two standards (or matrix CRM) and the results should agree within the acceptance criteria. The acceptance criteria should be set through the use of historical data, but the maximum deviation should not exceed 10%.

7. **PROCEDURE**

7.1. GENERAL ANALYTICAL PARAMETERS

The analytical parameters will depend on the sample size and matrix, and are instrument specific. It is important to follow the guidelines from the instrument manufacturer. There are three time to set: drying, decomposition and waiting.

Some typical recommended conditions below:

Drying time:

Sample type	Dry (s)	Comments
Liquid	0.7 x injected Volume (µl)	
Dry inorganic	10	
Organic liquid	50–300	To be optimized[1]
Dry organic (i.e. fat)	50–200	To be optimized[1]
Wet (i.e. fresh)	0.7 x weight x % moisture	Example: 100 mg with 45% moisture 0.7 x 100 x 0.45= 31.5s (35)

[1] In the case of organic, there is a risk of explosion especially with organic liquid; to optimize set the instrument at: 300s dry/ 150s decomposition/ 45s wait, do the measurement and check for possible small explosion, note the time of the phenomenon and add to the drying time 10s more.

Decomposition time:

Sample type	Decomposition (s)	Comments
Liquid	150–400	To be optimized[1]
Solid inorganic	120 + 0.4 x sample (mg)	To be optimized[1]
Solid organic	120	

[1] Set the instrument to XX (see above) dry/ 400s decomposition/ 45s wait, run a sample and observe the results. Decrease the decomposition time by 30s and repeat measurement. Continue until you observe a significant decrease, note that time and add to the decomposition time 30s more.

Waiting time:

It is recommended to use 40–45s, except for long decomposition time (over 200s) when it is beneficial to add 10s of waiting for every 100s of decomposition.

Note: These indications above are recommended by ALTECH (AMA 254).

7.2. ANALYSIS OF A SOLID SAMPLE

Weight a sample accurately onto a tare boat, insert the boat into the instrument, set the appropriate parameters (see 7.1) and start the measurements. The results can be records on absorbance, quantity or concentration depending on the instrument software. See 9: Calculation of results.

7.3. ANALYSIS OF BLANK FOR SOLID MEASUREMENT

Analyse an empty sample boat using the same instrument settings than for the sample.

7.4. ANALYSIS OF A LIQUID SAMPLE

Dose a known volume of the sample onto a sample boat, set the appropriate parameters (see 7.1) and start the measurements. The results can be records on absorbance, quantity or concentration depending on the instrument software. See the calculation section (see 9).

7.5. ANALYSIS OF BLANK FOR LIQUID

Repeat 7.4 with the same volume of blank solution (solution that contain the same reagent and chemical than the sample).

8. QUALITY CONTROL

8.1. For every day of analysis, the CALIBRATION SHOULD BE VALIDATED by doing a daily calibration (see 6.10) before starting the measurements. The results of the daily calibration should be recorded for quality control purposes.

8.2. CERTIFIED REFERENCE MATERIAL

At least one certified reference material of a representative matrix should be measured with each batch of the sample, the calculated results should fall in the value of the certificate and within the coverage uncertainty (Linsinger, 2010), to show evidence of unbiased results. The results for the CRM should be recorded for quality control purpose and plotted in a control chart (UNEP/IOC/IAEA 1994).

8.3. A DUPLICATE OR TRIPLICATE SAMPLE should be processed on a routine basis.

A duplicate sample should be processed with each analytical batch or for every 10 samples.

8.4. A SPIKED SAMPLE should also be included, whenever a new sample matrix is being analysed, especially if no certified reference material is available for that matrix. Measure a spiked sample by adding a known volume of standard solution (prepared as in paragraph 6.2) to the sample in the boat. Keep the spike volume small enough not to overspill. The recovery of spike calculated with the equation 2 should be 85–115% (this limits should be reset after collection of historical data). If the test fails, it is recommended to check the calibration (see 6.10) and/or to revise the instrument parameters (see 7.1).

$$\text{Spike (ng)} = \text{Concentration of standard (ng/ml)} \times \text{Volume of spike (ml)} \qquad \text{Equation 1}$$

$$\text{Recovery (\%)} = \frac{\text{Spiked sample (ng)} - \text{Unspiked sample (ng)}}{\text{Spike (ng)}} \times 100 \qquad \text{Equation 2}$$

To be valid the quantity of Spike (equation 1) should be in the range of 50–150% the quantity of unspiked sample.

9. CALCULATION OF RESULTS

9.1. SOLID SAMPLE RESULTS are calculated using equation 3

$$w(Hg) = \frac{(\rho 1 - \rho 0)}{\mathrm{m}} \times R \qquad \text{Equation 3}$$

Where:

w(Hg) is the mass fraction of element m in the sample, expressed in mg kg^{-1};

ρ1 is the quantity of mercury, expressed in ng as measured in the sample;

ρ0 is the quantity of mercury expressed in ng as measured in the blank (see 7.3);

R is the recovery calculated using the CRM (see 8.2) or spike (see 8.4);

m is the amount of sample in mg.

Note: ρ1 and ρ0 are calculated using calibration curve equation (usually done by software).

9.2. LIQUID SAMPLE RESULTS are calculated using equation 4

$$w(Hg) = \frac{\frac{(\rho 1 - \rho 0)}{Vi} \times V}{\mathrm{m}} \times f \times R \qquad \text{Equation 4}$$

Where:

w(Hg) is the mass fraction of mercury in the sample, expressed in mg kg^{-1};

ρ1 is the quantity of mercury, expressed in ng as measured in the sample solution;

ρ0 is the quantity of mercury expressed in ng as measured in the blank solution (see 7.4);

R is the recovery calculated using the CRM (see 8.2) or spike (see 8.4);

Vi is the injected volume (should be the same in sample and blank solution) in ml;

m is the amount of sample in mg;

V is the volume of solution in ml;

f is the dilution factor.

Note: ρ1 and ρ0 are calculated using calibration curve equation (usually done by software).

10. EXPRESSION OF RESULTS

The rounding of values will depend on the uncertainty reported with the results; in general for this method two or three significant figures should be reported.

The uncertainty component should be reported with all results. (ISO 2005, Nordtest 2004)

Example: w(Hg) = 0.512 ± 0.065 mg kg $^{-1}$.

11. REFERENCES

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Annex VII:

RECOMMENDED METHOD FOR THE DETERMINATION OF TOTAL MERCURY IN SAMPLES OF MARINE ORIGIN BY COLD VAPOUR ATOMIC ABSORPTION SPECTROMETRY

REPORT

RECOMMENDED METHOD FOR THE DETERMINATION OF TOTAL MERCURY IN SAMPLES OF MARINE ORIGIN BY COLD VAPOUR ATOMIC ABSORPTION SPECTROMETRY

IAEA/NAEL Marine Environmental Studies Laboratory in co-operation with UNEP/MAP MED POL

December 2012

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RECOMMENDED METHOD ON THE DETERMINATION OF TOTAL MERCURY IN SAMPLES OF MARINE ORIGIN BY COLD VAPOUR ATOMIC ABSORPTION SPECTROMETRY

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<u>NOTE:</u> This method is not intended to be an analytical training manual. Therefore, the method is written with the assumption that it will be used by formally trained analytical chemists.

In addition, the IAEA recommended methods are intended to be guidance methods that can be used by laboratories as a starting point for generating their own standard operating procedure. If performance data are included in the method they must not be used as absolute QC acceptance criteria.

1. SCOPE

This method describes a protocol for measurement of total mercury by cold vapour atomic absorption spectrometry (CV-AAS). The method is simple, rapid and applicable to a large number of environmental samples. This method is applicable when the element content in the digested solution is above the method limit (~ 0.15 ng ml^{-1} depending on instrument). The typical working range is 0.25–100 ng ml^{-1} for direct injection of cold vapour, using "batch system"; FIAS or amalgamation accessory will give better sensitivity.

2. PRINCIPLE

The sediment or biological samples are mineralized with strong acids. The inorganic mercury is reduced to its elemental form with stannous chloride. The cold mercury vapour is then passed through the quartz absorption cell of an atomic absorption spectrometer (AAS), where its concentration is measured. The light beam of Hg hallow cathode lamp is directed through the quartz cell, into a monochromator and onto a detector that measures the amount of light absorbed by the atomized vapour in the cell. The amount of energy absorbed at the characteristic wavelength is proportional to the concentration of the element in the sample.

3. REAGENT

The reagents used shall meet the purity requirement of the subsequent analysis:

3.1. WATER

Reagent water (referenced also as water in the text) should be free of contamination.

3.2. NITRIC ACID 65%

3.3. HYDROCHLORIC ACID (37%)

3.4. HYDROGEN PEROXIDE

3.5. VANADIUM PENTOXIDE (V_2O_5)

3.6. SILICON ANTI-FOAMING

3.7. HYDROXYLAMINE HYDROCHLORIDE ($NH_2OH.HCl$)

Dissolve 12.0 g of $NH_2OH.HCl$ in 100 ml reagent water. This solution may be purified by the addition of 0.1 ml of $SnCl_2$ solution and purging 1 hour with Hg-free argon.

3.8. POTASSIUM DICHROMATE OXIDIZING SOLUTION (10% w/v)

Weight 25 g of $K_2Cr_2O_7$ in a 250 ml glass bottle, fill it up to 250 ml with water, and shake until total dissolution of the solid. Keep the bottle tightly closed in a double plastic bag, and in an Hg free environment (i.e. laminar flow hood). This solution is stable indefinitely and rarely becomes contaminated.

3.9. BrCl OXIDIZING SOLUTION

Weigh accurately 11 g of KBrO3 and 15 g of KBr into a clean 1 liter glass bottle. Add 200 ml of Milli-Q water; add carefully 800 ml of concentrated HCl. The dilution has to be carried out in a well-ventilated fume hood to prevent exposure to toxic fumes released during dissolution of KBrO3. Keep the bottle wrapped in aluminium foil, tightly closed in a double plastic bag, and in an Hg free environment (i.e. laminar flow hood). This solution is stable indefinitely but can become contaminated.

3.10. STANNOUS CHLORINE SOLUTION 20% (w/v) in 20% (w/v) HCl

Weigh 20 g of $SnCl_2$ in a 100 ml volumetric flask; add 20 ml of concentrated HCl; dissolve the $SnCl_2$ (if needed heat at 60°C for a few minutes on a hot plate); complete to 100 ml with water. This solution might be purified by bubbling with Hg-free argon for 15 minutes. The obtained solution should be clear and transparent, cloudy or yellow solution indicates a bad quality $SnCl_2$. This solution should be prepared fresh every day preferably, if not it should be kept in the fridge.

Note: The concentration of this solution is dependent on the type of accessory use for vapour generation, and can vary between 5 and 30%, the recommendation of the manufacturer

should be followed (i.e. the solution above is recommended for a VGA-70 from Varian). The validity of the solution (i.e. shelf-life) should be defined during method validation.

3.11. COMMERCIAL STANDARD SOLUTION 1000 µg ml^{-1}

Use a certified reference material solution; this solution should be accompanied by a certificate stipulating at minimum the traceability of the certified concentration, as well as the expiry date. The density of the solution, or the certified content in mg kg^{-1} should also be defined, to allow for the preparation of the calibration solution by weighing. Stock solutions should be kept at 5°C.

3.12. ARGON

Use of a gas purifier cartridge for removing mercury, oxygen and organic compounds is recommended.

4. MATERIAL

This section does not list the common laboratory glassware.

4.1. ATOMIC ABSORPTION SPECTROPHOTOMETER

Instrument equipped with an appropriate cold vapour generation system and a quartz or glass tube atomizer. Use a hollow cathode lamp or, preferably, an electrodeless discharge lamp (which gives a greater and more stable light intensity), operated at a current recommended for the lamp and by the instrument manufacturer. An AAS system with background correction device is recommended.

4.2. GLASSWARE

All the glassware, polypropylene, or fluorocarbon (PFA or TFM) containers, including the sample bottles, flasks and pipettes tips, should be washed in the following sequence:

- 24 hrs soaking in a laboratory soap (or 10% alcohol);
- followed by 24 hrs soaking in 10% nitric acid;
- followed by 10% soaking in water;
- final rinse in water; and
- drying under a laminar flow hood.

The cleaned items should be kept in a double sealed plastic bag. It is better to avoid storage of low level (< 5 ng ml^{-1}) solution in plastic, and for this purpose glass or Teflon is recommended.

If it can be documented, through an active analytical quality control program, using spiked samples and method blanks, then certain steps in the cleaning procedure would not be needed for routine samples, those steps may be eliminated from the procedure (i.e. for the levels

measured by flame AAS, some sterile plastic containers are sufficiently free of contamination for certain analytes).

4.3. PIPETTES

Some microliter pipettes size ranging from 50 to 10000 µl are needed. The accuracy and precision of the pipettes used should be checked as a routine every 6 months, and the results obtained should be compared with the individual certificates.

4.4. VOLUMETRIC CONTAINERS of suitable precision and accuracy.

5. INTERFERENCES

5.1. IODIDE, GOLD AND SILVER are known interferences for mercury determination by cold vapour. In samples from marine origin (biota or sediment), the levels of those elements are low, and consequently, do not interfere in the measurement process.

5.2. WATER VAPOUR (moisture) should be avoided in the measurement cell, always follow the manufacturer's protocol (e.g. use of membrane drying tube, correct position of gas separator…) and check for absence of moisture in the measurement cell.

5.3. When using GOLD AMALGAMATION, and with certain batch systems, the excess of oxidant can cause interference or damage the gold amalgamator, it is then recommended to pre-reduce the samples with hydroxylamine ammonium (see 3.7). This is important when using large amount of digested solution in "batch system".

5.4. Some samples (i.e. plants or large amount of mussels) might produce FOAM during the reduction reaction. If the amount of foam is important, it can interfere with gas liquid separation, and/or leak in the measurement cell, this phenomenon can be overcome by using silicon anti-foaming inside the gas liquid separator and/or in the "batch" system. Another option is to use vanadium pentoxide during digestion (see 6.4).

5.5. REDUCTION of inorganic mercury will induce loss, so it is important to stabilise all the solutions by using a strong oxidant as dichromate or BrCl (see 3.8 or 3.9).

6. SAMPLE PREPARATION

6.1. The sample should be prepared according to the recommended method for digestion of marine samples for the determination of trace metal (IAEA, 2011), but before diluting to the final volume (or weight) with water, add an adequate volume of potassium dichromate or BrCl to get the final concentration of 2% or 1% respectively. All the samples from marine origin (sediment or biota) can be prepared using the acid mixture recommended for fish, as Hg is not attached to silicates. For microwave digestion of sample size above 0.8 g, it is strongly recommended to do cold digestion for at least 5 hours and to use a long ramping time (i.e. 25 minutes) to avoid strong reactions in the microwave vessels.

6.2. If other trace elements have to be determined in the digested solution prepared according to the recommended method for digestion of marine samples for the determination of trace metal (IAEA, 2011), following the dilution to the final volume or weight, transfer a quantitatively sufficient amount of digested solution (i.e. at least 10 ml) into a separate container (preferably glass or Teflon), and add an oxidising solution 1% (v/v) of BrCl (see 3.9) or 2% (v/v) of potassium dichromate (see 3.8). Record the amount of oxidising solution added in order to calculate the dilution factor (i.e. dilution factor =1.01 for 0.1 ml of dichromate in 10 ml).

6.3. Alternatively, the samples can be digested using a mixture of 5 ml of HNO_3 and 2 ml of H_2O_2 at 90°C for 4 hours on a hot plate. It is recommended to leave the samples in acid at room temperature, for at least 1 hour before heating. The digestion can be performed either in a Teflon or glass closed containers. After cooling, add an adequate volume of potassium dichromate or BrCl to get the final concentration of 2% or 1% respectively, and dilute to the final volume with water (i.e. for 50 ml final volume, add 1 ml of potassium dichromate or 0.5 ml of BrCl solution). This procedure can be used with bigger sample size if needed (i.e. 2 g); in this case, the volume of nitric acid should be increased to obtain a liquid mixture.

6.4. In the case that the digested solution produces foam during the reduction process (see 5.4), 45 mg of vanadium pentoxide should be added in the digestion vessels before addition of the acid mixture, then follow either paragraph 6.1 or 6.3.

7. PROCEDURE

7.1. SAMPLE SOLUTION

Use the sample prepared with one option as described in section 6.

7.2. BLANK SOLUTION

Prepare at least two blank solutions with each batch of sample, using the same procedure than for the samples.

7.3. PREPARATION OF CALIBRATION SOLUTIONS

7.3.1. Before each batch of determination, prepare by the appropriate dilution of 1000 µg ml^{-1} stock standard solution (see 3.11), at least 4 standard solutions and one calibration blank solution, covering the appropriate range of the linear part of the curve. The calibration standards and calibration blank should be prepared using the same type of acid and oxidising solution than in the test portion (the final concentration should be similar).

7.3.2. Calibration solutions should be prepared fresh each day.

7.3.3. If the necessary intermediate stock standard solutions can be prepared in 5% nitric acid and 1% BrCl or 2% $K_2Cr_2O_7$, these solutions should be prepared monthly.

7.3.4. All volumetric material (pipettes and containers) should be of appropriate precision and accuracy, if not available standard solution can be prepared by weighing.

7.4. INSTALLATION OF VAPOUR GENERATOR ACCESSORY

7.4.1. Install the accessory according to the manufacturer's instructions. Certain systems (i.e. VGA from Varian) are designed to be used for hydride generation as well, and require in the instructions to aspirate an extra HCl solution, in the case of stannous chlorine reduction this solution is to be replaced by water. It is recommended to separate the systems used for hydride and for $SnCl_2$ (i.e. use a spare gas liquid separator and Teflon tubing).

7.4.2. Switch on the argon. For on-line system: start the pump, check the aspiration, and verify the gas liquid separator. If needed replace the pump tubing, clean the gas liquid separator by sonication in diluted detergent.

7.4.3. Clean the system by aspirating reagent and 10% nitric acid as a sample for about 10 minutes. For batch system, perform two cycles with 10% nitric acid.

7.4.4. Set up the atomic absorption spectrometer according to the manufacturer's instructions, at the appropriate wavelength, using the appropriate conditions, and with the suitable background correction system in operation.

7.4.5. Optimise the position of the measurement cell to get the maximum signal.

7.4.6. Connect the vapour generation system to the measurement cell.

7.5. CALIBRATION

7.5.1. Adjust the response of the instrument to zero absorbance whilst aspirating water.

NOTE: if the instrument zero reading is more than 0.002 ABS, the system should be clean again and reagent should be checked.

7.5.2. Aspirate the set of calibration solutions in ascending order, and as a zero member, the blank calibration solution. After the last standard, aspirate 10% nitric acid for 1 minute to rinse the system.

NOTE: The calibration curve is automatically plotted by the instrument software. The obtained curve should be linear with r>0.995.

To correct for the instrumental drift, the calibration should be performed every 20 samples or if the calibration verification has failed (see 7.8.1).

7.6. ASPIRATE SAMPLE BLANK (see 7.2) AND SAMPLE SOLUTIONS (see 7.1)

Record their concentrations as calculated by the software using the calibration curve. Rinse the system by aspirating 10% nitric acid for at least 30 s between samples.

7.7. IF THE CONCENTRATION OF THE TEST PORTION EXCEEDS THE CALIBRATION RANGE, dilute the test portion with the blank solution accordingly.

NOTE: After the measurement of high level (or over calibration) sample, measure a sample blank or water to check the absence of memory effect. If necessary, clean the system for 1 minute with 10% nitric acid.

7.8. QUALITY CONTROL SOLUTIONS

The quality control solutions as described below should be measured during the run.

7.8.1. Calibration Verification CV

After the initial calibration, the calibration curve must be verified by the use of initial calibration verification (CV) standard.

The CV standard is a standard solution made from an independent (second source) material, at/or near midrange. This solution as a calibration standard should be prepared using the same type of acid and oxidising solution than in the test portion (the final concentration should be similar).

The acceptance criteria for the CV standard must be ±10% of its true value.

If the calibration curve cannot be verified within the specified limits, the causes must be determined and the instrument recalibrated before the samples are analysed. The analysis data for the CV must be kept on file with the sample analysis.

The calibration curve must also be verified at the end of each analysis batch and/or after every 10 samples. If the calibration cannot be verified within the specified limits, the sample analysis must be discontinued, the causes must be determined and the instrument recalibrated. All samples following the last acceptable test must be reanalysed.

7.8.2. **Blank solution** (see 7.2)

The maximum allowed blank concentration should be well documented, and if the blank solution exceeds this value all samples prepared along the contaminated blank should be prepared again and reanalysed.

7.8.3. Post digestion spike

Each unknown type of sample should be spiked to check for potential matrix effect.

This spike is considered as a single point standard addition, and should be performed with a minimum dilution factor. The recovery of spike calculated with equation 1 should be 85-115%. If this test fails, it is recommended to run analysis with standard addition method.

<u>Spike solution</u>: mix a fix volume (V1) of the sample solution, and a known volume (V2) of a standard solution with known concentrations (Cstandard).

<u>Unspike solution</u>: mix the same fix volume (V1) of sample solution, and the same volume (V2) of reagent water.

Measure the concentration C (mg l^{-1}) in both solutions on the calibration curve (see 7.6), and calculate recovery as:

$$Cspike = \frac{Cstandard \times V2}{(V1+V2)} \qquad \text{Equation 1}$$

$$R = \frac{C\ Spike\ Solution - C\ Unspike\ solution}{Cspike} \times 100 \qquad \text{Equation 2}$$

To be valid, the concentrations of spiked and unspiked solutions should be in the linearity range of the calibration curve, and the spiked concentration (equation 1) should be in the range of 50-150% of the concentration of the unspiked solution.

7.8.4. Dilution test

If the analyte concentration is sufficiently high (minimally, a factor of 10 above the lower limit of the quantitation following dilution), an analysis of a 1:5 dilution should agree within

±10% of the original determination. If not, then a chemical or physical interference effect should be suspected, and method of standard addition is recommended.

7.8.5. Certified Reference Material

At least one certified reference material of a representative matrix should be prepared with each batch of sample, the calculated result should fall in the value of the certificate and within the coverage uncertainty (Linsinger, 2010), to show evidence of an unbiased result.

The results for the CRM should be recorded for quality control purpose and plotted on a control chart (UNEP/IOC/IAEA 1994).

An example of sequence order with recommended criteria and actions is given in table 1.

TABLE 1. EXAMPLE OF AN ANALYTICAL SEQUENCE:

Solutions Description	Performance	Action
Calibration blank	< maximum allowed calibration blank value	Stop until resolve
Standard solution 1–4	r>0.995	recalibrate in the linearity range
CV	±10% of the true value	Stop until resolve
Sample blank	< maximum allowed blank value	
CRM	Fall in the certificate value within coverage uncertainty, or fall within acceptable criteria of the QC chart	Stop until resolve, check Matrix spike and run again with standard addition method if necessary
Matrix Spike	recovery 100% ± 15%	switch to standard addition, keep records for future analysis of the same matrix
Dilution Test	sample 1 = 5x sample 1 diluted 5x within 10%	switch to standard addition, keep records for future analysis of the same matrix
Unknown Sample 1–10	should ≥ standard 1 and ≤ standard 4	report as <minimum quantification limit or dilute
CV	±10% of the true value	Stop until resolve
Unknown Sample 11–20	should ≥ standard 1 and ≤ standard 4	report as <minimum quantification limit or dilute
Calibration blank	< maximum allowed calibration blank value	Stop until resolve
Standard solution 1–4	r>0.995	recalibrate in the linearity range
CV	±10% of the true value	Stop until resolve
Etc….		

8. CALCULATION OF RESULTS

Results are calculated using equation 3

$$w(m) = \frac{(\rho 1 - \rho 0)}{m} \times f \times V \times R \qquad \text{Equation 3}$$

Where:

w(m) is the mass fraction of element m in the sample, expressed in mg kg^{-1};

ρ1 is the concentration of element m, expressed in mg/l as measured in the sample solution;

ρ0 is the concentration of element m expressed in mg/l as measured in the blank solution;

F is the dilution factor calculated as follow:

$$f = \frac{final\ volume}{initial\ volume}$$

or equal to 1 if ρ1 is determined in undiluted solution;

R is the recovery calculated using the CRM (see 7.8.5) or the post digestion spike.

m is the mass of sample in g

V is the volume of solution in ml

9. EXPRESSION OF RESULTS

The rounding of values will depend on the uncertainty reported with the result; in general for this method two or three significant figures should be reported.

Uncertainty component should be reported with all results. (ISO 2005, Nordtest 2004)

Example: w(Hg) = 0.512 ± 0.065 mg kg^{-1}.

10. REFERENCES

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Annex VIII:

HELCOM

Manual for marine monitoring in the COMBINE programme
ATTACHMENT 1. TECHNICAL NOTE ON THE DETERMINATION OF TOTAL MERCURY IN MARINE BIOTA BY COLD VAPOUR ATOMIC ABSORPTION SPECTROSCOPY

HELCOM Manual for marine monitoring in the COMBINE programme

ATTACHMENT 1. TECHNICAL NOTE ON THE DETERMINATION OF TOTAL MERCURY IN MARINE BIOTA BY COLD VAPOUR ATOMIC ABSORPTION SPECTROSCOPY

1. POSSIBILITIES OF USING COLD VAPOUR ATOMIC ABSORPTION SPECTROMETRY IN TOTAL MERCURY ANALYSIS

The most widely used method for the determination of total mercury in biological tissues is cold vapour atomic absorption spectrometry (CV-AAS), based on a technique elaborated in detail by Hatch and Ott (1968). In this method, (divalent) ionic mercury is reduced to its metallic form (Hgo) in acidic solution using a powerful reducing agent. Subsequently, the elemental mercury is volatilized (purged) by a carrier gas and transported into an absorption cell, where the 253.65 nm wavelength absorbance of mercury atoms is measured.

CV-AAS analysis can be performed manually using batch CV-AAS or automatically using flow injection (FI) techniques. FI is a very efficient approach for introducing and processing liquid samples in atomic absorption spectrometry. The FI technique, combined with a built-in atomic absorption spectrometer optimised for mercury determination, reduces sample and reagent consumption, has a higher tolerance of interferences, lower determination limits and improved precision compared with conventional cold vapour techniques.

The efficiency of various flow injection mercury systems has been reported by several groups (Tsalev *et al*., 1992a, 1992b; Welz *et al*., 1992; Guo and Baasner, 1993; Hanna and McIntosh, 1995; Kingston and McIntosh, 1995; Lippo *et al*., 1997). Better sensitivities of both conventional CV-AAS and FI-CV-AAS can be obtained by collecting mercury vapour released from the sample solution on a gold adsorber (Welz and Melcher, 1984). This so-called amalgamation technique eliminates kinetic interferences due to a different vaporization rate or a different distribution function of the elemental mercury between the liquid and the gaseous phases. The amalgamation ability of the gold adsorber must be carefully and regularly checked. Volatile compounds (in particular sulfur-containing compounds) evaporating together with the elemental mercury from the sample solution may deactivate the adsorber surface. This means an increased risk of underestimation, as unknown quantities of mercury are not collected by the adsorber.

2. SAMPLE PRETREATMENT

It is generally agreed that oxidative conversion of all forms of mercury in the sample to ionic Hg(II) is necessary prior to reduction to elemental Hg and its subsequent measurement by CV-AAS. Therefore, the initial procedural step in mercury analysis is a sample pretreatment, which is aimed at liberating the analyte element from its chemical bonding to the organic matrix and thus transforming all of the analyte species into a well-defined oxidation state. For this purpose, a wide variety of combinations of strong acids (HCl, H2SO4, HNO3) and oxidants (H2O2, KMnO4, K2Cr2O7, K2S2O8) have been tested and recommended (Kaiser *et al*., 1978; Harms, 1988; Vermeir*et al*., 1989; Ping and Dasgupta, 1989; Baxter and Frech, 1990; Landi *et al*., 1990; Navarro *et al*., 1992; Lippo *et al*., 1997).

A suitable sample pretreatment, which implies the complete transformation of all organomercury species into inorganic mercury ions, requires the following:

- oxidation mixtures with a high oxidation potential;
- rapid oxidation (usually promoted by high reaction temperatures), preferably in closed systems;

- compatibility with CV-AAS techniques;
- stability of sample solutions during storage (at least short term);
- no formation of solid reaction products.

On-line sample pretreatment is of particular interest in total mercury determinations because it allows reduction of the well-known problems associated with the inherent risk of contamination, and volatilization and adsorption losses. At present, suitable procedures for on-line pretreatment of solid biological samples are lacking. However, several authors (Tsalev *et al*., 1992a 1992b; Welz *et. al*., 1992; Guo and Baasner, 1993) have demonstrated that microwave digestion coupled with FI-CV-AAS can successfully be applied to the analysis of liquid samples.

3. CONTROL OF CONTAMINATION AND ANALYTE LOSSES

Major difficulties arise due to the mobility and reactivity of mercury and its compounds, respectively, during sample preparation, sample pretreatment, and analysis. Therefore, the stability of samples and standard solutions is of prime importance, and it is advisable to test the stability of typical standard and sample solutions under typical laboratory conditions.
Mercury can disappear from solution due to several mechanisms, including volatilization of mercury compounds, reduction of such compounds followed by volatilization of elemental (metallic) mercury, adsorption on container walls, adsorption onto colloids or particles, incorporation into stable chemical complexes, or incorporation, upon reduction, into stable amalgams.

Thermodynamic considerations of Toribara *et al*. (1970) showed that loss of mercury from a solution containing the element in the monovalent form may occur readily through disproportion and subsequent loss of metallic mercury. Because of the high oxidation potential of the mercury(II)-mercury(I) system, almost any reducing substance could convert some divalent mercury ions into monovalent mercury ions, which then spontaneously disproportion into mercury(II) and mercury(o). The latter escape as metallic vapour from the solution into the gas phase. Because of the almost impossibility of preventing the introduction of small amounts of reducing substances by reagents or solvents, the more dilute mercury(II) solutions would be less stable and lose mercury more readily. The only practical method for stabilizing such solutions is to add a small excess of an oxidising substance (such as permanganate), which has a higher oxidation potential than the mercury(II)-mercury(I) system.
Similarly, Feldman (1974) concluded from his experiments that solutions with 0.1 µg divalent Hg dm-3 in distilled water could be stored in glass vials for as long as five months without deteriorating if the solutions contained 5 % (v/v) HNO3 and 0.01 % Cr2O72-. Storage of such solutions was safe in polyethylene vials for at least 10 days if the solutions contained 5 % (v/v) HNO3 and 0.05 % Cr2O72- . The efficiency of this mixture was probably due to its ability to prevent the hydrolysis of dissolved mercury and prevent its reduction to valencies lower than +2.

4. REDUCING REAGENTS

Tin(II) chloride and sodium tetrahydroborate are predominantly used as reducing reagents for the determination of total mercury by CV-AAS. Sodium tetrahydroborate has been found advantageous for several applications owing to its higher reducing power and faster reaction (Toffaletti and Savory, 1975). In addition, this reductant has been successfully used even in the presence of interfering agents such as iodide and selenium (Kaiser *et al*., 1978). However, potential interferences can occur from metal ions (e.g., Ag(I), Cu(II), Ni(II)), which are themselves reduced to the metallic state and so may occlude mercury through amalgamation.
Welz and Melcher (1984) showed that sodium tetrahydroborate could more readily attack those organic mercury compounds which were not reduced to metallic mercury by tin(II) chloride.

However, they stated that sodium tetrahydroborate could not be recommended as the reducing reagent for the amalgamation technique. They found that, due to the rather violent reaction with sodium tetrahydroborate, fine droplets of the sample solution were carried by the gas stream and contaminated or deactivated the adsorber surface. Further, they considered even more important the fact that not only mercury but all gaseous hydride-forming elements (e.g., arsenic, antimony, selenium) were volatilized when sodium tetrahydroborate was used as reductant. These hydrides reacted with the adsorber material and deactivated its surface, thus no longer permitting a sensitive and reproducible determination of mercury.

5. INTERFERENCES

Interferences by volatile nitrogen oxides in the determination of mercury by FI-CV-AAS were studied by Rokkjaer et al. (1993). The main symptom of the interference effects was a suppression, broadening or even splitting of the mercury signal. The authors postulated that volatile nitrogen oxides formed as reaction products of nitric acid during sample decomposition scavenged the reducing agent and concomitantly inhibited the reduction of mercury(II). The rate of the reaction of nitrogen oxides with the reducing agent was considered to be so fast that it was consumed before the reduction of mercury was complete. Rokkjaer et al. (1993) demonstrated that the interference could easily be remedied by purging the sample solution with an inert gas prior to the introduction of the reducing agent. Lippo et al. (1997) concluded from their experiments that nitrogen mono- and dioxide, having molecular absorption bands at 253.63 nm and 253.85 nm, respectively, might cause unspecific absorption at the specific mercury wavelength of 253.65 nm, leading to enhanced and broadened mercury signals if not properly compensated for by adequate instrumental background correction.

6. INTERNAL (ROUTINE) QUALITY CONTROL

In order to demonstrate that the analytical method applied is fit for the purpose of the investigations to be carried out, control materials should be regularly analysed alongside the test materials (cf. Chapter B.5 of the Manual).

The control materials - preferably certified reference materials (CRM) - should be typical of the test materials under investigation in terms of chemical composition, physical properties and analyte concentration. Fitness for purpose is achieved if the results obtained from the analysis of the control materials are within the defined limits of permissible tolerances in analytical error (see Chapters B.3.5, B.4.2.5 and B.4.2.5.2b of the Manual).

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Annex IX:

Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment

UNITED NATIONS ENVIRONMENT PROGRAMME **November 2011**

Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment

Recommended Methods For Marine Pollution Studies 71

Prepared in co-operation with

NOTE: This recommended method is not intended to be an analytical training manual. Therefore, the method is written with the assumption that it will be used by formally trained analytical chemists. Several stages of this procedure are potentially hazardous; users should be familiar with the necessary safety precautions.

For bibliographic purposes this document may be cited as:

UNEP/IAEA: Sample work-up for the analysis of selected chlorinated hydrocarbons in the marine environment. Reference Methods for Marine Pollution Studies No 71, UNEP, 2011.

PREFACE

The Regional Seas Programme was initiated by UNEP in 1974. Since then, the Governing Council of UNEP has repeatedly endorsed a regional approach to the control of marine pollution and the management of marine and coastal resources and has requested the development of regional action plans. The Regional Seas Programme at present includes thirteen regions and has over 140 coastal States participating in it (1).

One of the basic components of the action plans sponsored by UNEP in the framework of the Regional Seas Programme is the assessment of the state of the marine environment, its resources and the sources and trends of the pollution and its impact on human health, marine ecosystems and amenities. In order to assist those participating in this activity and to ensure that the data obtained through this assessment can be compared on a world-wide basis and thus contribute to the Global Environment Monitoring System (GEMS) of UNEP, a set of Reference Methods and Guidelines for marine pollution studies are being developed as part of a programme of comprehensive technical support which includes the provision of expert advice, reference methods and materials, training and data quality assurance (2). The Methods recommended for adoption by Governments participating in the Regional Seas Programme.

The methods and guidelines are prepared in co-operation with the relevant specialised bodies of the United Nations system as well as other organisations and are tested by a number of experts competent in the field relevant to the methods described.

In the description of the methods and guidelines, the style used by the International Organisation for Standardisation (ISO) has been followed as closely as possible.

The methods and guidelines published in UNEP's series of Reference Methods for Marine Pollution Studies are not considered as definitive. They are planned to be periodically revised taking into account the new developments in analytical instrumentation, our understanding of the problems and the actual need of the users. In order to facilitate these revisions, the users are invited to convey their comments and suggestions to:

Marine Environmental Studies Laboratory
IAEA Environment Laboratories
4, Quai Antoine 1er
MC 98000 MONACO

which is responsible for the technical co-ordination of the development, testing and inter-calibration of Reference Methods.

References:

(1) www.unep.org/regionalseas (2011)

(2) UNEP/IAEA/IOC: Reference Methods and Materials: A Programme of comprehensive support for regional and global marine pollution assessment. UNEP, 1990.

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1. SCOPE AND FIELD OF APPLICATION

This reference method is intended for use in monitoring programmes and pilot research studies. The document describes procedures for the isolation of purified fractions amenable for the determination of DDTs and PCBs in marine sediments and marine organisms by capillary GC/ECD. It is assumed that most of the participants in the UNEP Regional Seas Programmes are equipped with advanced high resolution capillary gas chromatographs and will be able to implement most, if not all, of the procedures described in Reference Method No 40, "Determination of DDTs and PCBs by capillary gas chromatography and electron capture detection" (UNEP 1988). Assuming consistent results are routinely being obtained with these methods by the analytical laboratory, the determination of specific compounds (as opposed to generic mixture of PCBs) opens up the possibility not only of identifying environmental "hot spots", but also for characterising sources, elucidating transport pathways and developing data of greater toxicological relevance. The organisation and content of this document, however, deserves further comment. Under the sections devoted to SEDIMENTS and ORGANISMS, subsections are provided relating to procedures for: 1) Sampling, 2) Extraction and 3) Clean-up and fractionation. In each subsection, several alternative procedures are described. These various procedures have been previously tested and are provided to accommodate the range of capabilities in participating laboratories. For example, laboratories which have access to an HPLC may consider the benefits of using HPLC fractionation procedures in lieu of more conventional low pressure column chromatographic method. Participants are generally encouraged to implement the most effective procedures within the constraints of their individual laboratories.

Several other halogenated pesticides and other electron capturing organic compounds may be present in environmental samples and many of these compounds could also be isolated by the methods described here. However, not all residues will be stable to the clean-up procedures applied for the determination of PCBs and DDTs. Consequently, every analyst must test for analyte recovery and analytical reproducibility prior to applying these methods for other analytes on a routine basis. Primary emphasis should be placed on obtaining the cleanest possible purified fraction for capillary GC/ECD analysis so that interferences and misidentification are minimised, if not eliminated.

2. PRINCIPLES

Following collection of sediment or biota samples using appropriate techniques, samples are stored in trace organic free vessels at -20°C until analysis. For analysis, the samples are prepared for solvent extraction. To achieve a satisfactory recovery of the chlorinated hydrocarbons, samples are dried by either desiccation with anhydrous sodium sulphate or by freeze-drying. Lipids are then Soxhlet extracted from sediments using hexane and dichloromethane, and from biota using hexane or petroleum ether. Following initial clean-up treatments (removal of sulphur from sediment extracts and treatment of biota extracts with concentrated sulphuric acid to destroy some interfering lipids), extracts are fractionated using column chromatography. Detailed protocols for absorption chromatographic fractionation are described for both low and high pressure systems, using Florisil and silica gel respectively. (Additional information concerning alternative techniques including gel permeation chromatography is provided).

3. REAGENTS, SOLVENTS, STANDARDS

3.1. Reagents

3.1.1. List of reagents

- Demineralized distilled water produced by distillation over potassium permanganate (0.1 g/l $KMnO_4$) or equivalent quality, demonstrated to be free from interfering substances.
- Detergent.
- Potassium dichromate.
- HCl. 32%.
- Concentrated H_2SO_4 (d 20°C: 1.84 g/ml).
- Sulfochromic cleaning solution made from concentrated sulphuric acid and potassium

dichromate.
- KOH.
- Anhydrous sodium sulphate.
- Copper fine powder (particle size 63µm).
- Carborundum boiling chips.
- Hg.
- Glass wool
- Alumina (200-240).
- Silica gel (60-100).
- Florisil PR (60-100).
- Bio-Beads SX-3 (200-400).
- Sephadex LX-20.

Solvents:
- Hexane, Dichloromethane, Methanol, Pentane, Cyclohexane, Toluene and Ethyl Acetate, all "distilled in glass" quality.

Standards:
- PCB congeners: 29, 30, 121, 198.
- ε HCH.
- Endosulfan Id_4.
- n-C_{14} d_{30}, n-C_{19} d_{40}, n-C_{32} d_{66}.
- Naphthalene d_8.
- Hexamethylbenzene.
- Cadalene: 1, 6-dimethyl-4-(1-methylethyl)naphthalene.
- DDT reference solutions - Prepare a stock solution of the DDT series (pp' DDT, op DDT, pp' DDD, op DDD, pp' DDE, op DDE) by dissolving 50 mg of each compound in 100 ml of hexane. Store stock solution in sealed glass ampoules.
- Other reference solutions - should be prepared if other residues are to be quantified in these procedures.

NOTES:

Working solutions obtained from the stock reference solutions should be prepared on a regular basis depending on their use and stored in clean glass volumetric flasks tightly capped with non-contaminating materials such as Teflon or glass. Extreme care must be taken to ensure that the concentrations of the standards have not altered due to solvent evaporation.

In order to achieve acceptable accuracy for the standard solutions, at least 50 mg of pure individual compound should be weighed and dissolved into 100 ml of hexane. This will give stock solutions of 500ng/µl.

Example of preparation of stock solutions:

Preparation of a stock solution of pp' DDE at approximately 500ng/µl:

The pp' DDE stock solution is prepared by dissolving approximately (but weighed accurately) 50 mg of pp' DDE in hexane in a 100 ml volumetric flask and bringing the volume to exactly 100 ml with hexane. If the actual weight of pp' DDE is 52 mg, then

$$\frac{52\,\text{mg DDE}}{100\,\text{ml solvent}} \times \frac{1000\,\mu\text{g}}{\text{mg}} \times \frac{\text{ml}}{1000\,\mu\text{l}} = \frac{52\,\text{mg DDE}}{100\,\text{ml of solution}}$$

52 mg/100 ml ⇒ 0.52 mg/ml⇒ 520 µg/ml ⇒ 520 ng/µl

The concentration of the stock solution will be: 520ng/µl

Preparation of an intermediate solution:

Use the stock solution to prepare the intermediate solution. The concentration of pp' DDE intermediate solution should be approximately 5ng/µl. To prepare the 5ng/µl intermediate solution, transfer 1 ml of the pp' DDE stock solution into a 100 ml volumetric flask and dilute with hexane to 100 ml.

$$\frac{1\,\text{ml DDE stock solution}}{100\,\text{ml final volume}} \times \frac{520\,\text{ng DDE}}{\mu l} = \frac{5.2\,\text{ng}}{\mu l\ \text{intermediate solution}}$$

The concentration of the intermediate solution will be: 5.2 ng/µl

Preparation of the working solution:

Use the intermediate solution to prepare the working solution. The concentration of pp' DDE in the working solution could be approximately 50pg/µl.

To prepare the 50 pg/µl working solution, transfer 1 ml of the pp' DDE intermediate solution into a 100 ml volumetric flask and dilute with hexane to 100 ml.

$$\frac{1\,\text{ml DDE intermediate solution}}{100\,\text{ml final volume}} \times \frac{5.2\,\text{ng}}{\mu l} \times \frac{1000\,\text{pg}}{\text{ng}} = \frac{52\,\text{pg}}{\mu l\ \text{working solution}}$$

The concentration of the working solution will be: 52 pg/µl

3.1.2. Cleaning of solvents

All reagents, including the distilled water should be of analytical quality. Commercially available solvents like acetone, acetonitrile, dichloromethane, hexane and pentane are invariably contaminated with ECD-active substances; their concentrations vary from batch to batch and with supplier. Reagent quality should be checked by injection of 2 µl of a 100 ml batch of solvent, after concentration to 50 µl in a rotary evaporator. No peak in the GC-ECD chromatogram (90 - 250 °C) should be larger than that for 1pg of lindane. Otherwise, the solvent must be distilled. The following procedure has been found to be both efficient and cost effective, as it allows the use of technical grade solvents as the basic material (reducing the cost by one order of magnitude). 130 - 150 cm height columns are required; the packing material must be glass (to allow subsequent cleaning with an oxidising acid). The entire equipment is cleaned prior to use by 2 consecutive distillation procedures with 500 ml water in each case. It is essential that a current of nitrogen gas (15 ml/min) flows from the distillation flask during distillation of the organic solvents: the condenser serves as exhaust. Ambient air is not in contact with the solvent in this way. Problems are associated with other methods of excluding room air (e.g., active carbon or molecular sieves), the most important one being discontinuity. The condensate is distilled into a 1 litre flask at a 1:20 ratio. This large volume allows for direct transfer into the appropriate solvent containers which should be made of glass and of a sufficient size to provide solvent for not more than 6 analyses. A bottle with sufficient solvent for 10 - 15 analysis has to be opened and closed many times and even when kept closed, when not in use, contamination from the surrounding atmosphere takes place. For more detailed information, consult the Reference Method No 65: UNEP/IOC/IAEA: Reagent and laboratory ware clean-up procedures for low-level contaminant monitoring.

3.1.3. Cleaning of reagents and adsorbents

3.1.3.1. Cleaning of reagents

Powdered or crystalline reagents, such as anhydrous sodium sulfate (Na_2SO_4)*, potassium hydroxide (KOH), glass wool * and carbon or carborundum boiling chips *, must be thoroughly cleaned before use. They should be extracted with hexane in a Soxhlet apparatus for 8 hours and then with methanol or dichloromethane for another 8 hours. For those items indicated by an *, this will require pre-combustion in a muffle furnace at approximately 400°C.

3.1.3.2. Cleaning of adsorbents

Silica gel, alumina and Florisil have to be solvent extracted. Each reagent is first refluxed with methanol or dichloromethane in a Soxhlet apparatus for 8 hours, then with n-hexane for the same period. The solvent is removed by a rotary evaporator operating at low speed, until the sorbent starts falling down as fine particles. Reagents are dried in a drying oven at 0.01 mbar. If this is not available, they are dried in a normal oven at 120°C for 4 hours. This serves to activate silica and alumina. Florisil has to be activated at 130°C for 12 hours. The sorbent is allowed to cool in the oven (if possible under vacuum to avoid uptake of contaminants from the atmosphere) or alternatively, in a dessicator. As active sorbents attract water and contaminants from the atmosphere, controlled deactivation should be carried out by adding water to the fully active sorbent (5% by weight to silica, 2% by weight to alumina, and 0.5% by weight to Florisil). The deactivation procedure should be carried out by adding the water to the sorbent and mixing by gentle shaking for a few minutes. The equilibration takes one day. The activity can be maintained for longer periods of time by sealing the required amount of sorbent in glass ampoules. Otherwise, the activation/deactivation has to be done the day before use.

3.2. Apparatus and equipment

The laboratory used for organic trace analysis must be a dedicated facility, isolated from other projects that could be sources of contamination. It must be properly constructed with fume hoods and benches with electric sockets that are safe for use with flammable solvents. The laboratory must have extractors and rotary evaporators cooling water to run the stills. In tropical regions and in dry climates, a refrigerated re-circulating system should be used to reduce temperatures to the required levels and/or to conserve water. Stainless steel or ceramic tiles make good non-contaminating surfaces. If necessary, benches can be coated with a hard epoxy resin and walls can be painted with epoxy paint. A sheet of aluminium foil on the workbench provides a surface which can be cleaned with solvent. A vented storage facility for solvents is essential. Benches must be fitted with frames to hold stills, extractors, etc. The emergency cut-off switch should be accessible from both inside and outside the laboratory. Fire fighting equipment should be mounted in obvious places and laboratory personnel trained in their use.

3.2.1. List of materials

- A coring device with liners and plunger or a grab sampler (thoroughly cleaned with detergents and solvents before use).
- Glass jars and aluminium foil, stainless steel knives, scoops, forceps, labels, marking pens, logbook.
- Insulated plastic boxes for transporting samples. Ice or dry ice.
- Deep freezer (-18 to -20°C) for sample preservation (frost free type freezers heat to above zero during frost removal cycles and they cannot be used for long term storage).
- Rotary evaporator.
- Kuderna-Danish (or similar) concentrator and heater.
- Soxhlet extraction apparatus and heaters.
- Glassware including boiling flasks, ground glass stoppers, beakers, Erlenmeyer flasks, separatory funnels, centrifuge tubes, weighing bottles, pipettes, tissue grinders.
- Drying oven (temperature range up to at least 300°C) for determining sample dry weights, baking of contaminant residues from glassware and reagents.

Note: A muffle furnace is better for baking materials at greater than 300°C, if required.

- Centrifuge and tubes.
- Freeze-dryer and porcelain pestle and mortar.
- Analytical balance with an accuracy of 0.1 mg and an electro-balance with an accuracy of at least 1 µg.
- Stainless steel tweezers and spatulas.

- Dessicator - completely free of organic contamination and with no grease applied to sealing edges.
- Supply of clean, dry nitrogen.
- Columns for silica gel, alumina and Florisil chromatography.
- Mechanical blender (food mixer).
- Vacuum pump (water-jet air pump).

3.2.2. Cleaning of glassware

Scrub all glassware vigorously with brushes in hot water and detergent. Rinse five times with tap water and twice with distilled water. Rinse with acetone or methanol followed by hexane or petroleum ether. Bake overnight in an oven at 300 °C. All glassware should be stored in dust free cabinets and tightly sealed with pre-cleaned aluminium foil when not in use. Ideally glassware should be cleaned just before use.

For more detailed information, consult Reference Method No 65: UNEP/IOC/IAEA: Reagent and laboratory ware clean-up procedures for low level contaminant monitoring.

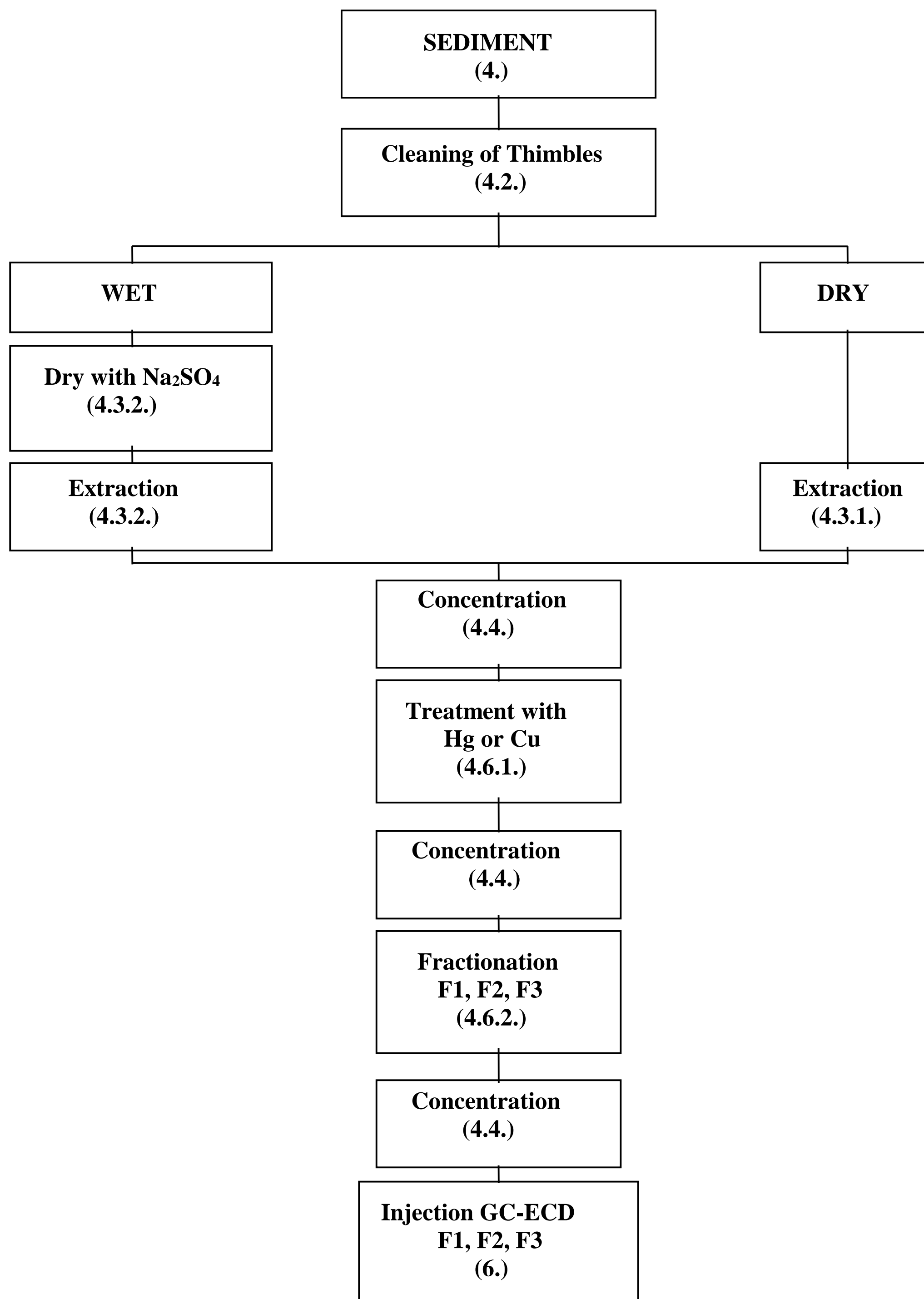


Diagram of the extraction procedure for sediment samples.

4. SEDIMENTS

4.1. Sampling

For the preparation of the samples (including selection of sites, collection of samples and storage) the reader should refer to the Reference Method Nº 58: Guidelines for the use of sediments for the marine pollution monitoring programmes, to the Reference Method Nº 20: UNEP/IOC/IAEA: Monitoring of petroleum hydrocarbons in sediments and to UNEP(DEC)/MEDW.C282/Inf.5/Rev1: Methods for sediment sampling and analysis (2006).

4.2. Cleaning of extraction thimbles

Paper extraction thimbles should be cleaned prior to sample extraction. For use in the extraction of sediment samples, the extraction can be performed in the Soxhlet apparatus with 250 ml of a mixture hexane / dichloromethane (50:50) for 8 hours cycling the solvent through at a rate of 4 to 5 cycles per hour. Add into the solvent a few carborundum boiling chips to get a regular ebullition.

The use of disposable paper thimbles for the extraction procedure rather than re-usable glass fibre thimbles is recommended due to the difficulties encountered in cleaning the latter.

4.3. Extraction of sediments

4.3.1. Extraction of freeze-dried samples

Select a 50-100 g sub-sample of the sediment, weigh this sub-sample and freeze-dry it. When dried, re-weigh it and calculate the dry to wet ratio. Then pulverise the sample using a pestle and mortar and sieve it using a 250 µm stainless steel sieve. Accurately weigh about 20 g of ground sample and place it in the pre-cleaned extraction thimble. Add 1 ml of a solution of 25 pg/µl of 2,4,5 trichlorobiphenyl (PCB Nº 29), 20.9 pg/µl of 2,2',3,3',4,5,5',6 octachlorobiphenyl (PCB Nº 198), 20 pg/µl of ε HCH and 21 pg/µl of Endosulfan Id_4 as internal standards and extract for 8 hours in a Soxhlet apparatus with 250 ml of a mixture hexane / dichloromethane (50:50), cycling the solvent through at a rate of 4 to 5 cycles per hour, add into the solvent a few carborundum boiling chips to get a regular ebullition. Alternatively (or in addition), PCB congeners No 30, 121, or octachloronaphthalene and PCB congeners can be used as internal standards. Prepare a procedural blank by extracting an empty thimble using the same procedure as for the samples.

4.3.2. Extraction of wet samples

The sediment is thawed, sieved at 250 µm and homogenised manually with a stainless steel spatula or clean glass rod. A sub-sample of 1-2 g is weighed into a flask and placed in a drying oven at 105 °C for 24 hours, then allowed to cool to room temperature and re-weighed. Calculate the dry to wet ratio and discard the dry sediment (unless it is being used for other analysis e.g. TOC, total organic carbon).

Place a 30-40 g sub-sample of thawed, homogenised sediment into a blender. Slowly, add 100g of anhydrous sodium sulphate (desiccant) and blend the mixture at high speed for 10 minutes. Transfer the dried sample quantitatively to the pre-cleaned extraction thimble in the Soxhlet apparatus, add the internal standard solution (see above) and apply the same extraction procedure as above. Extract the same amount of sodium sulphate as a procedural blank, making sure to add an appropriate amount of internal standard solution.

4.3.3. Example of determination of percent moisture

Many environmental measurements require the results to be reported on a dry weight basis. The percent moisture or water content in the sample is determined by weighing an aliquot, not used for analysis, of the sample before and after drying. The drying can be done by heating a few grams (1-2 g) of the sample in an oven to constant weight.

Weigh an empty glass beaker that will be used to hold the sample while it is dried.
Empty beaker weight = 10.4417 g

Add the wet sample to the beaker and reweigh. Calculate the wet weight of the sample.
Empty beaker weight + wet sample = 12.2972 g
Wet sample weight = 12.2972 g - 10.4417 g = 1.8555 g

Dry the sample to constant weight: dry the sample for 24 hours, weigh it, dry again for 12 hours, re-weigh it, when the difference in weight is less than 5%, it means that the sample is dried.
Empty beaker weight + dry sample weight = 10.9396 g
Dry sample weight = 10.9396 g - Empty beaker weight
Dry sample weight = 10.9396 g - 10.4417 g = 0.4979 g

Calculate the percent dry sample weight.

$$\% \text{ Sample weight} = \frac{\text{Sample dry weight}}{\text{Sample wet weight}} \times 100$$

$$= \frac{0.4979}{1.8555} \times 100 = 26.8\ \%$$

Calculate the percent moisture.

Water content = wet weight - dry weight
= 1.855 g - 0.4979 g = 1.3576 g

$$\% \text{ Moisture} = \frac{\text{Sample water weight}}{\text{Sample wet weight}} \times 100$$

$$\% \text{ moisture} = \frac{1.3576}{1.8555} \times 100 = 73.2\ \%$$

4.4. Concentration of the extract

For both extraction procedures, the extracts are concentrated in a rotary evaporator to about 15 ml. Under good vacuum conditions the temperature of the water bath must not exceed 30 °C. Dry the extract with anhydrous sodium sulphate (when the sodium sulphate moves freely in the flask it means that the extract is dried). Collect the dried extract in the graduated tube of a Kuderna-Danish concentrator. Concentrate the extract to approximately 5 ml with the Kuderna-Danish concentrator and adjust the volume to exactly 1 ml by evaporating excess solvent under a gentle stream of clean dry nitrogen. The sample extract will be analysed gravimetrically for extractable organic matter (EOM) content at the 1 ml volume as a starting point. If measurements of the EOM are outside the calibration range of the balance, the total volume of the extract is adjusted accordingly using either dilution with hexane or evaporating under a stream of nitrogen gas.

4.5. Extractable organic matter

Before carrying out the clean-up procedure, it is advisable to determine the extractable organic matter.

The EOM is determined in the following manner. On the weighing pan of an electro-balance, evaporate a known volume of the sediment or biota extract (up to 100 µl) and weigh the residue with a precision of about ± 1 µg. If the residue is less than 2 µg, pre-concentration of the original extract is required. The quantity of EOM is:

$$\text{EOM } (\mu g/g) = \frac{\text{Weight of residue } (\mu g) \text{ x volume of the extract (ml) x } 1000}{\text{Volume evaporated } (\mu l) \text{ x quantity of sample extracted (g)}}$$

Note that extreme care must be taken to ensure balance and pans are clean, dry and stable to obtain accurate readings at the ± 1 µg level. A small hot plate is used to warm pans and forceps and thus keep these instruments dry after solvent cleaning. If no electro-balance is available, a known volume of the extract can be transferred into a clean pre-weighed beaker. The solvent is evaporated with dry and clean nitrogen until a constant weight of about 1 mg is reached. Calculate the amount of "lipids" in the sample taking into account the volume of the lipid extract which was dried.

Example of calculation of E.O.M.

The extractable organic matter content of a sample is operationally defined as the weight of material extracted with the solvent employed (H.E.O.M. in case hexane is used as solvent). An aliquot of the sample extract is taken (few µl), the solvent is evaporated and the residue is weighed to determine the quantity of lipids extracted in the aliquot and from it to the total sample. The results are normally reported in mg lipids per gram dry weight extracted.

A 1 µl aliquot is removed from a 2.5 ml sample extract for determination of E.O.M. The 1 µl aliquot is evaporated on the pan of an electro-balance and the residue is weighed. Three determinations are made and the average taken.

Measurements:
Sample dry weight extracted: 4.443 g
Total volume of the extract: 2.5 ml
Sample aliquot removed: 1 µl
(1) Weight of a 1 µl aliquot after solvent evaporation: 32.2 µg
(2) Weight of a 1 µl aliquot after solvent evaporation: 32.1 µg
(3) Weight of a 1 µl aliquot after solvent evaporation: 32.3 µg
Average weight of a 1 µl aliquot : 32.2 µg

Total volume of the extract: 2.5 ml

Total quantity of lipids in the sample:

$$32.2\ \mu g/\mu l \text{ x } 2.5 \text{ ml x } \frac{1000\ \mu l}{\text{ml}} = 80500\ \mu g \text{ or: } 80.5 \text{ mg}$$

With 4.443 g of sample extracted:
80.5 mg/ 4.443 g = 18.1 mg lipids/g

4.6. Clean-up procedure and fractionation

Purposes of the clean-up: removal of lipids, whenever present at a significant amount; removal of elementary sulphur and sulphur compounds. Both these compound classes can interfere with the gas-chromatographic separation.

4.6.1. Sulphur and sulphur compounds removal

Elementary sulphur and sulphur compounds such as mercaptans should be removed from the extract. This could be done by using either mercury or activated copper.

a) Mercury method.

Add one drop (a few ml) of mercury to the sediment extract and shake vigorously for one minute. Centrifuge and carefully recover and transfer the extract in another tube with a Pasteur pipette. If the mercury is still tarnished, repeat the treatment with another drop of mercury, shake, transfer the hexane into another tube. Repeat this treatment until the mercury stays brilliant in the extract. Rinse the mercury with 5 ml of hexane and combine the extracts. Then, concentrate the resulting solution to ca. 1 ml with a gentle stream of nitrogen.

Cleaning of mercury:

Caution: When removing mercury from the sample, always use a plastic tray to keep the glassware in and work under a fume hood.

Fit a folded filter paper in a 10 cm diameter conical glass funnel and fix the funnel over a 250 ml glass beaker. Using a needle, make a small hole in the bottom of the filter paper. Carefully put the mercury onto the funnel. The mercury flows through the small hole in the filter paper leaving the solid impurities on its surface. The mercury collected is washed three times by shaking it carefully with dichloromethane and by removing dichloromethane layer with the help of a clean glass syringe. Allow the rest of dichloromethane evaporate and store the clean mercury in a thick walled glass bottle with a ground glass stopper. In order to avoid escape of mercury vapour, store the mercury under methanol.

Another way of cleaning the mercury involves sucking the dirty mercury through a capillary tube, such as a Pasteur pipette, connected to a guard-flask and then to a vacuum pump. The mercury will pass through the Pasteur pipette and will be collected and cleaned in the guard-flask. Then it should be transferred into a thick wall glass bottle with a ground glass stopper. The mercury is covered with a layer of methanol to protect it from oxidation.

b) Activated copper method.

Transfer about 20 grams of the copper powder in an Erlenmeyer. Add enough concentrated HCl to cover the copper powder, agitate. Sonicate for 10 min., agitate, put again in ultrasonic bath and sonicate for 10 min. Throw the used HCl, add some fresh HCl, transfer in ultrasonic bath and sonicate for 20 min. repeat that procedure four times in total. Wash with distilled water, agitate, discard, add water again, transfer in ultrasonic bath and sonicate for 15 min., discard the used water, repeat that procedure again, up to pH neutral. Wash with acetone, agitate, transfer in ultrasonic bath and sonicate for 15 min. repeat that procedure four times in total. Then use the same procedure with hexane as a solvent.
Keep in hexane (use it immediately, avoids Cu to be in contact with air).

Transfer 3 to 4 Pasteur pipettes per sample in the flasks containing the hexane extracts. Let the copper react all night. The presence of sulphur compounds in the sample will be detected by the tarnishing of the copper powder. Then, concentrate the resulting solution to ca. 1 ml with a gentle stream of pure nitrogen.

4.6.2. Fractionation

An adsorption chromatography step is used to remove interfering lipids and to fractionate the extract into classes of compounds. Many variations of adsorption chromatography clean-up procedures have been published to date. Four procedures are reported here in order of increasing complexity.

Preparation of the columns: Glass burettes (1 cm diameter) with Teflon stopcocks make convenient adsorption columns. The column is plugged with pre-cleaned cotton or glass wool. Prepare separate columns for each sample and blank determination. The column is partially filled with hexane. The appropriate amount of sorbent is mixed with hexane in a small beaker to form a slurry. A glass funnel and a glass rod are used to pour the adsorbent into the column. Several rinses with hexane are necessary to fill the column to the desired height. Tap with a pencil or a hard silicone tube against the column in order to settle the adsorbent into an even bed. Flush the material adhering to the wall of the column down to the bed with solvent. Prepare each column freshly immediately before use. Never let the column get dry.

4.6.2.1. Florisil

A Florisil column is used for this fractionation, which is prepared in the following way. The Florisil should be pre-extracted in the Soxhlet apparatus to remove any contaminants, using methanol or dichloromethane for 8 hours, followed by hexane for another 8 hours. It is then dried in an oven. Activation is achieved by heating the dried Florisil at 130°C for 12 hours. It is then partially deactivated with 0.5% water by weight and stored in a tightly sealed glass jar with ground glass stopper. The water should be well mixed into the Florisil and the mixture should be allowed to equilibrate for one day before use. The activation/deactivation procedure should be carried out one day before use. A 1 cm burette with Teflon stopcock is plugged with pre-cleaned glass wool. A column with a sintered glass disk could also be used. 17 grams of Florisil are weighed out in a beaker and covered with hexane. A slurry is made by agitation and poured into the glass column. The Florisil is allowed to settle into an even bed and any Florisil adhering to the column is rinsed down with hexane. The solvent is drained to just above the Florisil bed. It should be rinsed with a further 5 ml of hexane; one gram of anhydrous sodium sulphate is added to the top of the column in order to protect the surface of the Florisil from any disturbance. The column should never run dry. Individual columns should be prepared immediately before use and a new column of Florisil used for each sample.

The extract, reduced to 1 ml, is put onto the Florisil column. It is carefully eluted with 65 ml of hexane and the first fraction collected. Then the column is eluted with 45 ml of a mixture containing 70 % of hexane and 30 % of dichloromethane and the second fraction collected. The third fraction will be eluted with 60 ml of pure dichloromethane.

Fraction one will contain the PCBs, pp' and op DDE and some other pesticides such as HCB, aldrin, heptachlor, DDMU.

Fraction two will contain the DDTs, DDDs, most of the toxaphene, and some pesticides such as the HCH isomers and chlordane components.

Fraction three will contain mainly dieldrin, endrin, heptachlor epoxide and endosulfan components. Typical chromatograms obtained are shown below.

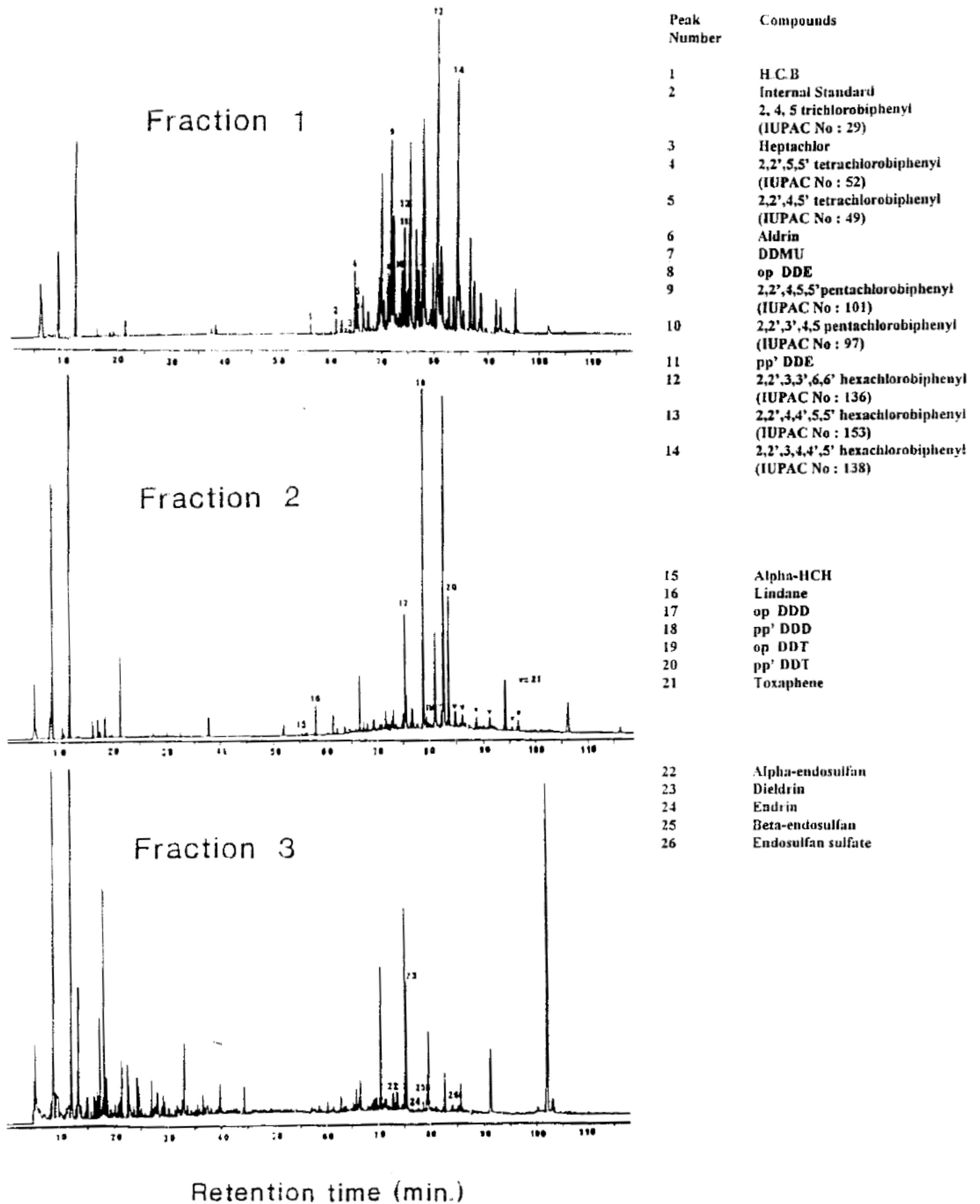


Figure 2: GC-ECD organohalogen analyses

4.6.2.2. Gel permeation chromatography

Low pressure GPC can be used as an alternative clean-up technique to remove high molecular weight co-extractable lipidic material from polycyclic aromatic compounds and halogenated aromatics. Concurrently, elemental sulphur could be also removed from the whole organic extract.

The main feature of the semi-preparative-GPC as a clean-up technique relies on the compatibility of this analytical procedure with labile components of the extract (i.e. DDTs, chlorinated cyclohexadiene derivatives), which are not stable in other types of extract clean-up procedures. Further, GPC as a clean-up technique has already been automated, enabling a high sample throughput, taking into account the short analysis time involved.

The GPC retention mechanism may involve adsorption, partition and size exclusion mechanisms. The predominance of one mechanism over the others is largely determined by the choice of the mobile phase and the pore size of the packing. In the case of GPC packings with large pore size (1000-2000 daltons) size exclusion and adsorption mechanisms prevail (Bio-Beads SX-3 using cyclohexane, dichloromethane-hexane, dichloromethane-cyclohexane, toluene-ethylacetate and ethylacetate-cyclohexane) (Ericksson *et al*., 1986). On the other hand, when smaller pore sizes (400 daltons) are used in combination with highly polar solvents, (THF, DMF) size exclusion predominates (Lee *et al*., 1981). While using the first approach, a chemical class fractionation could be obtained, however, if smaller pore sizes are used it should be combined with another fractionation technique (i.e. adsorption chromatography) to achieve this selectivity. It has yet to be demonstrated that using GPC as a single clean-up step produces a completely clean extract for GC-ECD determination. Nevertheless, taking into account the increasing availability of high-resolution low molecular weight exclusion packings, they could definitively integrate fractionation and clean-up in a single step.

Low resolution packing (Sephadex LH and Bio-Beads SX, 200-400 mesh size) are the most widely used because they are inexpensive and afford relatively high sample loading (500 mg in 10 mm i.d. columns). The implementation of low resolution GPC requires a solvent delivery system and a UV detector and may be useful. For method development, it is advisable to inject a broad range of standard compounds covering the whole range of molecular weights of the analytes to be determined in order to determine the cut-off points to fractionate real samples. Reported recoveries of PCBs and PAHs range from 60 to 80 % for the concentration level (ng) injected. (Fernandez and Bayona, 1992).

4.6.2.3. Alumina and HPLC (silica column)

The first step in this clean-up procedure is an adsorption step using an alumina column to remove most of the lipid material. Prepare an alumina column (4 x 0.5 cm i.d., made from a Pasteur pipette). Apply the concentrated extract to the top of the column and elute with 10 ml hexane. Concentrate the eluate to about 200 µl. It is followed by a second step to more completely remove interfering compounds and at the same time to separate the compounds of interest into different fractions, containing aliphatics, PCBs, PAHs, pesticides and toxaphene. Between 20 and 200 µl of the extract (after alumina clean-up) are eluted on a stainless steel column (200 x 4 mm i.d.), packed with Nucleosil 100-5 with n-pentane, 20 % dichloromethane in n-pentane and finally dichloromethane. The eluate is collected in fractions containing 1) n-hydrocarbons, 2) PCBs, 3) PAHs and toxaphene, 4) pesticides and toxaphene and 5) acids, etc. (polar compounds). The size of the fractions has to be determined with standard solutions containing the compounds of interest, collecting the eluate in 0.5 ml fractions. Each fraction is then analysed by GC-ECD. Full details have been given in the literature (Petrick *et al*., 1988 and IOC, 1993).

4.6.2.4. High pressure chromatography

High pressure liquid chromatography (HPLC) columns packed with microparticles are available and have the advantages of high reproducibility, low consumption of solvents, high efficiency and high sample loading capacity.

This method can be used to separate fractions containing aliphatic hydrocarbons, PCBs and aromatic hydrocarbons from interfering compounds. These fractions can then be analysed separately for their constituents by GC-FID and/or GC-ECD.

HPLC methods have been developed using synthetic solutions of n-alkanes, PAHs, pesticides, PCBs and toxaphene and have been applied to samples in which interfering substances were present in such high concentrations as to render the analysis of HC and PCBs extremely difficult without this clean-up procedure (e.g. sediments and biological tissues with OCs in the ng/g range). The samples are eluted with n-hexane, subjected to clean-up over alumina, concentrated down to 20-200 µl and treated by HPLC. With the use of n-hexane, n-pentane and 10 %, 20 % and 50 % dichloromethane in n-hexane, respectively, the following five fractions are obtained : 1) n-hydrocarbons and alkenes, 2) PCBs and alkylbenzenes, 3) PAHs and toxaphene, 4) pesticides, 5) acids, etc.(polar compounds). (Petrick *et al*. 1988).

5. BIOTA

5.1. Sampling

Organisms accumulate many contaminants from their environment (i.e., from sea water, suspended particulate matter, sediment and food). Field and laboratory studies have shown that contaminant concentrations in some marine plants and animals reflect concentrations in their environment. Scientists use this process (termed bio-accumulation) to assess marine contamination resulting from human activity (e.g., pipeline discharges, dumping from ships).

There are problems with using biota as bio-accumulators (bio-indicators). For example, tissues from individuals of a species exposed to the same contaminant concentration may contain different levels of contamination after the same exposure time. These deviations reflect individual differences in factors such as age, sex, size, and physiological and nutritional states. Also, various species show different contaminant concentrations following identical exposure; differences in elimination rates may partially account for this. These factors must be considered when planning a monitoring programme in order to control their effects on the precision of the analysis (by reducing the variances). Variance reduction is necessary in order to detect smaller differences in mean contaminant concentrations observed in monitoring programmes.

For proper sampling and sample preparation, refer to Reference Method No 6 “Guidelines for monitoring chemical contaminants in the sea using marine organisms” and Reference Method No 12 Rev.2 “ Sampling of selected marine organisms and sample preparation for the analysis of chlorinated hydrocarbons”.

5.2. Cleaning of extraction thimbles

As for extraction of sediment samples, thimbles should be extracted first with the same solvent used for the extraction of the sample. As the extraction of biota sample is achieved with hexane, a pre-extraction of these thimbles is made with 250 ml of hexane for 8 hours in the Soxhlet apparatus, cycling the solvent through at a rate of 4 to 5 cycles per hour. Add into the solvent a few carborundum boiling chips to get a regular ebullition.

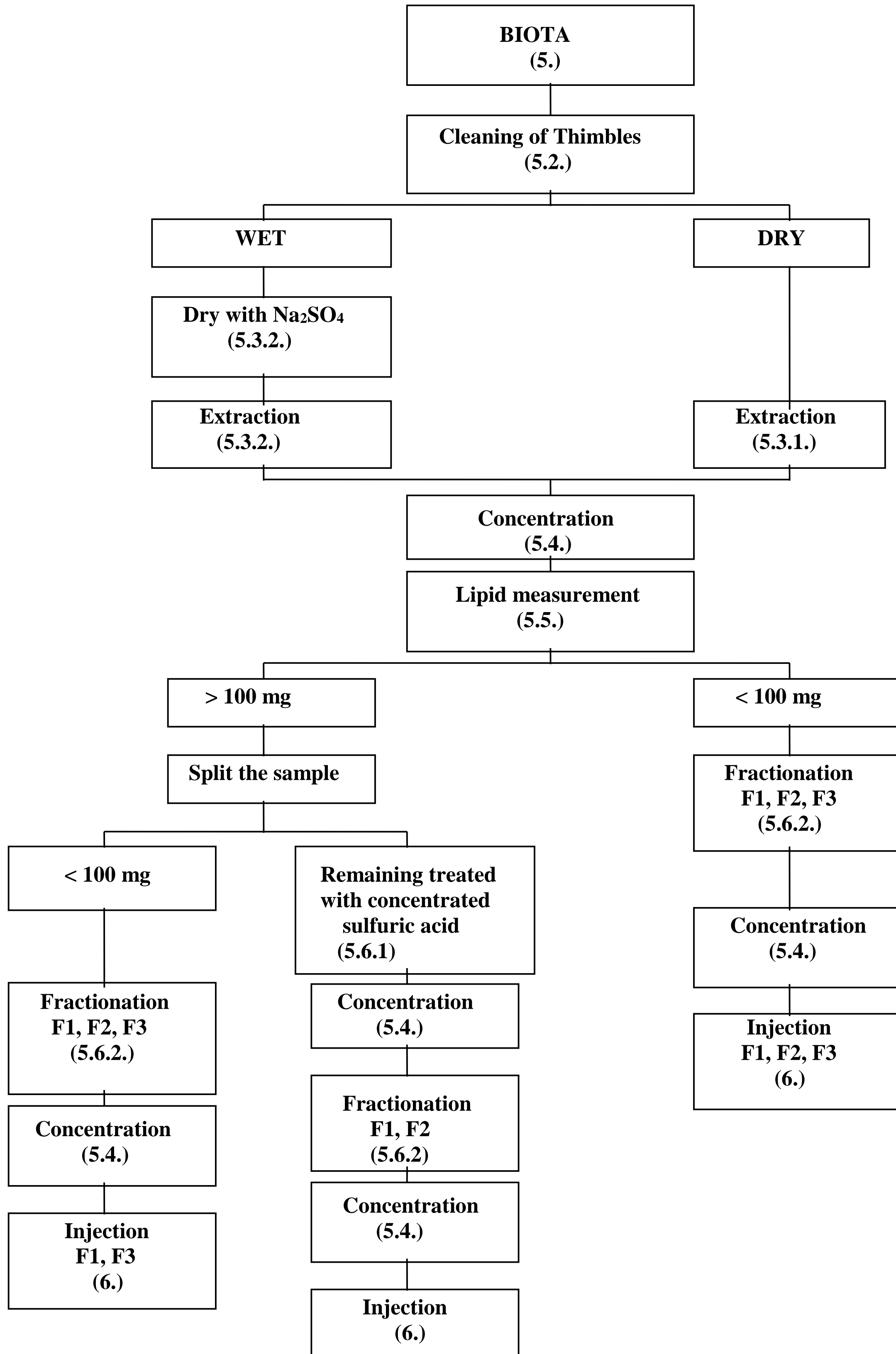


Figure 3: Diagram of the extraction procedure for biota samples.

5.3. Extraction of tissues

5.3.1. Extraction procedure for freeze-dried samples.

Take a 50 to 100 g fresh weight sub-sample from the sample. Weigh this sub-sample and freeze-dry it. When the sub-sample appears to be dry, re-weigh it and freeze-dry it for a further 24 hours and then re-weigh it. If the difference between the two dry weights is greater than 5%, continue the freeze-drying process. Special care must be taken to ensure that the freeze-drier is clean and does not contaminate the samples. The freeze drying procedure should be tested by drying 100 g Na_2SO_4 as a blank and extracting this as a sample. Pulverise the freeze-dried sub-sample carefully using a cleaned pestle and mortar. Accurately weigh about 5 to 10 g of this pulverised material, note the exact weight to be extracted, and place it into a pre-cleaned extraction thimble in a Soxhlet apparatus. The size of the sub-sample should be adjusted so that about 100 mg of extractable organic matter ("lipid") will be obtained. Smaller sub-samples should be used if residue concentrations are expected to be high. Add a known amount of internal standard to the sub-sample in the thimble before Soxhlet extraction. It is important to spike the sample at levels that are near to that of the analyte concentrations in the samples. If, in the end, the analyte and the internal standard concentrations do not fall within the established calibration range of the GC-ECD, the analysis must be repeated. Consequently, it may be advisable to perform range-finding analysis for samples of unknown character beforehand. Candidate internal standards are the same as for sediment samples (see 5.3.). Add about 200 ml of hexane or petroleum ether to the extraction flask with a few carborundum boiling chips, and extract the sample for 8 hours cycling the solvent through at a rate of 4 to 5 cycles per hour. Extract an empty thimble as a procedural blank, making sure to spike it with internal standards in the same fashion as the sample. If unacceptable procedural blanks are found, the source of contamination must be identified and eliminated rather than subtracting high blank values from the analytical results.

5.3.2. Extraction procedure without freeze-drying

Select a 25 to 100 g fresh weight sub-sample and place in a blender. Add anhydrous sodium sulphate to the sample, manually homogenise and determine whether the sample is adequately dried. If not, more sodium sulphate should be added until a dry mixture is obtained. Normally, 3 times by the sample weight used should be enough. Once this has been achieved, blend the mixture at high speed for 1 or 2 minutes until the mixture is well homogenised and the sample appears to be dry. Transfer the mixture to a pre-cleaned extraction thimble, add internal standards as described above and extract the dehydrated tissue with about 200 ml hexane or petroleum ether for 8 hours in a Soxhlet apparatus, cycling 4 to 5 times per hour. Extract the same amount of sodium sulphate as the procedural blank, making sure to add internal standards in the same fashion as the sample.

5.4. Concentration of the extract

Refer to section (4.4.)

5.5. Extractable Organic Matter (EOM)

Refer to section (4.5.)

5.6. Clean-up procedure and fractionation

5.6.1. Removal of lipids by concentrated sulphuric acid

If the lipid content of the extracts is higher than 100-150 mg, a preliminary step for the removal of the lipids is necessary before further sample purification. This can be carried out by using concentrated sulphuric acid. Treatment with sulphuric acid is used when chlorinated hydrocarbons are to be determined. However, sulphuric acid will destroy dieldrin and endrin so that an aliquot of the untreated extract must be set aside for the determination of these compounds.
CAUTION: During all this procedure it is very important to wear safety glasses.

Take an aliquot of the concentrated extract, containing about 200 mg of "lipids", transfer into a separatory funnel and add to this extract enough hexane in order to dilute the sample (40 to 50 ml should be enough), this will allow recovery of the hexane after acid treatment, because if the sample is too concentrated, the destroyed "lipids" will become almost solid and it will be difficult then to recover the hexane from this solid mass. Add 5 ml concentrated sulphuric acid to the extract and tightly fit the glass stopper and shake vigorously. Invert the funnel and carefully vent the vapours out through the stopcock. Repeat this procedure for several minutes. Place the separatory funnel in a rack and allow the phases to separate. Four or five samples and a spiked blank are convenient to process at one time. The extract should be colourless. Recover the hexane phase into a glass beaker. Dry with sodium sulphate and transfer the hexane into a Kuderna-Danish concentrator. Reduce the volume of the extract by evaporating the solvent with a gentle stream of pure nitrogen to about 1 ml.

5.6.2. Fractionation

Refer to section (4.6.2.)

6. CAPILLARY GAS CHROMATOGRAPHIC DETERMINATIONS

6.1. Gas chromatographic conditions

- Gas chromatograph with a split/splitless injection system, separate regulation system for inlet and column pressures and temperatures; multi-ramp temperature programming facilities (preferably microprocessor controlled), electron capture detector interfaced with the column with electronic control unit and pulsed mode facilities. An integrator with a short response time (0.25 s) is essential.

- Narrow-bore (0.22 mm internal diameter), 25 m long, fused silica open tubular column, coated with SE-54 (0.17 µm film thickness, preferably chemically bonded) with sufficient resolution to separate the relevant peaks in the standards provided for PCB analysis.

- Carrier gas should be high purity H_2. If this is not available or if the GC is not equipped with a special security system for hydrogen leak, He may be used. Gas purification traps should be used with molecular sieves to remove oxygen, moisture and other interfering substances.

- High purity nitrogen gas (99.995 %) as ECD make-up gas can be used (Argon/methane high purity gas is another option).

Conditions:

- H_2 or He carrier gas at inlet pressure of 0.5 to 1 Kg/cm^2 to achieve a flow rate of 1 to 2 ml/min.

- Make-up gas N_2 or Ar/CH_4 at the flow rate recommended by the manufacturer (between 30 and 60 ml/min.).

- ECD temperature: 300°C

6.2. Column preparation

Fused silica columns are the columns of choice for their inertness and durability (they are extremely flexible). They are made of material that is stable up to 360 °C. The 5 % phenyl methyl silicone gum (SE-54) liquid phase, is present as a thin, (0.17 µm), uniform film which can tolerate temperatures up to 300 °C. SE-54 is relatively resistant to the detrimental effects of solvents, oxygen and water, at least at low temperatures. These columns are even more resistant and durable if the liquid phase is chemically bonded to the support by the manufacturer.

For GC/MS work, it is advised to restrict the film thickness to 0.17 µm because with thicker films some of the phase could be released, resulting in an increase of the noise signal in the GC/MS.

The flexible fused silica columns can be conveniently connected directly to the inlet and outlet systems without the transfer lines used in conventional glass capillary chromatography which often lead to increased dead volume. Low bleed graphite or vespel ferrules provide a good seal.

The presence of extraneous peaks and elevated baseline drift will result in poor detector performance. This can be caused by components which elute from the column, such as residual solvents and low molecular weight liquid phase fractions on new columns and build-up of later eluting compounds on old columns. Conditioning is a necessary step to remove these contaminants. New columns are connected to the inlet (while left unconnected to the detector). Columns are flushed with carrier gas at low temperature for 15 min. to remove the oxygen, then heated at 70-100 °C for 30 min. and finally at 170 °C overnight. The column can be then connected to the detector. Old columns can be heated directly to elevated temperatures overnight. The final temperature is selected as a compromise between time required to develop a stable baseline and expected column life. Thus, it may be necessary for older columns to be heated to the maximum temperature of the liquid phase resulting in shorter column life. The temperature of the ECD, when connected to the column, should always be at least 50 °C higher than the column, in order to avoid condensation of the material onto the detector foil. It is essential that carrier gas flows through the column at all times when at elevated temperatures. Even short exposure of the column to higher temperature without sufficient flow will ruin the column.

CAUTION: if H_2 is used as a carrier gas, position the column end outside of the oven to avoid explosion risk.

6.3. Column test

When the column has been connected to the detector, the carrier gas flow is set to 30 ml per minute for a column with 4 mm internal diameter. The column performance is then measured according to the criteria of the "number of theoretical plates" for a specific compound and can be achieved according to the following procedure.

- Set injector and detector temperatures at 200 and 300°C respectively and the column oven temperature at 180 °C.
- Inject pp' DDT standard and measure the retention time (Tr). Adjust the column temperature to get a pp' DDT retention time relative to Aldrin of 3.03.
- Measure the width of the pp' DDT peak at its half height ($b_{1/2}$), in minutes and the retention time (Tr) also in minutes.
- Calculate the number of theoretical plates using the formula:

$$\mathrm{N} = 5.54\left(\frac{Tr}{b_{1/2}}\right)^2$$

- A parameter which is independent of the column length is the height equivalent to a theoretical plate (HETP):

$$\mathrm{HEPT} = \frac{\mathrm{L}}{\mathrm{N}}$$

Where L is the column length. Adjust the flow rate of the carrier gas to obtain optimum performance. The HETP should be as low as possible (i.e. the number of theoretical plates should be as great as possible).

The column remains in optimum condition as long as the liquid phase exists as a thin, uniform film. The quality of the film at the inlet side may be degraded as a result of repeated splitless

injections. Decreased column quality may be remedied by the removal of the end of the column (10 to 20 cm) at the inlet side. Chemically bonded liquid phases require less maintenance.

6.4. Electron capture detector

High-energy electrons, emitted by a radioactive source within the detector (e.g. a ^{63}Ni foil), are subject to repeated collisions with carrier gas molecules, producing secondary electrons. These electrons, upon returning to their normal state, can be captured by sample molecules, eluting from a GC column. The resulting reduction in cell current is the operating principle of an electron capture detector. The detector current produced is actually a non-linear function of the concentration of electron-capturing material. However, the useful linear range of an ECD may be greatly improved if the instrument is operated at a constant current, but in a pulsed mode, i.e. with short voltage pulses being applied to the cell electrodes. The current in the cell is kept constant by varying the frequency of the pulses.

Contamination of the detector (and thus lower sensitivity) may result from high-boiling organic compounds eluting from the column. Periodic heating to 350°C may overcome this problem. The ^{63}Ni ECD can be used at 320°C under normal operational conditions, in order to limit such contamination.

The optimum flow for an ECD (30 to 60 ml/min.) is much higher than carrier gas flow through the column of one or two ml/min. Thus an additional detector purge flow is necessary (N_2 or Ar/CH_4). Once leaving the outlet of the column, the compounds have to be taken up into an increased gas flow in order to avoid extra-volume band broadening within the detector. Thus, the detector purge flow also serves as the sweep gas.

6.5. Quantification

The most widely used information for identification of a peak is its retention time, or its relative retention time (i.e., the adjusted retention time relative to that of a selected reference compound). Retention behaviour is temperature dependent and comparison of retention times obtained at two or more temperatures may aid in determining a peak's identity. However, retention times are not specific and despite the high resolution offered by capillary columns, two compounds of interest in the same sample may have identical retention times.

One way of using retention indexes could be to inject di-n-alkyl-phthalates such as a mixture containing di-n-methyl-phthalate, di-n-ethyl-phthalate, di-n-propyl-phthalate, di-n-butyl-phthalate, di-n-hexyl-phthalate and di-n-heptyl-phthalate, which will cover the elution range from 70°C to 260°C. An arbitrary index of 100 is given to the di-n-methyl phthalate, 200 to the di-n-ethyl phthalate, and so on up to 700 to the di-n-heptyl phthalate; it is possible to identify all chlorinated pesticides by a proper retention index. This will be used also for unknown compounds which can be found easily on the GC/MS using the same index and so, identified. (Villeneuve J.P. 1986).

PCBs represent a complex mixture of compounds that cannot all be resolved on a packed column. Also there is no simple standard available for their quantification. Each peak in a sample chromatogram might correspond to a mixture of more than one individual compound. These difficulties have led to the recommendation of various quantification procedures. The usual method to quantify PCBs is to compare packed-column chromatograms of commercially available industrial formulations (Aroclors, Clophens, Phenoclors) with the sample chromatogram. Most commonly, it is possible to match one single formulation, such as Aroclor 1254 or Aroclor 1260 with the sample chromatogram. An industrial formulation (or mixture of formulations) should be chosen to be as close a match as possible and in the case of sample extracts from sediment or organisms, Aroclor 1254 and Aroclor 1260 are most frequently chosen.

For the second fraction obtained on Florisil separation, it is possible to quantify DDTs after comparison with the retention times of peaks in the sample chromatogram to those in the corresponding standard, the peak heights (or peak areas) are measured and related to the peak height (or peak area) in the standard according to the formula:

$$[\text{Concentration}] = \frac{h \times C \times V \times 1000}{h' \times V(inj) \times M \times R} \text{ ng/g (or pg/g)}$$

Where:

V	=	total extract volume (ml)
M	=	weight of sample extracted (g)
H	=	peak height of the compound in the sample
h’	=	peak height of the compound in the standard
C	=	quantity of standard injected (ng or pg)
V (inj)	=	volume of sample injected (µl)
R	=	Recovery of the sample

7. COMPUTERIZED GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS)

7.1. Operating conditions

The chemical ionisation source of a mass spectrometer can be used to produce negative ions by electron capture reactions (CI-NI-MS) using a non-reactive enhancement gas such as methane or argon. CI-NI has the advantage of being highly selective, permitting the detection of specific compounds in complex matrices. Under CI-NI conditions, methane (99.99 %) is used as the reagent gas. Samples are introduced through a SE-54, 30 m x 0.25 mm i.d., fused silica column. The film thickness used is 0.17 µm in order to minimise the bleeding of the phase into the system. Helium is used as carrier gas with an inlet pressure of 13 psi, which gives a carrier flow of 1.5 ml/min. or a gas velocity of 44 cm/sec.

The temperature of the injection port is held at 250°C.

The temperature of the source is set at 240°C, the quadrupole at 100°C and the interface at 285°C.

Injections of 1-3 µl are made in the splitless mode.

The temperature programme of the oven starts at 70°C, for 2 minutes, then it is increased at 3°C/min. to 260°C and kept under isothermal conditions for 40 minutes.

```
File        : C:\HPCHEM\1\DATA\AR1254.D
Operator    : jpv
Acquired    : 12 Jul 95   8:02 am using AcqMethod OC
Instrument  :   5989B
Sample Name: standard ar1254
Misc Info   :
Vial Number: 1
```

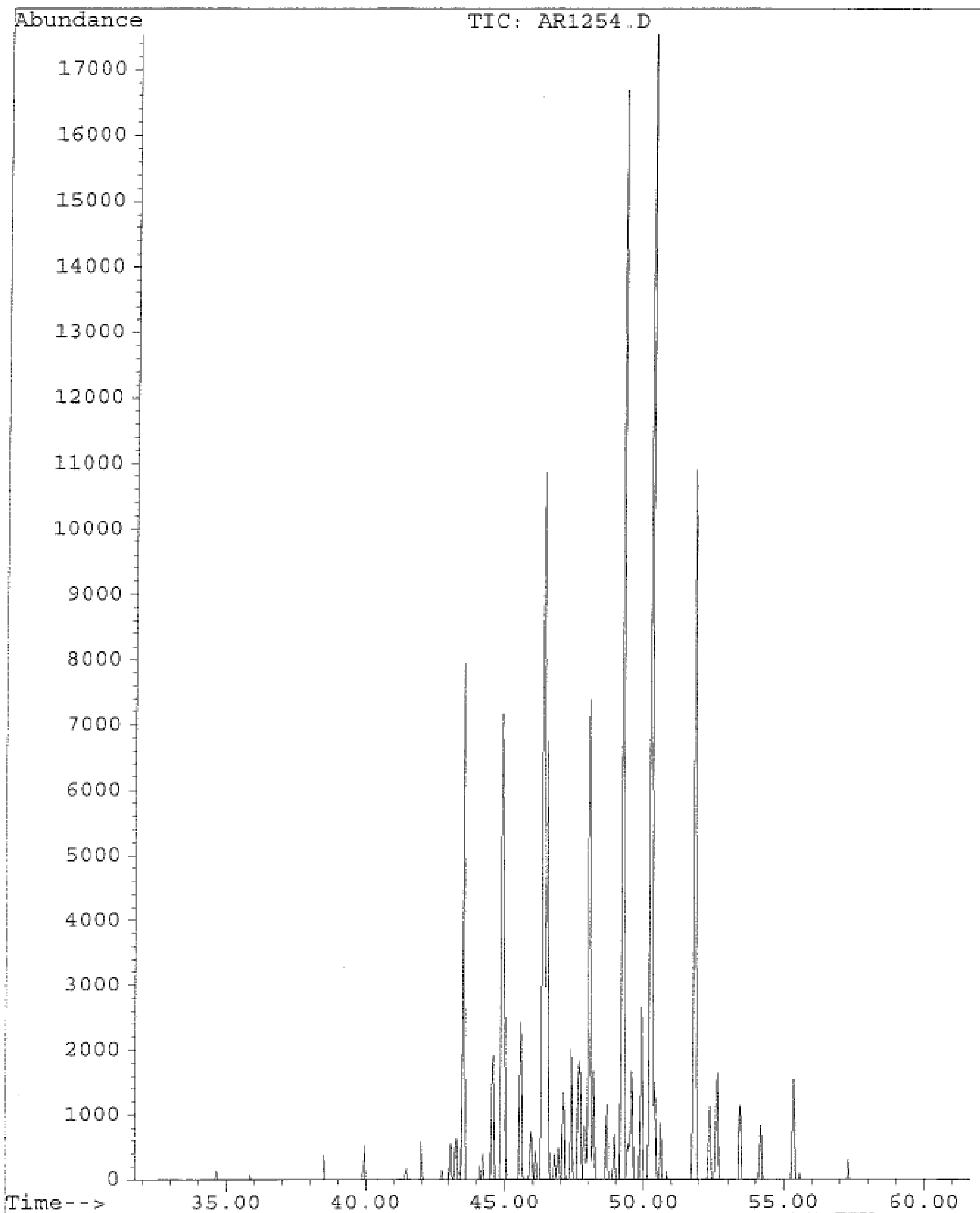


Figure 4: TIC of Aroclor 1254

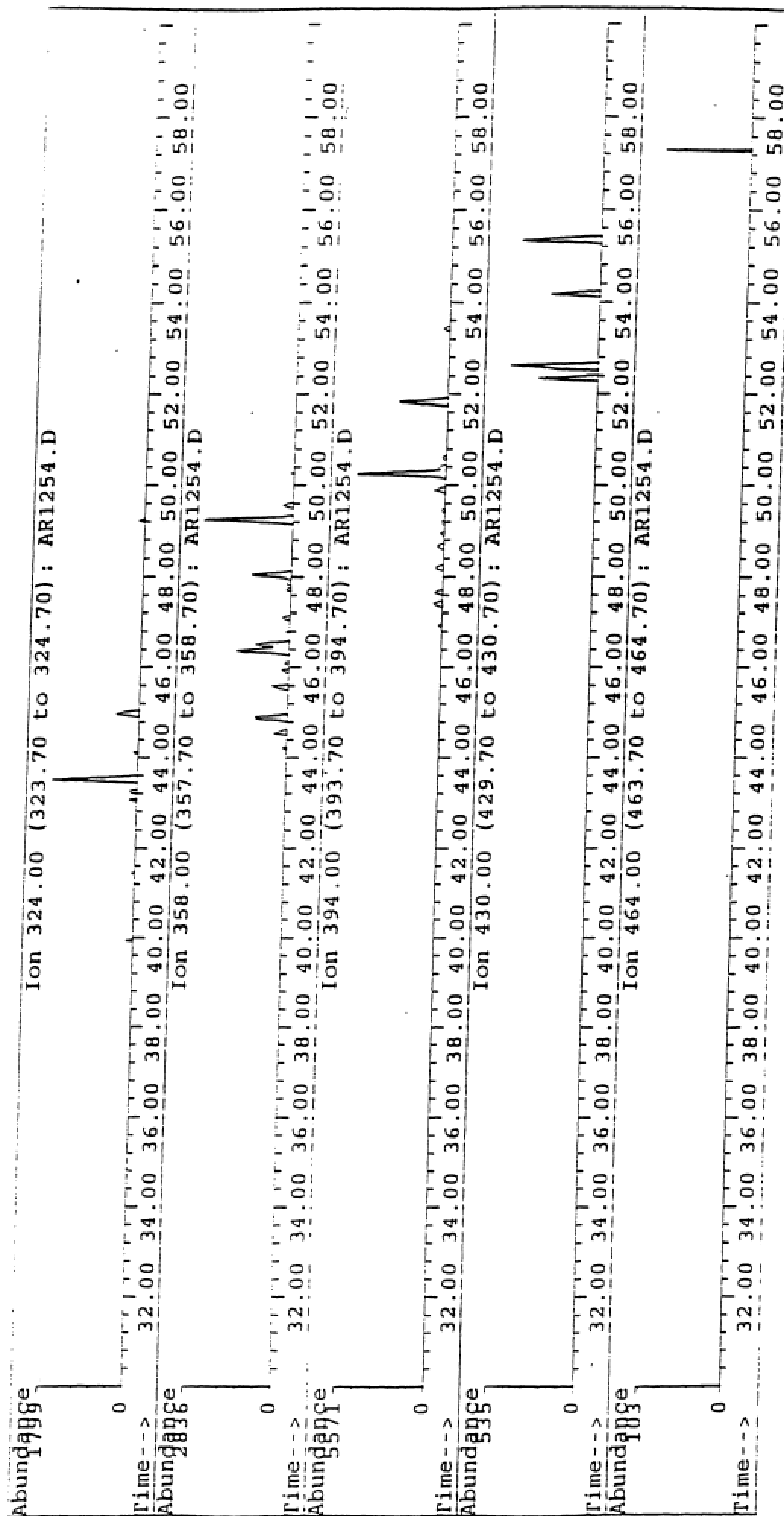


Figure 5: RIC of Aroclor 1254 main compounds

```
File        : C:\HPCHEM\1\DATA\AR1260.D
Operator    : jpv
Acquired    : 12 Jul 95   9:42 am using AcqMethod OC
Instrument  :   5989B
Sample Name: standard ar1260
Misc Info   :
Vial Number: 1
```

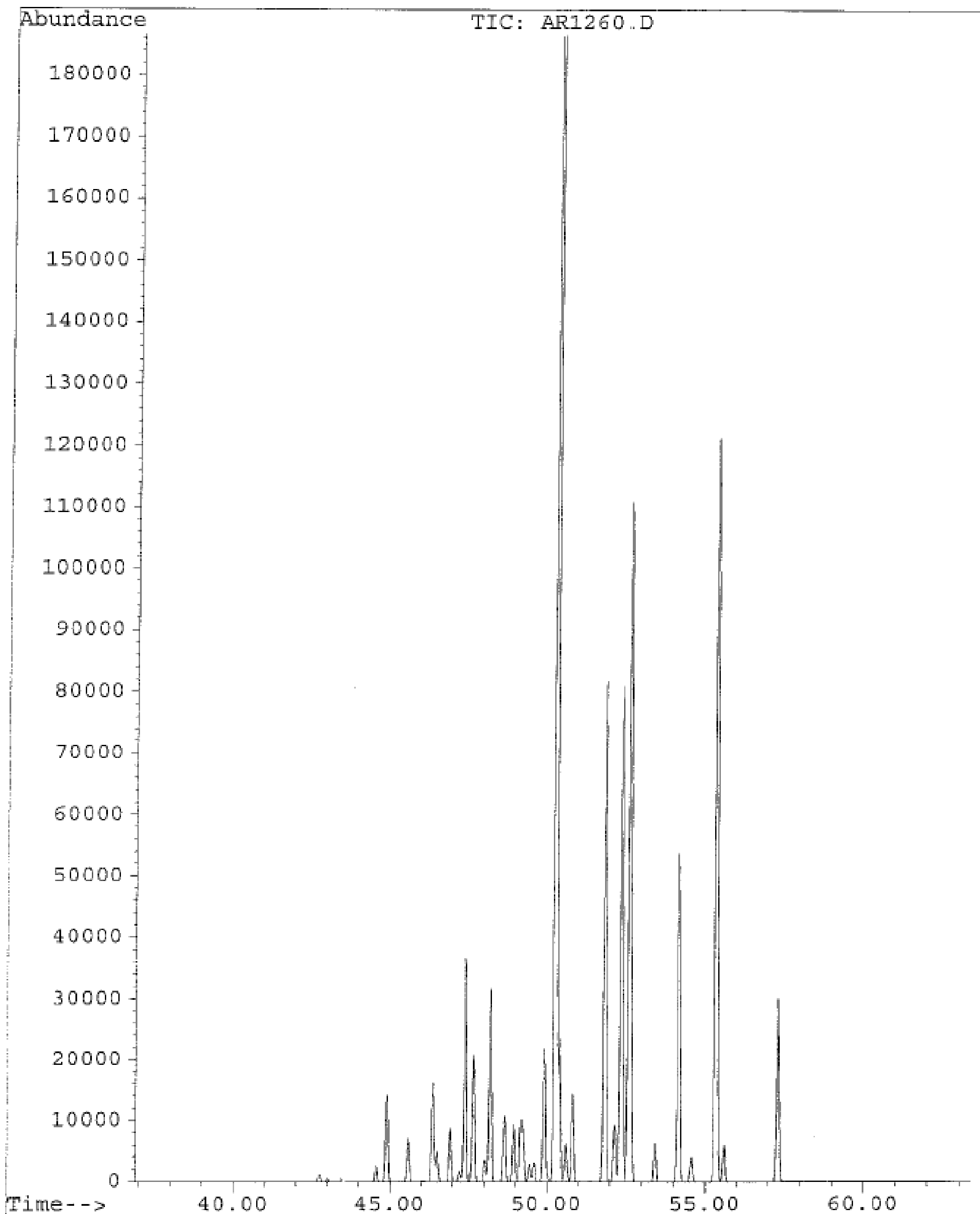


Figure 6: TIC of Aroclor 1260

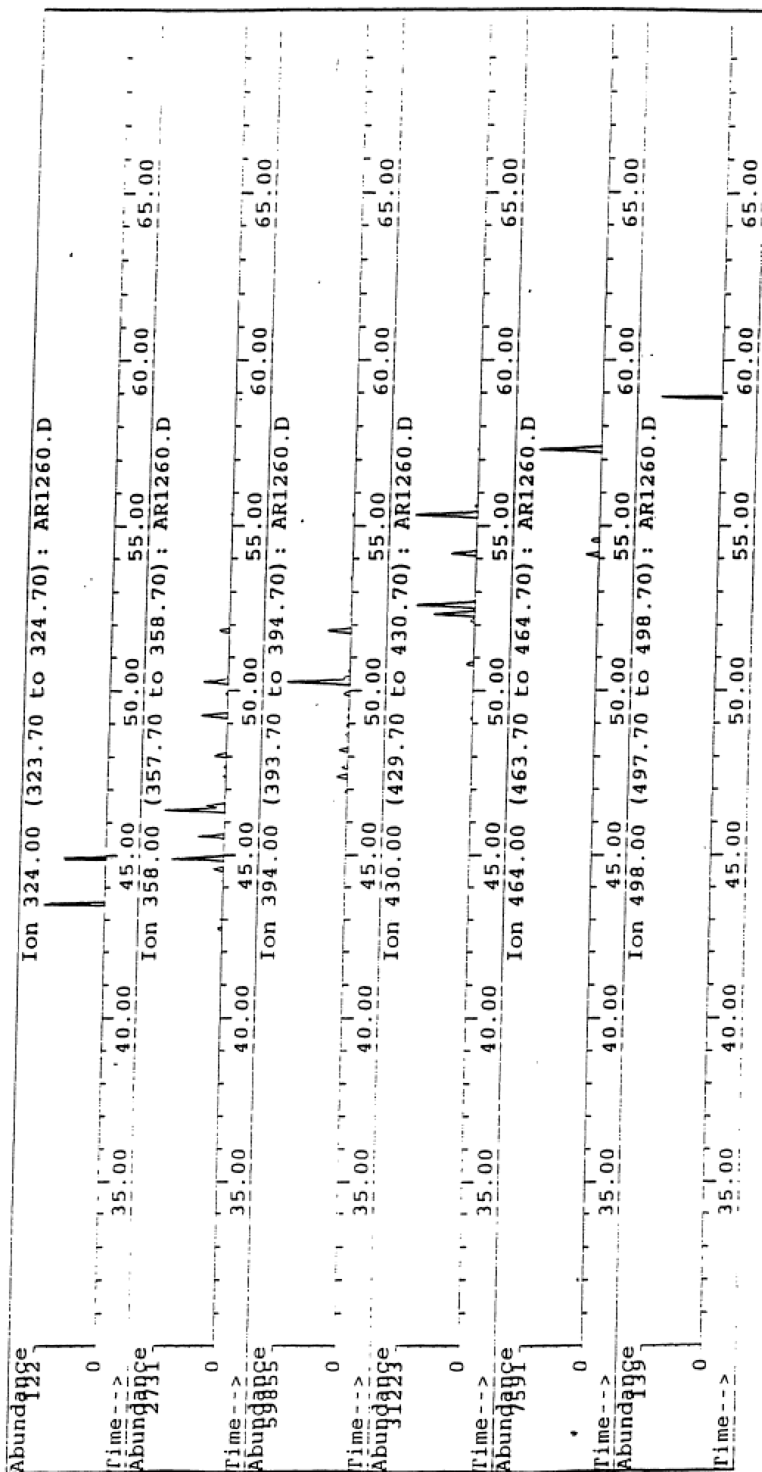


Figure 7: RIC of Aroclor 1260 main compounds

7.2. Example of a selected ion monitoring programme useful for quantitative analysis of chlorinated compounds.

Compounds	Fraction N° on Florisil	Retention Time (min.)	Target Ion (daltons)
HCB	1	37-38	284
Heptachlor	1	44-45	266
Aldrin	1	46-48	237
op DDE	1	51-53	246
Transnonachlor	1	52-54	444
pp' DDE	1	53-55	281
PCBs			
3 Cl	1		258
4 Cl	1		292
5 Cl	1	40-55	324
6 Cl	1	40-55	358
7 Cl	1	45-55	394
8 Cl	1	45-60	430
9 Cl	1	50-60	464
10 Cl	1	58-60	498
α HCH	2	37-39	255
β HCH	2	39-41	255
γ HCH (Lindane)	2	39-41	255
δ HCH	2	41-43	255
γ Chlordane	2	51-53	410
α Chlordane	2	52-54	266
op DDD	2	54-56	248
pp' DDD	2	56-58	248
op DDT	2	56-58	246
pp' DDT	2	58-60	283
Heptachlor epoxide	3	49-51	318
α Endosulfan	3	52-54	406
Dieldrin	3	53-55	346
Endrin	3	55-57	346
β Endosulfan	3	55-57	406
Endosulfan sulfate	3	58-60	386

8. NOTES ON WATER ANALYSIS

The levels of lipophilic compounds in tissues of aquatic organisms and organic fractions of sediments are determined to a large extent by the levels of these compounds in the surrounding water (marine mammals are an obvious exception). Data for CBs and hydrocarbons in sea water is therefore extremely useful for an understanding of the levels in organisms. However, the levels in sea water are extremely low and consequently, their determination needs considerable experience. Large volumes of water are required and extreme care has to be taken in order to avoid contamination during sampling, extraction and clean-up of the samples. Details are described in Manual and guide No 27 of IOC, 1993 and Villeneuve J.P. (1986).

9. ALTERNATIVE PROCEDURES

9.1. Combining sample preparation and extraction for chlorinated and petroleum hydrocarbons in sediment samples.

In the event that analyses for petroleum hydrocarbons and chlorinated compounds (and/or sterols) are of interest, the following extraction procedure can be used. To the freeze-dried sample introduce internal standards for each compound class. The following are suggested: 1) aliphatic hydrocarbons: - n-C_{14} d_{30}, n-C_{19} d_{40}, n-C_{32} d_{66}, 2) polycyclic aromatic hydrocarbons: Naphthalene d_8,

Hexamethylbenzene, Cadalene (deuterated PAHs are also useful), 3) organochlorine compounds: PCB congeners 29, 30, 121 or 198, ε HCH and Endosulfan Id_4, 4) sterols: 5 α (H)-androstan-3β-ol. These standards are used for quantifying the recovery of the total procedure. Samples are Soxhlet extracted for 8 hours with 250 ml of a mixture hexane / dichloromethane (50:50), cycling the solvent through at a rate of 4 to 5 cycles per hour. The solvent extract is concentrated by rotary evaporation down to 15 ml and transferred to a Kuderna-Danish tube. It is then further concentrated down to 5-6 ml under nitrogen gas. Following removal of sulphur and water, the extract is separated into aliquots: 1/3 for petroleum hydrocarbons and sterols and 2/3 for chlorinated hydrocarbons.

Note: Mercury method should be used only if chlorinated pesticides and PCBs are analysed. If the combined method is used for petroleum and chlorinated hydrocarbons, then the copper method should be used instead of mercury that will destroy some of the PAHs.

9.2. Supercritical fluid extraction (SFE) of marine samples

Sample preparation is probably the most time-consuming and labor-intensive analytical task performed in a laboratory. Studies shows that 60 % of the overall sample analysis time is spent in sample preparation which is the main source of error and of contamination. In addition, the amount of hazardous chemicals used for sample preparation is a continuous source of concern. Due to safe handling and disposal requirements, the reduction of their use is a priority for laboratories worldwide.

Supercritical fluids are gases (i.e. N_2O and CO_2) at room temperature and pressures above the critical point. The SFE technique allows an efficient extraction of a variety of contaminants with considerable reduction in the analysis cost, sample amount and allows the extraction of the thermal sensitive substances, reducing the amount of environmentally hazardous solvents.

A small change in the pressure of a supercritical fluid results in a big change in its density and the solvent strength of the fluid changes with changing density. As a result, one supercritical fluid easily performs the work of many solvents. If this is not enough, it is possible to add a modifier, such as methanol (a few per cent) to increase the solvating range of the fluid. Therefore, SFE should speed up the sample preparation process, minimising the wastes associated with the analysis.

Until now, the main fields of analytical applications of SFE are related to environmental studies and to the food-processing industry (Hawthorne, 1990, Bayona, 1993). A method using carbon dioxide (80°C-340 atm) for the extraction of total petroleum hydrocarbons has been approved as an EPA standard method. The extraction efficiency of modified CO_2 for the recovery of 41 organochlorine and 47 organophosphorus pesticides spiked on sand at different pressures and temperatures were higher than 80%. Furthermore, by increasing the extraction temperature up to 200°C, PCBs and PAHs can be extracted from naturally occurring samples with neat CO_2. Nam *et al.* (1991), have developed a method for rapid determination of polychlorinated organics in complex matrices. The method is based on direct coupling of supercritical fluid extraction with tandem supercritical fluid chromatography and gas chromatography. The on-line system permits simultaneous extraction and analysis with high reproducibility and accuracy.

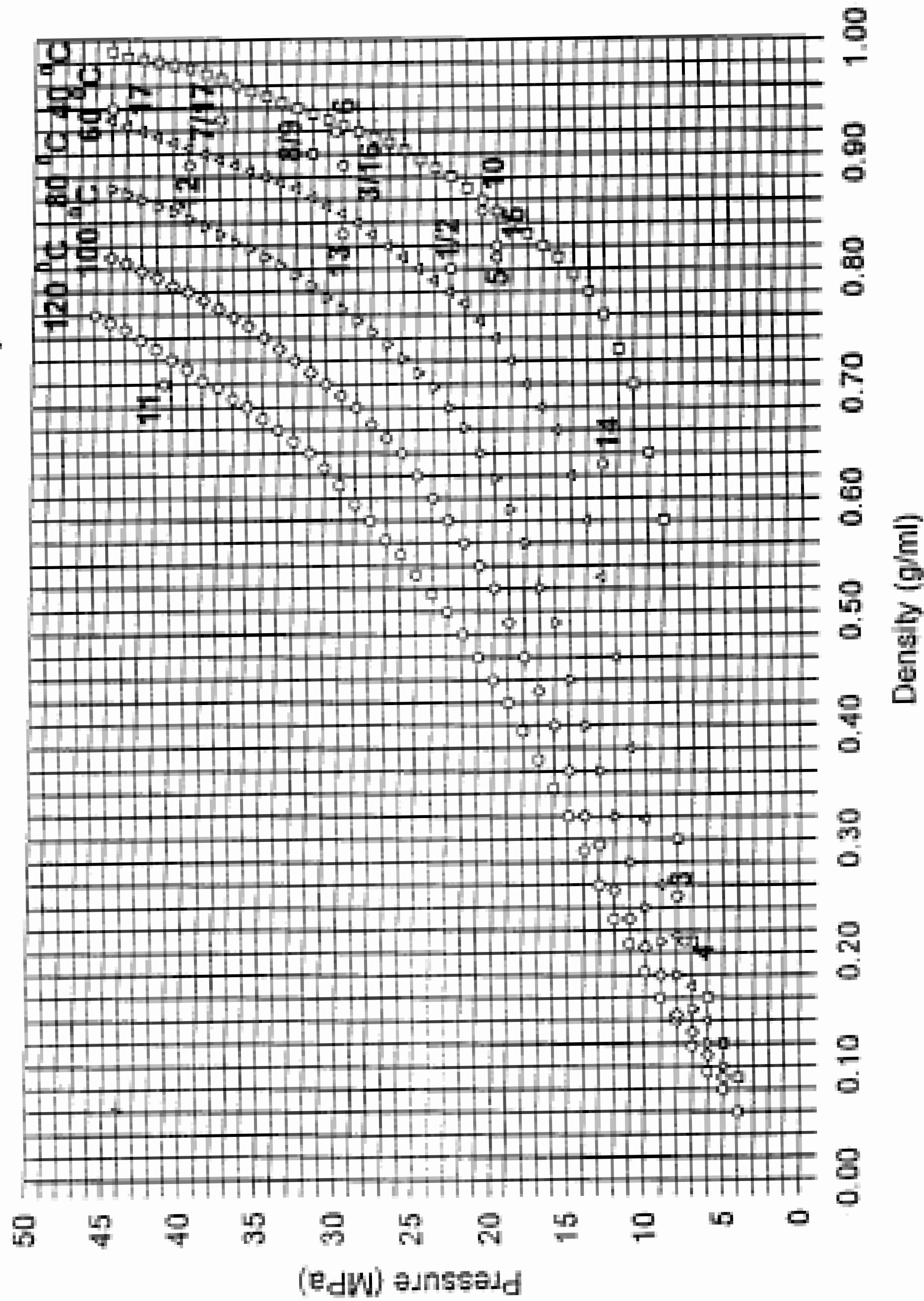


Figure 8: Guide for CO_2 extractions

9.3. Microwave assisted extraction for marine samples

9.3.1 Sediment

Another alternative method for the extraction of chlorinated hydrocarbons in sediment samples (or combined extraction for chlorinated hydrocarbons and petroleum hydrocarbons) is the use of the Microwave oven instead of the Soxhlet extractor. The main advantage of the microwave oven is the fact that, for one sample, only 40 ml of solvent mixture are used instead of 250 ml for clean-up of extraction thimbles and 250 ml for the extraction itself.

10 to 15 grams of freeze-dried sediment sample, ground and sieved at 250 µm, are put in the glass tube of the reactor. Appropriate internal standards (for OCs and/or PHs, see10.1.) are added to the sample for recovery and samples are extracted with 40 ml of a mixture of hexane / dichloromethane (50:50).

Extraction is realised within the following cycle:

- Power of the microwaves: 1200 watts
- Temperature increase to 115 °C in 10 minutes.
- Extraction maintained at 115 °C for 30 minutes
- Cooling to ambient temperature within one hour.

The carrousel containing 14 reactors, 12 samples could be extracted together with one blank and one Reference Material within 1 and half hour and with 10 times less solvent mixture than the standard Soxhlet extraction.

After cooling down to room temperature the solvent mixture is recovered in a 100 ml glass flask. The sediment is poured in a glass funnel containing a plug made of glass wool. The extracted sediment is washed with 10 - 20 ml of hexane. The extract follows then the procedure of clean-up and fractionation.

9.3.2 Biota

3 to 8 grams of freeze-dried biota sample is accurately weighted, the weight to be extracted is noted, and it is placed into the pre-cleaned glass tube of the reactor. A known amount of internal standard is added to the sub-sample in the tube before extraction. Candidate internal standards are the same than for sediment samples refers to section (5.3.1.)

Extraction is realized with 30 ml of a mixture hexane / acetone (90:10) within the following cycle:

- Power of the microwaves: 1200 watts
- Temperature increase to 115 °C in 10 minutes.
- Extraction maintained at 115 °C for 20 minutes
- Cooling to ambient temperature within one hour.

The carrousel containing 14 reactors, 12 samples could be extracted with one blank and one Reference Material within 1 and half hour and with 10 times less solvent mixture than the standard Soxhlet extraction.

After cooling down to room temperature the solvent mixture is recovered in a 100 ml glass flask. The powder of biota is poured in a glass funnel containing a plug made of glass wool. The extracted biota is washed with 10 - 20 ml of hexane. The extract is then concentrated with rotary evaporator and ready for E.O.M, clean-up and fractionation procedure.

10. DATA INTERPRETATION

10.1. DDT

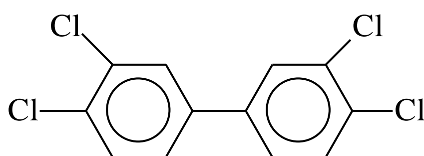
The residence time of total DDT in the environment is relatively short (t1/2 = 3-5 years), so, at least 75-80 % of the current total DDT should be in the form of DDE or DDD if it was introduced into the environment before the 1975 ban. Values of Henry's law constant indicate that these compounds can reach the troposphere as vapour. These vapours are little adsorbed by airborne particulate matter and represent the major component in atmospheric chlorinated hydrocarbon levels. Vapour movements of these pollutants suggest that restrictions and regulations operating in the more technically advanced countries could only be partially effective on a worldwide basis.

The presence of the op DDT together with anomalous pp' DDT values in environmental samples indicates a recent treatment with this insecticide.

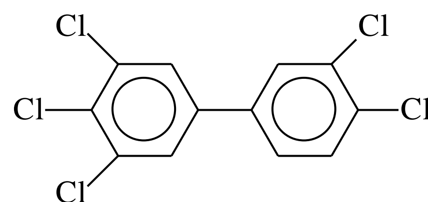
10.2. PCBs congeners

Among the 209 possible PCB congeners, seven of them: 28, 52, 101, 118, 138, 153 and 180, were selected as the most relevant because of their distribution in the chromatogram and in the chlorination range.

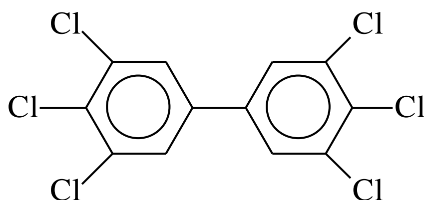
Recently, attention has been paid to congeners having 2 para-chlorines and at least 1 meta-chlorine. These congeners are called "coplanar" PCBs. Among the 209 congeners, 20 members attain coplanarity due to non-ortho chlorine substitution in the biphenyl ring. Three of these show the same range of toxicity as the 2,3,7,8 tetrachlorodibenzo-p-dioxin and the 2,3,7,8 tetrachlorodibenzofuran, these are the IUPAC Nº: 77, 126 and 169. These compounds should be identified and quantified in the environmental samples with high priority. They can be separated using fractionation with carbon chromatography (Tanabe *et al*., 1986).



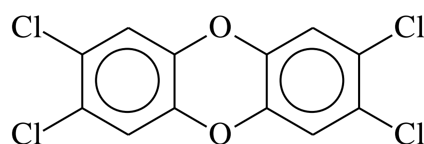
3,3',4,4' tetrachlorobiphenyl
IUPAC Nº: 77



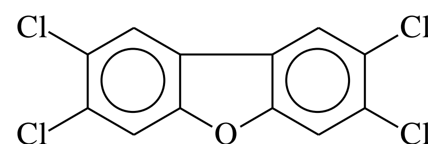
3,3',4,4',5 pentachlorobiphenyl
IUPAC Nº: 126



3,3',4,4',5,5' hexachlorobiphenyl
IUPAC Nº: 169



2,3,7,8 tetrachlorodibenzo-p-dioxin



2,3,7,8 tetrachlorodibenzofuran

10.3. Typical profiles of commercial mixtures

Formulations available in different countries are slightly different in their composition (Aroclor in USA, Kanechlor in Japan, Clophen in Germany, Phenoclor in France, Fenclor in Italy or Sovol in Russia). For the same global composition, such as Aroclor 1254, KC-500 or Phenoclor DP-5, the composition of individual congeners differs by 5-10 %. If a sample is collected on the French coast (therefore, contaminated with DP-5), and is quantified with DP-5 and Aroclor 1254, the difference observed in concentration could be in the order of 5-10 %. This shows the importance of choosing one common standard for the quantification of global industrial formulations or the importance of quantifying with individual congeners.

Percent contribution of individual chlorobiphenyls to Clophen A 50 and Aroclor 1254.

PCB N°	Clophen A50	Aroclor 1254	PCB N°	Clophen A50	Aroclor 1254
17	0	0.19	115	0.28	0.3
18	0	0.41	118	10.9	6.39
28	0.05	0.25	119	0.19	0.14
31	0.05	0.22	122	0.19	0.5
33	0.11	0.14	123	0.85	0.81
40	0.28	0.2	126	0.08	0
41	0.83	0.64	128	3.04	2.07
42	0.13	0.23	129	0.83	0.23
44	2.46	2.03	130	0.83	0.63
47	0.18	0.11	131	0.06	0.16
48	0.17	0.14	132	2.57	1.98
49	1.96	1.64	134	0.52	0.49
52	5.53	5.18	135	1.61	1.62
53	0.06	0.09	136	0.91	1.12
56	0.44	0.58	137	0.25	0.25
60	0.34	0.54	138	3.61	3.2
63	0.15	0.05	141	0.98	1.04
64	0.71	0.45	146	0.8	0.83
66	0.5	0.59	149	4.5	2.21
67	0.13	0.09	151	1.22	1.17
70	3.85	3.21	153	4.17	4.26
74	1.35	0.78	156	1.43	1.62
82	1.05	0.95	157	0.31	0
83	0.53	0.45	158	0.98	0.77
84	2.08	1.95	167	0.35	0.21
85	1.85	1.66	170	0.65	0.31
87	4.22	3.78	171	0.5	0.5
90	0.85	0.93	172	0.09	0.05
91	0.92	0.83	173	0.09	0.09
92	1.53	1.58	174	0.37	0.34
95	6	6.02	175	0.11	0.05
96	0.05	0.08	176	0.43	0.32
97	2.8	2.55	177	0.21	0.21
99	4.06	3.6	178	0.19	1.35
100	0.15	0.1	179	0.2	0.21
101	7.72	7.94	180	0.53	0.38
105	1.9	3.83	183	0.21	0.17
107	0.94	0.72	187	0.3	0.32
110	6.27	5.85	190	0.05	0.08
			201	0.6	0.68

11. QUALITY ASSURANCE / QUALITY CONTROL

Guidelines on the QA/QC requirements for analysis of sediments and marine organisms are detailed in Reference Method No 57, "Contaminant monitoring programs using marine organisms: Quality assurance and good laboratory practice". Brief descriptions of issues that must be addressed in the course of understanding the procedures described here are given below.

11.1. Precision

The precision of the method should be established by replicate analysis of samples of the appropriate matrix. Estimate the precision of the entire analytical procedure by extracting five sub-samples from the same sample after homogenisation. Alternatively, perform replicate analysis of an appropriate certified reference material (RM; see below) containing the analytes of interest. The principal advantage of using a RM is that the material permits the simultaneous evaluation of accuracy while offering a well homogenised sample. Precision should be evaluated as a matter of course during the initial implementation procedure just before initiation of sample analysis.

11.2. Accuracy

The accuracy of the methods described here must be confirmed by analysis of a suitable RM (i.e. appropriate matrix, analytes) prior to initiation of sample analysis. Agreement between measured and certified concentrations for any individual analyte should be within 35 % and on average within 25%. It is advisable to introduce RMs on a regular basis (e.g. every 10-20 samples) as a method of checking the procedure. Further description of the preparation of control charts and criteria for data acceptance are discussed in Reference Method Nº 57.

11.3. Blanks

Blanks represent an opportunity to evaluate and monitor the potential introduction of contaminants into samples during processing. Contributions to the analyte signal can arise from contaminants in the reagents, those arising from passive contact between the sample and the environment (e.g. the atmosphere) and those introduced during sample handling by hands, implements or glassware. It is essential to establish a consistently low (i.e. with respect to analytes) blank prior to initiating analysis or even the determination of the method detection limit. In addition, it is necessary to perform blank determinations on a regular basis (e.g. every batch of samples).

11.4. Recovery

Recovery reflects the ability of the analyst to fully recover surrogate compounds introduced to the sample matrix or blank at the beginning of the procedure. The primary criteria for selection of compounds to be used for testing recovery are that they: 1) have physical (i.e. chromatographic/partitioning) properties similar to and if necessary spanning those of the analytes of interest, 2) do not suffer from interferences during gas chromatographic analysis, 3) are baseline resolved from the analytes of interest.

Recovery should be tested on all samples and blanks as a routine matter of course. Recoveries below 70% are to be considered unacceptable. Recoveries in excess of 100 % may indicate the presence of interference.

11.5. Archiving and reporting of results

Every sample should have an associated worksheet which follows the samples and the extracts through the various stages of the procedure and upon which the analyst notes all relevant details. An example of such a worksheet is given below. Each laboratory should construct and complete such a worksheet. Relevant chromatograms should be attached to the worksheet. Analyses should be grouped

and composite or summary analysis sheets archived with each group. Final disposal of the data will depend on the reasons for which it was collected but should follow the overall plan model.

All processed samples should be archived at all steps of the procedure:

- deep frozen (in the deep-freezer as it was received).
- freeze-dried (in sealed glass container kept in a dark place).
- extracted (after injection on the GC, sample extracts should be concentrated down to 1 ml and transferred into sealed glass vials, a Pasteur pipette sealed with a butane burner is adequate and cheap).

Sample: IAEA-357 : Marine Sediment

wet wt.
-------- = ..., % water in freeze dried sample determined by drying at 105°C : .....
dry wt.

.......g freeze-dried wt. extracted with hexane in Soxhlet extractor for 8 hours.

.......pg PCB Nº29, ...pg PCB Nº198, ...pg ε HCH and ….. pg Endosulfan Id_4 were added as internal standard.

The ....ml extract was reduced by rotary evaporator to approximately ......ml.

This was treated with sodium sulfate to dry the extract. Then treated with mercury to remove sulphur. This was further reduced to ....ml for lipid determinations. Corrected dry wt. : ....g.

Lipid determinations:

.............ml total extract;

10 µl aliquots weighed on micro-balance: ....mg; ....mg; ....mg.

HEOM = ...mg/g dry weight.

...........mg lipid subjected to column chromatography fractionation on Florisil.

F1: ....ml hexane

F2: ....ml hexane/dichloromethane (70:30)

F3: ....ml dichloromethane

GC determinations:

PCB Nº29 : ....ng recovered in F1 : ....% Recovery.

PCB Nº198 : ....ng recovered in F1 : ....% Recovery.

ε HCH : ....ng recovered in F2 : ....% Recovery.

Endosulfan Id_4: ....ng recovered in F3 : ....% Recovery.

Attach tabulation of individual compounds quantified in sample.

Sample worksheet for analysis of chlorinated compounds in marine sediments.

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ANNEX

PREPARATION OF THE SOLUTION OF INTERNAL STANDARDS: PCB No 29, PCB No 198, ε HCH and Endosulfan I d4

Stock Solution of PCB No 29:

1 ml from the original vial (250ng/µl) should be transferred into a 100 ml volumetric flask, and then the volume is adjusted to 100 ml with hexane. This stock solution contains:

2.5 ng/µl of PCB No 29

Stock Solution of Endosulfan I d4:

1 ml from the original vial (250ng/µl) should be transferred into a 100 ml volumetric flask, and then the volume is adjusted to 100 ml with hexane. This stock solution contains:

2.5 ng/µl of Endosulfan I d_4

Working solution of internal standards:

0.5 ml from the stock solution of PCB No 29 (2.5 ng/µl) should be transferred into a 50 ml volumetric flask, then, 0.5 ml from the stock solution of Endosulfan I d4 (2.5 ng/µl) should be transferred into the volumetric flask, then 1 ml from the original vial (1ng/µl) of ε HCH should be transferred into that volumetric flask, then 0.5 ml from the concentrated solution (2ng/µl) of PCB No 198, and the volume adjusted to 50 ml with hexane. This working solution contains:

25 pg/µl of PCB No 29
20 pg/µl of PCB No 198
20 pg/µl of ε HCH
25 pg/µl of Endosulfan I d4

CAUTION: VIALS SHOULD BE COOLED AT 20°C PRIOR TO OPENING

Preparation of the Aroclor 1254 solution

Preparation of the stock solution:

1 ml from the original vial should be transferred into a 100 ml volumetric flask, then, the volume is adjusted to 100 ml with hexane. This stock solution contains:

6.5 ng/µl of Aroclor 1254

Preparation of the working solution:

1 ml from this stock solution should be transferred into a 50 ml volumetric flask and the volume adjusted to 50 ml with hexane. This working solution contains :

0.13 ng/µl of Aroclor 1254

CAUTION : VIAL SHOULD BE COOLED TO 20 °C PRIOR TO OPENING

Preparation of the Aroclor 1260 solution

Preparation of the stock solution:

1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains:

5.44 ng/µl of Aroclor 1260

Preparation of the working solution:

1 ml from the stock solution should be transferred into a 50 ml volumetric flask, then the volume is adjusted to 50 ml with hexane. This working solution contains

0.1088 ng/µl of Aroclor 1260

CAUTION: VIAL SHOULD BE COOLED TO 20 °C PRIOR TO OPENING

Preparation of the pp’ DDE, pp’ DDD and pp’ DDT solution

pp’ DDE:

Stock solution: 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This stock solution contains:

5 ng/µl of pp’ DDE

pp’ DDD:

Stock solution: 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains:

5 ng/µl of pp’ DDD

pp’ DDT:

Stock solution: 1 ml of the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This stock solution contains:

5 ng/µl of pp’ DDT

Working solution: pp’ DDE, pp’ DDD and pp’ DDT together.

1 ml from the stock solution of pp’ DDE, 2 ml of the stock solution of pp’ DDD and 3 ml of the stock solution of pp’ DDT should be transferred into a 100 ml volumetric flask and the volume adjusted to 100 ml with hexane. This solution contains

- pp’ DDE : 50 pg/µl
- pp’ DDD : 100 pg/µl
- pp’ DDT : 150 pg/µl

NOTE: Further dilution may be necessary depending on the sensitivity of the EC Detector.

CAUTION: VIAL SHOULD BE COOLED TO 20 °C PRIOR TO OPENING

Preparation of Aldrin, Diedrin and Endrin standard solutions:

Aldrin:

Stock solution: 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains:

5 ng/µl of Aldrin

Dieldrin:

Stock solution: 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains:

5 ng/µl of Dieldrin

Endrin:

Stock solution: 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains:

5 ng/µl of Endrin

Working solution: Aldrin, Dieldrin and Endrin together.

1 ml from the stock solution of Aldrin, 1 ml from the stock solution of Dieldrin and 1 ml from the stock solution of Endrin are transferred into a 100 ml volumetric flask and the volume is adjusted to 100 ml with hexane. This working solution contains:

Aldrin	:	50 pg/µl
Dieldrin	:	50 pg/µl
Endrin	:	50 pg/µl

NOTE: Further dilution may be necessary depending on the sensitivity of the detector.

CAUTION: VIALS SHOULD BE COOLED TO 20 °C PRIOR TO OPENING

Preparation of the HCB and Lindane standard solutions:

HCB:

Stock solution: 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains:

5 ng/µl of HCB

Lindane:

Stock solution: 1 ml from the original vial should be transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains:

5 ng/µl of lindane

Working solution:

1 ml from the stock solution of HCB and 1 ml from the stock solution of Lindane are transferred into a 100 ml volumetric flask, then the volume is adjusted to 100 ml with hexane. This solution contains:

HCB	:	50 pg/µl
Lindane	:	50 pg/µl

NOTE: further dilution may be necessary depending on the sensitivity of the EC Detector.

CAUTION: VIALS SHOULD BE COOLED TO 20 °C PRIOR TO OPENING

Preparation of the PCB congeners solution

In a 100 ml volumetric flask, transfer 1 ml from the original vial. Adjust to 100 ml with hexane in order to obtain the working solution with the following concentrations:

CB Nº:	Compounds:	Concentrations (pg/µl)
8	2,4’	17.50
18	2,2’,5	12
31	2,4’,5	10.6
28	2,4,4’	4.6
52	2,2’,5,5’	8.6
49	2,2’,4,5’	12.1
44	2,2’,3,5’	10.7
66	2,3’,4,4’	5.5
95	2,2’,3,5’,6	5.7
101	2,2’,4,5,5’	9.3
110	2,3,3’,4’,6	11.1
149	2,2’,3,4’,5’,6	12.1
118	2,3’,4,4’,5	8.5
153	2,2’,4,4’,5,5’	8.4
138	2,2’,3,4,4’,5’	13.8
183	2,2’,3,4,4’,5’,6	10.3
174	2,2’,3,3’,4’,5,6’	9.4
177	2,2’,3,3’,4’,5,6	9.5
180	2,2’,3,4,4’,5,5’	16.3
170	2,2’,3,3’,4,4’,5	13.4
199	2,2’,3,3’,4,5,5’,6’	9.3
194	2,2’,3,3’,4,4’,5,5’	12.6

Separate into 10 volumetric flasks of 10 ml, seal with Teflon tape and keep in refrigerated place in order not to evaporate them.

<u>CAUTION: VIAL SHOULD BE COOLED TO 20 °C PRIOR TO OPENING</u>

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Annex X:

HELCOM

Manual for marine monitoring in the COMBINE programme
ANNEX B-12, APPENDIX 3. TECHNICAL NOTE ON THE DETERMINATION OF CHLORINATED BIPHENYLS AND ORGANOCHLORINE PESTICIDES IN BIOTA

HELCOM Manual for marine monitoring in the COMBINE programme

ANNEX B-12, APPENDIX 3. TECHNICAL NOTE ON THE DETERMINATION OF CHLORINATED BIPHENYLS AND ORGANOCHLORINE PESTICIDES IN BIOTA

1. INTRODUCTION

The analysis of chlorinated biphenyls (CBs) and organochlorine pesticides (OCPs) in fish samples generally involves extraction from the respective matrix with organic solvents, followed by clean-up and gas chromatographic separation with electron capture (GC-ECD) or mass spectrometric (GC-MS) detection.

The analytical procedure is liable to systematic errors due to insufficiently optimized gas chromatographic conditions, determinant losses (evaporation, unsatisfactory extraction yield), and/or contamination from laboratory ware, reagents and the laboratory environment. It is therefore essential that the sources of systematic errors are identified and eliminated as far as possible.

In the following paragraphs, the guidelines drafted by the OSPAR Ad Hoc Working Group on Monitoring (OSPAR, 1996) have been taken into consideration.

2. PRE-TREATMENT OF LABORATORY WARE AND REAGENTS; CONTAMINATION CONTROL

Glassware, reagents, solvents, column adsorption materials and other laboratory equipment that come into contact with the sample material to be analysed should be free of impurities that interfere with the quantitative determination of CBs and OCPs.

For cleaning purposes, the following procedures should be followed:

1. Glassware should be thoroughly washed with detergents, dried with acetone and rinsed with a non-polar solvent such as *n*-pentane, and heated to > 100 $_{o}$C prior to use.

2. Glass fibre Soxhlet thimbles should be pre-extracted with an organic solvent. The use of paper Soxhlet thimbles should be avoided. Alternatively, glass fibre thimbles or full glass Soxhlet thimbles, with a G1 glass filter at the bottom, are recommended.

3. Solvents should be checked for impurities using GC after concentrating the volume normally used in the procedure to 10 % of the final volume. If necessary, solvents can be purified by controlled re-distillation and rectification over KOH in an all-glass distillation column.

4. Reagents and column adsorption materials should be checked for contamination before use by extraction with an organic solvent (e.g., *n*-pentane) and analysis by GC, using the detector which will also be used for the final determination (ECD or MS).

5. Laboratory air can also be contaminated with CBs, OCPs or compounds interfering with the CB/OCP analysis. A good estimation of the contamination of the air can be found by placing a petri dish with 2 grams of C18-bonded silica for two weeks in the laboratory. After this period, the material is transferred to a glass column and eluted with 10 ml of 10% diethylether in hexane. After concentrating the eluate, the CB concentrations can be measured. Absolute amounts of <1 ng show that the contamination of the air is at an acceptably low level in that laboratory (Smedes and de Boer, 1994).

3. SAMPLE PRETREATMENT

To ensure complete extraction of the lipophilic CBs and OCPs from biological sample matrices, it is essential to dry the material and disrupt the cell walls of the biological matrix to be analysed. This can be achieved by Ultra Turrax mixing or grinding of the sample with a dehydrating reagent, such as Na_2SO_4, followed by multiple solid/liquid extraction with a mixture of polar and non-polar solvents (e.g., acetone/hexane or methanol/dichloromethane). It is essential to allow complete binding of the water present in the sample with the dehydrating reagent (this requires at least several hours) prior to starting the extraction step. The extraction efficiency must be checked for different types and amounts of biological matrices to be investigated (see 'recovery section').

4. CLEAN-UP

The crude extract obtained from sample pretreatment requires a clean-up in order to remove co-extracted lipophilic compounds that interfere with the gas chromatographic determination of CBs and OCPs. Normal-phase solid/liquid chromatography, using deactivated Al_2O_3 or deactivated silica as adsorbents and hexane or iso-octane as solvents, is an appropriate technique for the separation of the determinands from lipids or other interfering compounds. Effective removal of high molecular weight compounds can be achieved by gel permeation chromatography (GPC). However, GPC does not separate CBs from other compounds in the same molecular range, such as organochlorine pesticides (OCPs). Therefore, additional clean-up may be required. Treatment of the OCP fraction with concentrated H_2SO_4 can improve the quality of the subsequent gas chromatogram. However, this treatment is not recommended if determinands of the dieldrin type or heptachloroepoxides, which are easily broken down by H_2SO_4, are to be determined.

5. DETERMINATION BY GAS CHROMATOGRAPHY

Because of the large number of organochlorine compounds to be determined, high resolution gas chromatography (GC) using, preferably narrow bore, fused silica wall-coated open-tubular (capillary) columns is necessary.

Carrier gas
Hydrogen is the preferred carrier gas and is indispensable for columns with very small inner diameters. For safety reasons, hydrogen should not be used without a safety module which is able to check for small hydrogen concentrations inside the GC oven coming from possible leakages. As a compromise to safety aspects, helium is also acceptable.

Columns
In order to achieve sufficient separation, capillary columns should have a length of >60 m, an internal diameter of < 0.25 mm (for diameters below 0.18 mm the elevated pressure of the carrier gas needs special instrumentation) and a film thickness of the stationary phase of < 0.25 µm. For routine work, the SE 54 (Ultra 2, DB 5, RTx 5, CP-Sil 8) phase (94 % dimethyl-, 5 % phenyl-, 1 % vinyl-polysiloxane) or medium polar columns (CP-Sil 19, OV-17, OV 1701, DB 17) have been shown to give satisfactory chromatograms. A second column with a stationary phase different, from that used in the first column, may be used for confirmation of the peak identification.

Injection
Splitless and on-column injection techniques may both be used. Split injection is not recommended because strong discrimination effects may occur. Other techniques such as temperature-programmed or pressure-programmed injection may have additional advantages, but should be thoroughly optimized before use.

In splitless injection, the volume of the liner should be large enough to contain the gas volume of the evaporated injected solvent. If the liner is too small, memory effects can occur due to contamination of the gas tubing attached to the injector. Very large liner volumes, in contrast, can cause a poor transfer of early eluting components.

A 1 µl injection normally requires a ca. 1 ml liner. The occurrence of memory effects should be tested by injection of iso-octane after analysis of a CB or OCP standard. The use of a light packing of silylated glass wool in the liner improves the response and reproducibility of the injection. However, some organochlorine pesticides such as DDT may disintegrate when this technique is used. In splitless injection, discrimination effects can occur.

The splitless injection time should therefore be optimized to avoid discrimination. This can be done by injecting a solution containing an early-eluting and a late-eluting CB, e.g., CB28 and CB180. Starting with a splitless injection time of 0.5 minutes, the peak height of the late-eluting compound will presumably increase relative to that of the first compound. The optimum is found at the time when the increase does not continue any further. The split ratio is normally set at 1:25 and is not really critical. The septum purge, normally approximately 2 ml min-1, should be stopped during injection. This option is not standard in all GCs.

Due to the variety of on-column injectors, a detailed optimization procedure cannot be given. More information on the optimization of on-column parameters may be obtained from Snell *et al.* (1987).

The reproducibility of injection is controlled by the use of an internal standard not present in the sample.

Detector
Quantitative analysis is performed by comparing the detector signal produced by the sample with that of defined standards. The use of an electron capture detector (ECD) sensitive to chlorinated compounds or - more generally applicable - a mass selective detector (MSD) or (even) a mass spectrometer (MS) is essential.

Due to incomplete separation, several co-eluting compounds can be present under a single detector signal. Therefore, the shape and size of the signal have to be critically examined. With a MSD or MS used as detector, either the molecular mass or characteristic mass fragments should be recorded for that purpose. If only an ECD is available, the relative retention time and the signal size should be confirmed on columns with different polarity of their stationary phases, or by the use of multi-dimensional GC techniques (de Boer *et al.*, 1995; de Geus *et al.*, 1996).

Calibration
Stock solutions of individual organohalogen compounds should be prepared using iso-octane as the solvent and weighed solid individual standard compounds of high purity (> 99 %). Stock solutions can be stored in measuring flasks in a refrigerator or in a dessicator with a saturated atmosphere of iso-octane, but losses can easily occur, particularly when storing in refrigerators (Law and de Boer, 1995). Loss of solvents in stock solutions can be controlled by recording the weight and filling up the missing amount before a new aliquot is taken. However, aliquots stored in sealed glass ampoules are much more appropriate and can normally be stored for several years. Fresh stock standard solutions should be prepared in duplicate and compared with the old standard solutions. Working standards should be prepared gravimetrically from stock solutions for each sample series. All manipulations with solvents, including pipetting, diluting and concentrating, should preferably be checked by weighing. Due to day-to-day and season-to-season temperature differences in laboratories and due to the heating of glassware after cleaning, considerable errors can be made when using volumetric glassware as a basis for all calculations.

The GC should be calibrated before each batch of measurements. Since the ECD has a non-linear response curve, a multilevel calibration is strongly advised. Megginson *et al*. (1994) recommend a set of six standard solutions for CB determination or five standard solutions for OCP determination. Standards used for multilevel calibration should be regularly distributed over the sample series, so that matrix and non-matrix containing injections alternate.
When concentrations of compounds in the sample fall outside either side of the calibration curve, a new dilution or concentrate should be made and the measurement repeated. Considerable errors can be made when measuring concentrations which fall outside the calibration curve.

For MS detection, a multi-level calibration is also recommended.

Recovery
For the purpose of determining recovery rates, an appropriate internal standard should be added to each sample at the beginning of the analytical procedure. The ideal internal standard is a CB which is not present in the sample and which does not interfere with other CBs. All 2,4,6-substituted CB congeners are, in principle, suitable. Alternatively, 1,2,3,4-tetrachloronaphthalene or the homologues of dichloroalkylbenzylether can be used. For GC with mass selective detection (GC-MSD), 13C-labelled CBs must be used as internal standards. With GC/MS, 13C-labelled CBs should preferably be used as internal standards.

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Annex XI:

OSPAR COMMISSION

CEMP Guidelines for Monitoring Contaminants in Sediments
(OSPAR Agreement 1999-02)
Technical Annex 8: Determination of chlorobiphenyls in biota

CEMP Guidelines for Monitoring Contaminants in Sediments

(OSPAR Agreement 1999-02)

Technical Annex 8: Determination of chlorobiphenyls in biota

1. Introduction

This technical annex provides advice on chlorobiphenyl (CB) analysis for all biota samples. The guideline is an update of an earlier version (OSPAR, 1999) taking into account evolutions in the field of analytical chemistry and also covering the determination of the planar CBs, i.e. the mono-*ortho* (CB105, CB114, CB118, CB123, CB156, CB157, CB167 and CB189) and non-*ortho* substituted CBs (CB81, CB77, CB126 and CB169). When reviewing the literature, it should be noted that planar, coplanar and dioxin-like CBs / PCBs are all equivalent terms.

The analysis of CBs in biota generally involves extraction with organic solvents, clean-up (removal of lipids and fractionation), and gas chromatographic separation with electron capture or mass-spectrometric detection. All stages of the procedure are susceptible to insufficient recovery and/or contamination. Where possible, quality control procedures are required in order to check the method's performance. These guidelines are intended to encourage and assist analytical chemists to reconsider their methods and to improve their procedures and/or the associated quality control measures where necessary. Due to the low concentrations of, particularly, non-*ortho* substituted CBs in biota compared to those of other CBs, their determination requires an additional separation and concentration step. Therefore, in the relevant sections a distinction will be made between the non-*ortho* CBs and the others.

These guidelines can also be used for several other groups of organochlorine compounds, e.g. DDTs and their metabolites, chlorobenzenes and hexachlorocyclohexanes. Recoveries in the clean-up procedures must be checked carefully. In particular, treatment with H_2SO_4 results in a loss of some compounds (e.g. dieldrin and endosulfan (de Boer and Wells, 1996)).

These guidelines are not intended as a complete laboratory manual. If necessary, further guidance should be sought from specialised laboratories. Whichever analytical procedure is adopted, the laboratory must demonstrate the validity of the procedure. Analyses must be carried out by trained staff.

2. Analysis

2.1. Precautionary measures

Solvents, chemicals and adsorption materials must be free of CBs or other interfering compounds. If not they should be purified using appropriate methods. Solvents should be checked by concentrating the volume normally used in the procedure to 10% of the final volume if practical and then analysing for the presence of CBs and other interfering compounds. If necessary, the solvents can be purified by

redistillation but this practice is not favoured by most analytical laboratories as they generally opt to purchase high quality solvents directly. Chemicals and adsorption materials should be purified by extraction and/or heating. Glass fibre materials (*e.g.* thimbles for Soxhlet extraction) should be pre-extracted. Alternatively, glass thimbles with a G1 glass filter at the bottom can be used. Generally, paper filters should be avoided in filtration and substituted for by appropriate glass filters. As all pre-cleaned materials are prone to contamination (*e.g.* by the adsorption of CBs and other compounds from laboratory air), materials ready for use should not be stored for long periods. All containers, tools, glassware *etc.* which come into contact with the sample must be made of appropriate material and must have been thoroughly pre-cleaned. Glassware should be extensively washed with detergents, heated at >250°C and rinsed immediately before use with organic solvents or mixtures such as hexane/acetone. In addition all glassware should preferably be covered with aluminium foil and stored in cupboards to keep out any dust. Old and scratched glassware is more likely to cause blank problems because of the larger surface and therefore greater chance of adsorption. Furthermore, scratched glassware can be more difficult to clean effectively, as shown during analysis of brominated flame retardants (QUASIMEME, 2007).

2.2 Lipid determination

The determination of the lipid content of tissues can be of use in characterising the samples and reporting concentrations in biota on a wet weight or lipid weight basis. The total lipid content of fish or shellfish should be determined using the method of Bligh and Dyer (1959) as modified by Hanson and Olley (1963) or an equivalent method such as Smedes (1999). Extractable lipid methods may be used, particularly if the sample size is small and lipid content is high. It has been shown that if the lipid content is high (> 5%) then the extractable lipid content will be comparable to that of the total lipid. If extraction techniques are applied which destroy or remove lipid materials (e.g., PLE with fat retainers), the lipid content should be determined on a separate subsample of the tissue homogenate. Other relevant information concerning lipid determination are provided by QUASIMEME, 1994 and Roose *et al*., 1996.

2.3. Dry weight determination

Dry weight determinations should be carried out by drying homogenised sub-samples of the material to be analysed to constant weight at 105°C.

2.4. Homogenisation and drying

Prior to analysis, the samples should be sufficiently homogenised. Homogenisation is generally carried out on fresh tissue. Care should be taken that the sample integrity is maintained during the actual homogenisation. When the analysis is undertaken, all fluids that may initially separate on thawing should be included with the materials homogenised. Homogenisation should be performed prior to extraction and clean-up procedures. When homogenising samples after drying, classical techniques using a ball mill can be used. Cryogenic homogenisation of dried or fresh materials at liquid nitrogen temperatures using a PTFE device (*cf.* Iyengar and Kasperek, 1977) or a similar technique is also possible (*cf.* Iyengar, 1976; Klussmann *et al*., 1985).

CBs can be extracted from either wet or dried samples, although storage, homogenisation and extraction are easier when the samples are dry. Drying the samples may, however, alter the CB concentrations e.g. by the loss of compounds through evaporation or by contamination. Potential losses and contamination should be checked as part of the method validation.

Chemical drying can be performed by grinding with e.g. Na_2SO_4 or $MgSO_4$ until the sample reaches a free-flowing consistency. It is essential that at least several hours elapse between grinding and extraction to allow for complete dehydration of the sample, as the presence of residual water will decrease the extraction efficiency.

Freeze-drying is also a popular technique, although its application should be carefully considered. Possible losses or contamination must be checked. Losses through evaporation are diminished by keeping the temperature in the evaporation chamber below 0°C. Contamination during freeze-drying can be reduced by putting a lid, with a hole of about 3 mm in diameter, on the sample container.

2.5. Extraction

Recovery standards should be added prior to extraction. When using Soxhlet extraction, a combination of polar and apolar solvents is recommended. Alternatively, saponification may be used. This technique is highly effective, but conditions should be controlled as saponification could result in the decomposition of some pesticides and, under certain conditions, of some CB congeners.

Although the use of binary non-polar/polar solvent mixtures and Soxhlet extraction is still the benchmark for CB extraction, there have been numerous attempts to find alternative procedures, which are less time-consuming, use less solvent and/or enable miniaturisation. Amongst these novel approaches are pressurised liquid extraction (PLE) and related subcritical water extraction (SWE), microwave-assisted extraction (MAE), matrix solid-phase dispersion (MSPD), ultrasound extraction (US) and supercritical fluid extraction (SFE).

From among the techniques mentioned, PLE or Accelerated Solvent Extraction (ASE) has – so far – been most successful. Soxhlet methods are easily translated into PLE as the same solvent compositions can be used. The method further allows interesting modifications that include in-cell clean-up of samples by adding fat retainers, such as acid-impregnated silica, florisil or alumina, to the cell. New promising techniques have been described, e.g. the use of a small carbon column in the extraction cell, which selectively adsorbs dioxin-like compounds (subsequently isolated by back-flushing with toluene), but these are not established for routine analysis (Sporring *et al.*, 2003). PLE and MAE have the shared advantage over SFE that they are matrix-independent, which facilitates method development and changing-over from the classical Soxhlet extraction. Recent years have also seen an increased use of ultrasound-based techniques for the isolation of analytes from solid samples. With most applications, extraction efficiency is satisfactory, and sonication time often is 30 min or less (Roose and Brinkman, 2005).

All the methods described above are in principle suitable for extracting CBs from biota. However, Soxhlet extraction is still the reference for alternative approaches.

2.6 Clean-up

The extraction procedures above will result in the co-extraction of lipids, which will need to be removed from the extract. Furthermore, tissue extracts will always contain many compounds other than CBs, and a suitable clean-up is necessary to remove those compounds which may interfere with the subsequent analysis. Different techniques may be used, either singly or in combination, and the choice will be influenced by the selectivity and sensitivity of the final measurement technique and also by the extraction method employed. Most CBs are stable under acid conditions; therefore treatment with sulphuric acid or acid impregnated silica columns may be used in the clean-up.

The most commonly used clean-up methods involve the use of alumina or silica adsorption chromatography, but gel permeation chromatography (GPC) is also employed. Any water residues in the extract should be removed prior to clean-up, e.g. by adding Na_2SO_4.

As CBs are apolar, clean-up using normal-phase chromatography is the most appropriate technique for their separation from other compounds. Using an apolar solvent (*e.g.* hexane or *iso*-octane) as an eluent, CBs normally elute very rapidly. All polar solvents used in the extraction should be removed before further clean-up. The last concentration step is usually performed by evaporation with a gentle stream of nitrogen. Evaporation to dryness should always be avoided but, for the analysis of planar CBs, very small final sample extract volumes might be necessary to achieve detectable concentrations.

Deactivated Al_2O_3 (5-10% water) is often used as a primary clean-up technique. Al_2O_3 can yield a sufficiently clean extract for a GC-ECD analysis of the sample. Al_2O_3 effectively removes lipid compounds from the extracts (although samples with a very high lipid content and low CB concentrations may require additional clean-up).

Deactivated silica (1-5% water) does not retain CBs (including non-*ortho* CBs) and only retains polycyclic aromatic hydrocarbons (PAHs) slightly when eluted with hexane or *iso*-octane. When organochlorine pesticides are also to be determined in the same extract, deactivation of the silica with a few percent of water is necessary.

For high activity silica (heated overnight at 180°C) the retention of CBs is negligible, while PAHs are more strongly retained. The CBs and a few other organochlorine compounds can be eluted with apolar solvents. More polar solvents (*e.g.* hexane/acetone) should be avoided as some interfering organochlorine pesticides would be eluted as well.

For the separation of CBs from lipids or oil components, reversed-phase HPLC can be used. In reversed-phase chromatography, CBs elute during a solvent gradient of 80 to 90% methanol together with numerous other compounds of the same polarity. Most of the above mentioned extraction methods and clean-up procedures yield an extract containing an apolar solvent. These cannot be injected directly for reversed-phase chromatography, and so compounds must be transferred between solvents several times *e.g.* before injection and after elution. When using polar solvents for extraction, reversed-phase columns could be used directly for clean-up. When eluting an acetonitrile extract from a C_{18} solid phase extraction (SPE) column with acetonitrile, high molecular hydrocarbons are strongly retained while CBs elute in the first few column volumes.

The above mentioned normal-phase chromatographic procedures on silica and Al_2O_3 can be transferred to HPLC having the advantages of higher resolution and better reproducibility.

When using GPC, the elution of CBs should be carefully checked. Two serial columns are often used for improved lipid separation. Solvent mixtures such as dichloromethane/hexane or cyclohexane/ethyl acetate can be used as eluents for GPC. However, a second clean-up step is often required to separate the CBs from other organohalogenated compounds and/or to remove residual lipids.

One advantage of using PLE extraction is that it is possible to combine the clean-up with the extraction, especially where mass spectrometry will be used as the detection method. If Soxhlet extraction is used for biota, then there is a much greater quantity of residual lipid to be removed than in the case of PLE with fat retainers. An additional clean-up stage may therefore be necessary. Methods have been developed for online clean-up and fractionation of dioxins, furans and CBs with PLE for food, feed and

environmental samples (Sporring *et al*., 2003), utilising a fat retainer for the on-line clean-up of fat. Silica impregnated with sulphuric acid, alumina and florisil have all been used as fat retainers. A non-polar extraction solvent such as hexane should be used if fat retainers are used during PLE.

Non-*ortho* CBs require a more specialised clean-up that is generally associated with the analysis of dioxins. Although initial clean-up may very well proceed along the lines described above, the larger sample intake results in the presence of even more co-extractive compounds and care has to be taken that the capacity of the adsorption columns is not exceeded and/or that lipids are adequately removed. Often, more rigorous procedures are applied to remove the excess material by e.g. shaking the sample with concentrated sulphuric acid. A more efficient alternative is to elute the sample over a silica column impregnated with sulphuric acid (40 % w/w).

Non-*ortho* CBs are nearly always separated from the other CBs using advanced separation techniques. One very efficient method is to inject the extracts (after concentrating them) into a HPLC system coupled to a PYE (2-(1-pyrenyl) ethyldimethylsilylated silica) column. Column dimensions are typically 4.6 x 150 mm, but combinations of several columns in-line are sometimes used. The use of PYE columns not only allow the separation of ortho, mono-ortho and non-ortho CBs from one another on the basis of structural polarity, but also from dibenzo-p-dioxins and dibenzofurans. The eluting solvent is an apolar solvent such as iso-hexane. Coupled to a fraction collector, the use of a HPLC system allows the automatic clean-up of a considerable number of samples at a time. Alternatively, HPLC systems equipped with porous graphitised carbon can be used. Column sizes are in the order of 50 x 4.7 mm and care has to be taken that the column is not overloaded. Similarly to PYE columns, they will separate non-*ortho* CBs from the other CBs and from dioxins. Fully automated systems, such as Powerprep™, that combine several steps are routinely used.

2.7 Pre-concentration

Evaporation of solvents using a rotary-film evaporator was, until recently, the most common method. However, evaporation of solvents using this technique should be performed at low temperature (water bath temperature of ≤ 40°C) and under controlled pressure conditions, in order to prevent losses of the more volatile CBs. To reduce the sample to the final volume, solvents can be removed by blowing-down with a gentle stream of nitrogen. Only nitrogen of a controlled high quality should be used.

Turbovap sample concentrators can also be used to reduce solvent volume. This is a rapid technique, but needs to be carefully optimised and monitored to prevent both losses (both of volatiles and solvent aerosols) and cross-contamination. The use of rotary-film evaporators is more time consuming but more controllable. Here also, evaporation to dryness should be avoided at all costs. Syncore™ parallel evaporators (Buchi, Switzerland) can be used with careful optimisation of the evaporation parameters. The Buchi Syncore™ Analyst also uses glass tubes but the system is sealed, avoiding contamination from the laboratory air during evaporation. It does not use a nitrogen stream, thus reducing the loss of volatiles and if the flushback module is fitted the sides of the tubes are rinsed automatically thus reducing the loss of the heavier components. Again water-bath temperatures should be minimised to prevent losses. When reducing the sample to the required final volume, solvents can be removed by a stream of clean nitrogen gas. Suitable solvents for injection into the gas chromatograph (GC) include hexane, heptane, toluene and *iso*-octane.

2.8 Calibration and preparation of calibrant solutions

Internal standards (recovery and quantification standards) should be added in a fixed volume or weight to all standards and samples. The ideal internal is a CB which is not found in the samples. All CBs with a 2,4,6-substitution (e.g. CB112, CB155, CB198) are, in principle, suitable for this purpose. Alternatively, 1,2,3,4-tetrachloronaphthalene or homologues of dichloroalkylbenzylether can be used. For GC analysis with mass selective detection (GC-MS), 13C labelled CBs should be used at each degree of chlorination. This is especially critical for the determination of the non-ortho CBs. If possible, the labelled calibrant solutions should correspond to the unlabelled determinants. For the non-ortho CBs, a labelled standard is available for each congener and use of all of them is recommended. When preparing a calibration solution for a new determinant for the first time, two independent stock solutions of different concentrations should always be prepared simultaneously to allow cross checking. A new calibration solution should also be cross-checked to the old standard solution. Crystalline CBs of known purity can be used for preparing calibration solutions but, for health and safety reasons, the purchase of solutions is recommended for planar CBs. In recent years, a lot of certified commercial custom made standards have become available and laboratories have been switching to these. If the quality of the standard materials is not guaranteed by the producer or supplier, it should be checked by GC preferably with mass spectrometric detection. Solid standards should be weighed to a precision of 10-5 grams. Calibration solutions should preferably be stored in ampoules in a cool and dark place. Commercially available screw-cap vials with a capillary opening (CertanTM) combine of advantages of ampoules and vials, and, have proven to be reliable. When stored in containers the weight loss during storage should be recorded.

2.9 Instrumental determination

2.9.1 Injection techniques

The two modes commonly used are splitless and on-column injection as, in split injection, strong discrimination effects may occur. The liner should possess sufficient capacity with respect to the injected volume after evaporation, but should not be oversized so as to avoid poor transfer to the column and losses by adsorption. Liners with a light packing of (silylated) glass wool may improve the performance for CBs, but may cause degradation of some organochlorine compounds like DDT, which are often included in national monitoring programmes.

Recently, other techniques such as temperature-programmed or pressure-programmed injection have become more prominent. They offer additional advantages such as an increased injection volume without the negative effects previously associated with that technique, but should be thoroughly optimised before use. Increasing the injection volume will allow either the elimination of an extra evaporation step or the lowering of the analytical detection limits, or both.

2.9.2 Carrier gas

Hydrogen is the preferred carrier gas and is indispensable for columns with very small inner diameters. Helium is also acceptable and is the standard carrier gas for use with GC-MS techniques.

2.9.3 Columns

Only capillary columns should be used. The following parameters are recommended:

Minimum Length	50 m (for microcolumns of internal diameter <0.1 mm, shorter columns can be suitable).

Maximum internal diameter	0.25 mm. Note that for diameters <0.15 mm the elevated pressure of the carrier gas needs special instrumental equipment as most of the instruments are limited to 400 kPa.
Film thickness	0.2 - 0.4 µm.

Columns which do not fulfil these requirements generally do not offer sufficient resolution to separate CB28, CB105 and CB156 from closely eluting CBs. A wide range of stationary phases can be used for CB separation. The chemical composition is different for columns from different producers and this influences the maximum temperature at which the column can be operated. Further advice may be found in the producers' catalogues, where compositions, applications and tables from which to compare products from different manufacturers are included.

In recent years, new chromatographic phases have become available that result in an improved separation of critical CB pairs. A good example is the HT-8 phase (1,7-dicarba-closo-dodecarborane phenylmethyl siloxane) (Larsen *et al.*, 1995) that shows a remarkable selectivity for CBs (Table 1). This column is currently recommended for CB analysis.

2.9.4 Detection

The electron capture detector (ECD) is still frequently used for CB analysis. Injection of chlorinated solvents or oxygen-containing solvents should be avoided when ECD is used due to the generation of large interfering signals. When using mass selective detectors (MSD), the electron-capture negative-ion chemical ionisation mode (ECNICI) is extremely sensitive for pentachlorinated to decachlorinated CBs, and is approximately ten fold more sensitive than ECD. However, the sensitivity of MS systems has improved considerably, allowing analysis also to be undertaken using electron impact ionisation (EI). Previously, the use of ECNICI was often necessary in order to detect the low concentrations of, in particular, the non-*ortho* CBs. Suggested target and qualifier ions for *ortho* CBs (including mono-*ortho* CBs) are shown in Table 1 and in Table 2 for non-*ortho* CBs.

Next to conventional GC-MS, the use of ion-trap with its tandem MS^2 option – i.e., yielding improved selectivity – is receiving increased attention. The use of GC-ITMS provides a less expensive alternative to high-resolution mass spectrometry (HRMS), which is commonly used to determine PCDD/Fs and, as such, is also ideally suited for the detection of all CB groups.

Table 1: Example of retention times for selected CB congeners using a 50 m HT8 column (0.25 mm i.d. and 0.25 µm film), along with possible target and qualifier ions. Temperature programme: 80°C, hold for 1 minute, ramp 20°C/minute, to 170 °C, hold 7.5 minutes, ramp 3 °C/minute to 300 °C, hold for 10 minutes.

CB congener	**MW**	**RT**	**Target Ion**	**Qualifier Ion**	**Number of chlorines**
^{13}C-CB28	**270**	28.371	268	270	3
CB31	**258**	28.071	256	258	3
CB28	**258**	28.388	256	258	3
^{13}C-CB52	**304**	30.317	304	302	4
CB52	**292**	30.336	292	290	4
CB49	**292**	30.698	292	290	4
CB44	**292**	32.024	292	290	4

CB congener	MW	RT	Target Ion	Qualifier Ion	Number of chlorines
CB74	**292**	34.881	292	290	4
CB70	**292**	35.199	292	290	4
^{13}C-CB101	**340**	36.612	338	340	5
CB101	**326**	36.630	326	328	5
CB99	**326**	37.062	326	328	5
CB97	**326**	38.267	326	328	5
CB110	**326**	39.277	326	328	5
CB123*	**326**	41.2	326	328	5
CB118*	**326**	41.563	326	328	5
CB105*	**326**	43.443	326	328	5
CB114*	**326**	42.2	326	328	5
^{13}C-CB153	**374**	42.567	372	374	6
CB149	**362**	40.328	360	362	6
CB153	**362**	42.584	360	362	6
CB132	**362**	42.236	360	362	6
CB137	**362**	43.744	360	362	6
13C-CB138	**374**	44.437	372	374	6
CB138	**362**	44.487	360	362	6
CB158	**362**	44.663	360	362	6
CB128	**362**	46.307	360	362	6
^{13}C-CB156	**374**	48.406	372	374	6
CB156*	**362**	48.366	360	362	6
CB167*	**362**	46.2	360	362	6
CB157*	**362**	48.698	360	362	6
^{13}C-CB180	**408**	48.829	406	408	7
CB187	**396**	44.787	394	396	7
CB183	**396**	45.264	394	396	7
CB180	**396**	48.846	394	396	7
CB170	**396**	50.684	394	396	7
^{13}C-CB189	**406**	53.182	406	408	7
CB189*	**396**	53.196	394	396	7
^{13}C - CB194	**442**	57.504	442	440	8
CB198	**430**	50.347	430	428	8
CB194	**430**	57.514	430	428	8

*mono-*ortho* CBs

Table 2: Possible target and qualifier ions for non-*ortho* CBs, including labelled internal standards

CB	Target ion (*m/z*)	Qualifier (*m/z*)	Qualifier (*m/z*)	Qualifier (*m/z*)
^{13}CB81	304	302	NA	NA
CB81	292	290	220	222
^{13}CB77	304	302	NA	NA
CB77	292	290	220	222
^{13}CB126	338	340	NA	NA
CB126	326	328	254	256
^{13}CB169	372	374	NA	NA
CB169	360	362	218	220

2.9.5 Separation, identification and quantification

When using GC-ECD and, to a certain extent, GC-MS, two columns with stationary phases of different polarity should be used, as column-specific co-elution of the target CBs with other CBs or other organochlorine compounds can occur on a single column. Using columns of differing selectivity's can resolve these co-elution problems. The temperature programme used must be optimised for each column to achieve sufficient separation of the CB congeners to be determined. An isothermal period in the programme around 200-220°C of approximately 30 minutes is recommended. Care should be taken that CBs of interest do not coelute with other CB congeners (for example CB28 and CB31). When using GC-ECD, compounds are identified by their retention time in relation to the standard solutions under the same conditions. Therefore GC conditions should be constant. Shifts in retention times should be checked for different areas of the chromatogram by identifying characteristic, unmistakable peaks (*e.g.* originating from the internal standard or higher concentrated CBs such as CB153 and CB138. Using a GC-MS system, the molecular mass or characteristic mass fragments or the ratio of two ion masses can be used to confirm the identity of resolved CBs. Since calibration curves for most CBs are usually non-linear when using GC-ECD, but should be linear when using GC-MS, a multilevel calibration of at least five concentrations is recommended. The calibration curve must be controlled and the best fit must be applied over the relevant concentration range. One should strive to work within the linear range of the detector. Analysis of the calibration solutions should be carried out in a mode encompassing the concentrations of the sample solutions (or alternatively by injecting matrix-containing sample solutions and matrix-free standard solutions distributed regularly over the series). When the chromatogram is processed with the help of automated integrators, the baseline may not always be set unambiguously and always needs to be inspected visually. When using GC-ECD, peak height is preferable to peak area for quantification purposes. From the two columns of different polarity the more reliable result (in terms of absence of co-elutions) should be reported.

Recent years have witnessed the emergence of so-called comprehensive two-dimensional gas chromatography (GC x GC) – a technique that can be used to considerably improve analyte/matrix as well as analyte/analyte separation. Briefly, a non-polar x (semi-)polar column combination is used, with a conventional 25–30 m long first-dimension, and a short, 0.5-1 m long, second-dimension column. The columns are connected via an interface called a modulator. The latter device serves to trap, and focus, each subsequent small effluent fraction from the first-dimension column and, then, to launch it into the second column. The main advantages of the comprehensive approach are that

the entire sample (and not one or a few heart-cuts, as in conventional multidimensional GC (Dallüge *et al*., 2003) is subjected to a completely different separation, that the two-dimensional separation does not take any more time than the first-dimension run, and that the re-focusing in the modulator helps to increase analyte detectability. The most interesting additional benefit for CBs is, that structurally related as CB congeners show up as so-called ordered structures in the two-dimensional GC x GC plane. The very rapid second-dimension separation requires the use of detectors with sufficiently high data acquisition rates. Initially, only flame ionisation detectors could meet this requirement. However, today there is also a micro-ECD on the market that is widely used for GC x GC-µECD of halogenated compound classes. Even more importantly, analyte identification can be performed by using a time-of-flight mass spectrometer (Dallüge *et al*., 2002) or – with a modest loss of performance, but at a much lower price – one of the very recently introduced rapid-scanning quadrupole mass spectrometers (Korytar *et al*., 2005; Adahchour *et al*., 2005). So far, the use of GC x GC has been limited to qualitative applications and still seems inappropriate for routine quantification of analytes.

3 Quality assurance

Planners of monitoring programmes must decide on the accuracy, precision, repeatability, and limits of detection and determination which they consider acceptable. References of relevance to QA procedures include HELCOM, 1988; QUASIMEME 1992; Wells *et al*., 1992; Oehlenschläger, 1994; Smedes *et al.*, 1994 and ICES, 1996.

3.1 System performance

The performance of the GC system should be monitored by regularly checking the resolution of two closely eluting CBs. A decrease in resolution points to deteriorating GC conditions. The signal-to-noise ratio yields information on the condition of the detector. A dirty ECD detector or MS ion source can be recognised by the presence of an elevated background signal together with a reduced signal-to-noise ratio. Chromatograms should be inspected visually by a trained operator.

3.2 Recovery

The recovery should be checked and reported. One method is to add an internal (recovery) standard to each sample immediately before extraction and a second (quantification) standard immediately prior to injection. If smaller losses occur in extraction or clean-up, or solutions are concentrated by uncontrolled evaporation of solvents (*e.g.* because vials are not perfectly capped), such losses can be compensated for by normalisation. If major losses are recognised and the reasons are unknown, the results should not be reported, as recoveries are likely to be irreproducible. A control for the recovery standard is recommended by adding the calibration solution to a real sample. Recoveries should be between 70 and 120%, if not, analysis of samples should be repeated.

3.3 Blanks

A procedural blank should be measured for each sample series and should be prepared simultaneously using the same chemicals and solvents as for the samples. Its purpose is to indicate sample contamination by interfering compounds, which will lead to errors in quantification. Even if an internal standard has been added to the blank at the beginning of the procedure, a quantification of peaks in the blank and subtraction from the values obtained for the determinands must not be performed, as

the added internal standard cannot be adsorbed by a matrix. An alternative may be using a CB-free oil as a matrix blank.

3.4 Accuracy and precision

Analysis of a Laboratory Reference Material (LRM) should be included, at least one sample within each batch of samples. The LRM must be homogeneous, well characterised for the determinands in question and stability tests must have shown that it produces consistent results over time. The LRM should be of the same type of matrix (*e.g.* liver, muscle tissue, fat or lean fish) as the samples, and the determinant concentrations should occur in a comparable range to those of the samples. If the range of determinant concentrations in the sample is large (greater than a factor of 5) it is preferable to include two reference materials within each batch of analyses to cover the lower and upper concentrations. It is good practice to run duplicate analyses of a reference material to check within-batch analytical variability. A quality control chart should be recorded for a selected set of CBs. When introducing a new LRM or when it is suspected from the control chart that there is a systematic error possibly due to an alteration of the material, a relevant Certified Reference Material (CRM) of a similar matrix to the material analysed should be used to check the LRM. Additionally a duplicate of at least one sample should be run with every batch of samples. Each laboratory should participate in interlaboratory comparison studies and proficiency testing schemes on a regular basis, preferably at an international level.

3.5 Data collection and reporting

The calculation of results and the reporting of data can represent major sources of error. Control procedures should be established in order to ensure that data are correct and to obviate transcription errors. Data stored on databases should be checked and validated, and checks are also necessary when data are transferred between databases. If possible data should be reported in accordance with the latest ICES reporting formats.

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Annex XII:

HELCOM

Manual for marine monitoring in the COMBINE programme
ANNEX B-12, APPENDIX 2. TECHNICAL NOTE ON THE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN BIOTA

HELCOM Manual for marine monitoring in the COMBINE programme

ANNEX B-12, APPENDIX 2. TECHNICAL NOTE ON THE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN BIOTA

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) consist of a variable number of fused aromatic rings. By definition, PAHs contain at least three fused rings, although in practice related compounds with two fused rings (such as naphthalene and its alkylated derivatives) are often determined and will be considered in these guidelines. PAHs arise from incomplete combustion processes and from both natural and anthropogenic sources, although the latter generally predominate. PAHs are also found in oil and oil products, and these include a wide range of alkylated PAHs formed as a result of diagenetic processes, whereas PAHs from combustion sources comprise mainly parent (non-alkylated) PAHs. PAHs are of concern in the marine environment for two main reasons: firstly, low-molecular weight (MW) PAHs can be directly toxic to marine animals; secondly, metabolites of some of the high-MW PAHs are potent animal and human carcinogens, benzo[a]pyrene is the prime example. Carcinogenic activity is closely related to structure, however, and benzo[e]pyrene and four benzofluoranthene isomers (all six compounds have a molecular weight of 252 Da) are much less potent. Some compounds (e.g., heterocyclic compounds containing sulphur, such as benzothiophenes and dibenzo-thiophenes) may also cause taint in commercially exploited fish and shellfish and render them unfit for sale.
PAHs are readily taken up by marine animals both across gill surfaces and from their diet, and may bioaccumulate, particularly in shellfish. Filter-feeding organisms such as bivalve molluscs can accumulate high concentrations of PAHs, both from chronic discharges to the sea (e.g., of sewage) and following oil spills. Fish are exposed to PAHs both via uptake across gill surfaces and from their diet, but do not generally accumulate high concentrations of PAHs as they possess an effective mixed-function oxygenase (MFO) system which allows them to metabolize PAHs and to excrete them in bile. An assessment of the exposure of fish to PAHs therefore requires also the determination of PAH-metabolite concentrations in bile samples, as turnover times can be extremely rapid. Thus, the analysis of PAHs in fish muscle tissue should normally only be undertaken for food quality assurance purposes (Law and Biscaya, 1994).

There are marked differences in the behaviour of PAHs in the aquatic environment between the low-MW compounds (such as naphthalene; 128 Da) and the high-MW compounds (such as benzo[ghi]perylene; 276 Da) as a consequence of their differing physico-chemical properties. The low-MW compounds are appreciably water soluble and can be bioaccumulated from the "dissolved" phase by transfer across gill surfaces, whereas the high-MW compounds are relatively insoluble and hydrophobic, and can attach to both organic and inorganic particulates within the water column. PAHs derived from combustion sources may actually be deposited to the sea already adsorbed to atmospheric particulates, such as soot particles. The majority of PAHs in the water column will eventually be either taken up by biota or transported to the sediments, and deep-water depositional areas may generally be regarded as sinks for PAHs, particularly when they are anoxic.

2. APPROPRIATE SPECIES FOR ANALYSIS OF PAHS

2.1 Benthic fish and shellfish

All teleost fish have the capacity for rapid metabolism of PAHs, thereby limiting their usefulness for monitoring temporal or spatial trends of PAHs. Shellfish (particularly molluscs) generally

have a lesser metabolic capacity towards PAHs, and so they are preferred because PAH concentrations are generally higher in their tissues.

For the purposes of temporal trend monitoring, it is essential that long time series with either a single species or a limited number of species are obtained. Care should be taken that the sample is representative of the population and that sampling can be repeated annually. There are advantages in the use of molluscs for this purpose as they are sessile, and so reflect the degree of contamination in the local area to a greater degree than fish which are mobile. The analysis of fish tissues is often undertaken in conjunction with biomarker and disease studies, and associations have been shown between the incidence of some diseases (e.g., liver neoplasia) in flatfish and the concentrations of PAHs in the sediments over which they live and feed (Malins et al., 1988; Vethaak and ap Rheinallt, 1992). The exposure of fish to PAHs can be assessed by the analysis of PAH-metabolites in bile, and by measuring the induction of mixed-function oxygenase enzymes which affect the formation of these metabolites. At offshore locations, the collection of appropriate shellfish samples may be problematic if populations are absent, sparse or scattered, and the collection of fish samples may be simpler. Generally, the analysis of PAHs in fish muscle tissue should only be considered for the purposes of food quality assurance.

Recent monitoring studies have indicated a seasonal cycle in PAH concentrations (particularly for combustion-derived PAHs) in mussels, with maximum concentrations in the winter prior to spawning and minimum concentrations in the summer. It is particularly important, therefore, that samples selected for trend monitoring and spatial comparisons are collected at the same time of year, and preferably in the first months of the year before spawning.

2.2 Fish

Fish are not recommended for spatial or temporal trend monitoring of PAHs, but can be useful as part of biological effects studies or for food quality assurance purposes. The sampling strategy for biological effects monitoring is described in the OSPAR Joint Assessment and Monitoring Programme (JAMP).

3. TRANSPORTATION

Live mussels should be transported to the laboratory for sample preparation. They should be transported in closed containers at temperatures between 5 °C and 15 °C, preferably below 10 °C. For live animals it is important that the transport time is short and controlled (e.g., maximum of 24 hours).

Fish samples should be kept cool or frozen (at a temperature of -20 °C or lower) as soon as possible after collection. Frozen fish samples should be transported in closed containers at temperatures below -20 °C. If biomarker determinations are to be made, then it will be necessary to store tissue samples at lower temperatures, for example, in liquid nitrogen at -196 °C.

4. PRETREATMENT AND STORAGE

4.1 Contamination

Sample contamination may occur during sampling, sample handling, pretreatment and analysis, due to the environment, the containers or packing materials used, the instruments used during sample preparation, and from the solvents and reagents used during the analytical procedures. Controlled conditions are therefore required for all procedures, including the dissection of fish organs on-board a ship (see ANNEX B-13, Appendix 1). In the case of PAHs, particular care must be taken to avoid contamination at sea. On ships there are multiple sources of PAHs, such as the

oils used for fuel and lubrication, and the exhaust from the ship's engines. It is important that the likely sources of contamination are identified and steps taken to preclude sample handling in areas where contamination can occur. A ship is a working vessel and there can always be procedures occurring as a result of the day-to-day operations (deck cleaning, automatic overboard bilge discharges, etc.) which could affect the sampling process. One way of minimizing the risk is to conduct dissection in a clean area, such as within a laminar-flow hood away from the deck areas of the vessel. It is also advisable to collect samples of the ship's fuel, bilge water, and oils and greases used on winches, etc., which can be used as fingerprinting samples at a later date, if there are suspicions of contamination in particular instances.

4.2 Shellfish

4.2.1 Depuration

Depending upon the situation, it may be desirable to depurate shellfish so as to void the gut contents and any associated contaminants before freezing or sample preparation. This is usually applied close to point sources, where the gut contents may contain significant quantities of PAHs associated with food and sediment particles which are not truly assimilated into the tissues of the mussels. Depuration should be undertaken under controlled conditions and in filtered sea water; depuration over a period of 24 hours is usually sufficient. The aquarium should be aerated and the temperature and salinity of the water should be similar to that from which the animals were removed.

4.2.2 Dissection and storage
When samples are processed, both at sea and onshore, the dissection must be undertaken by trained personnel on a clean bench wearing clean gloves and using clean stainless steel knives and scalpels. Stainless steel tweezers are recommended for holding tissues during dissection. After each sample has been prepared, all tools and equipment (such as homogenizers) should be cleaned.

4.3 Fish

4.3.1 Dissection and storage

The dissection of fish muscle and internal organs should be carried as soon as possible after collection. The details of fish muscle and liver dissection are given in ANNEX B-13, Appendix 1. If possible, the entire right side dorsal lateral fillet should be homogenized and sub samples taken for replicate PAH determinations. If, however, the amount of material to be homogenized would be too large, a specific portion of the dorsal musculature should be chosen. It is recommended that the portion of the muscle lying directly under the first dorsal fin is used in this case.

When dissecting the liver, care should be taken to avoid contamination from the other organs. If bile samples are to be taken for PAH-metabolite determinations, then they should be collected first. If the whole liver is not to be homogenized, a specific portion should be chosen in order to ensure comparability. Freeze-drying of tissue samples cannot be recommended for PAH determination, due to the contamination which may result from back-streaming of oil from the rotary pumps used to generate the vacuum.

If plastic bags or boxes are used, then they should be used as outer containers only, and should not come into contact with tissues. Organ samples (e.g., livers) should be stored in pre-cleaned containers made of glass, stainless steel or aluminium, or should be wrapped in pre-cleaned aluminium foil and shock-frozen quickly in liquid nitrogen or in a blast freezer. In the latter case, care should be taken that the capacity of the freezer is not exceeded (Law and de Boer,

1995). Cold air should be able to circulate between the samples in order that the minimum freezing time can be attained (maximum 12 hours). The individual samples should be clearly and indelibly labelled and stored together in a suitable container at a temperature of -20 °C until analysis. If the samples are to be transported during this period (e.g., from the ship to the laboratory), then arrangements must be made which ensure that the samples do not thaw out during transport. Sub samples for biomarker determinations should be collected immediately after death in order to minimize post-mortem changes in enzymatic and somatic activities, and stored in suitable vials in liquid nitrogen until analysis.

When samples are processed, both at sea and onshore, the dissection must be undertaken by trained personnel on a clean bench wearing clean gloves and using clean stainless steel knives and scalpels. Stainless steel tweezers are recommended for holding tissues during dissection. After each sample has been prepared, all tools and equipment (such as homogenizers) should be cleaned.

When pooling of tissues is necessary, an equivalent quantity of tissue should be taken from each fish, e.g., 10 % from each whole fillet.

5. ANALYSIS

5.1 Preparation of materials

Solvents, reagents, and adsorptive materials must be free of PAHs and other interfering compounds. If not, then they must be purified using appropriate methods. Reagents and absorptive materials should be purified by solvent extraction and/or by heating in a muffle oven, as appropriate. Glass fibre materials (e.g., Soxhlet thimbles) are preferred over filter papers and should be cleaned by solvent extraction. It should be borne in mind that clean materials can be re-contaminated by exposure to laboratory air, particularly in urban locations, and so storage after cleaning is of critical importance. Ideally, materials should be prepared immediately before use, but if they are to be stored, then the conditions should be considered critically. All containers which come into contact with the sample should be made of glass, and should be pre-cleaned before use. Appropriate cleaning methods would include washing with detergents, rinsing with water, and finally solvent-rinsing immediately before use. Heating of glassware in an oven (e.g., at 400°C for 24 hours) can also be useful in removing PAH contamination.

5.2 Lipid determination

Although PAH data are not usually expressed on a lipid basis, the determination of the lipid content of tissues can be of use in characterizing the samples. The lipid content should be determined on a separate subsample of the tissue homogenate, as some of the extraction techniques used routinely for PAH determination (e.g., alkaline saponification) destroy lipid materials. The total fat weight should be determined using the method of Smedes (1999) or an equivalent method.

5.3 Dry weight determination

Generally PAH data are expressed on a wet weight basis, but sometimes it can be desirable to consider them on a dry weight basis. Again, the dry weight determination should be conducted on a separate sub sample of the tissue homogenate, which should be air-dried to constant weight at 105 °C.

5.4 Extraction and clean-up

PAHs are lipophilic and so are concentrated in the lipids of an organism, and a number of methods have been described for PAH extraction (see, e.g., Ehrhardt *et al.,* 1991). The preferred methods generally utilize either Soxhlet extraction, or alkaline digestion followed by liquid-liquid extraction with an organic solvent. Microwave-assisted solvent extraction can be mentioned as one of the modern techniques being applied to PAH analysis (Budzinski *et al.,* 2000; During and Gaath, 2000; Vázquez Blanco *et al*., 2000; Ramil Criado *et al.,* 2002). In the case of Soxhlet extraction, the wet tissue must be dried by mixing with a chemical agent (e.g., anhydrous sodium sulphate), in which case a time period of several hours is required between mixing and extraction in order to allow complete binding of the water in the sample. Alkaline digestion is conducted on wet tissue samples, so this procedure is unnecessary. In neither case can the freeze-drying of the tissue prior to extraction be recommended, owing to the danger of contamination from oil back-streaming from the rotary pump (which provides the vacuum) into the sample. Non-polar solvents alone will not effectively extract all the PAHs from tissues when using Soxhlet extraction, and mixtures such as hexane/dichloromethane may be effective in place of solvents such as benzene and toluene, used historically for this purpose. Alkaline digestion has been extensively used in the determination of PAHs and hydrocarbons and is well documented. It is usually conducted in alcohol (methanol or ethanol), which should contain at least 10 % water, and combines disruption of the cellular matrix, lipid extraction and saponification within a single procedure, thereby reducing sample handling and treatment. For these reasons, it should be the method of choice. Solvents used for liquid-liquid extraction of the homogenate are usually non-polar, such as pentane or hexane, and they will effectively extract all PAHs.

Tissue extracts will always contain many compounds other than PAHs, and a suitable clean-up is necessary to remove those compounds which may interfere with the subsequent analysis. Different techniques may be used, both singly or in combination, and the choice will be influenced by the selectivity and sensitivity of the final measurement technique and also by the extraction method employed. If Soxhlet extraction was used, then there is a much greater quantity of residual lipid to be removed before the analytical determination can be made than in the case of alkaline digestion. An additional clean-up stage may therefore be necessary. The most commonly used clean-up methods involve the use of alumina or silica adsorption chromatography, but gel permeation chromatography and similar high performance liquid chromatography (HPLC) based methods are also employed (Nondek *et al.,* 1993; Nyman *et al.,* 1993; Perfetti *et al.,*1992). The major advantages of using HPLC-based clean-up methods are their ease of automation and reproducibility.

5.5 Pre-concentration

The sample volume should be 2 cm^3 or greater to avoid errors when transferring solvents during the clean-up stages. Evaporation of solvents using a rotary-film evaporator should be performed at low temperature (water bath temperature of 30 °C or lower) and under controlled pressure conditions, in order to prevent losses of the more volatile PAHs such as naphthalenes. For the same reasons, evaporation to dryness should be avoided. When reducing the sample to final volume, solvents can be removed by a stream of clean nitrogen gas. Suitable solvents for injection into the gas chromatograph (GC) or GC-MS include pentane, hexane, heptane and *iso*-octane, whereas for HPLC analyses acetonitrile and methanol are commonly used.

5.6 Selection of PAHs to be determined

The choice of PAHs to be analysed is not straightforward, both because of differences in the range of PAH compounds resulting from combustion processes and from oil and oil products, and also because the aims of specific monitoring programmes can require the analysis of different representative groups of compounds. PAHs arising from combustion processes are predominantly parent (unsubstituted) compounds, whereas oil and its products contain a much

wider range of alkylated compounds in addition to the parent PAHs. This has implications for the analytical determination, as both HPLC-based and GC-based techniques are adequate for the determination of a limited range of parent PAHs in samples influenced by combustion processes, whereas in areas of significant oil contamination and following oil spills only GC-MS has sufficient selectivity to determine the full range of PAHs present. The availability of pure individual PAHs for the preparation of standards is problematic and limits both the choice of determinands and, to some degree, the quantification procedures which can be used. The availability of reference materials certified for PAHs is also rather limited. A list of target parent and alkylated PAHs suitable for environmental monitoring is given in Table 1. In both cases, the list was concentrated on a subset of parent (predominantly combustion-derived) PAHs due to analytical limitations. This approach completely neglects the determination of alkylated PAHs, which allows the interpretation of PAH accumulation from multiple sources including those due to oil inputs. It will not be necessary for all of these PAH compounds and groups to be analysed in all cases, but an appropriate selection can be made from this list depending on the specific aims of the monitoring programme to be undertaken.

Table 1: Compounds of interest for environmental monitoring for which the guidelines apply

Compound	MW	Compound	MW
Naphthalene	128	C2-Phenanthrenes/Anthracenes	206
C1-Naphthalenes	142	C3-Phenanthrenes/Anthracenes	220
C2-Naphthalenes	156	Fluoranthene	202
C3-Naphthalenes	170	Pyrene	202
C4-Naphthalenes	184	C1-Fluoranthenes/Pyrenes	216
Acenaphthylene	152	C2-Fluoranthenes/Pyrenes	230
Acenaphthene	154	Benz[a]anthracene	228
Biphenyl	154	Chrysene	228
Fluorene	166	2,3-Benzanthracene	228
C1-Fluorenes	180	Benzo[a]fluoranthene	252
C2-Fluorenes	194	Benzo[b]fluoranthene	252
C3-Fluorenes	208	Benzo[j]fluoranthene	252
Dibenzothiophene	184	Benzo[k]fluoranthene	252
C1-Dibenzothiophenes	198	Benzo[e]pyrene	252
C2-Dibenzothiophenes	212	Benzo[a]pyrene	252
C3-Dibenzothiophenes	226	Perylene	252
Phenanthrene	178	Indeno[1,2,3-cd]pyrene	276
Anthracene	178	Benzo[ghi]perylene	276
C1-Phenanthrenes/ Anthracenes	192	Dibenz[ah]anthracene	278

5.7 Instrumental determination of PAHs

Unlike the situation for chlorobiphenyls (CBs), where GC techniques (particularly GC-ECD) are used exclusively, two major approaches based on GC and HPLC are followed to an equal extent in the analysis of PAHs. The greatest sensitivity and selectivity in routine analyses are achieved by combining HPLC with fluorescence detection (HPLC-UVF) and capillary gas chromatography with mass spectrometry (GC-MS). In terms of flexibility, GC-MS is the most capable technique, as in principle it does not limit the selection of determinands in any way, while HPLC is suited only to the analysis of parent PAHs. In the past, analyses have also been conducted using HPLC with UV-absorption detection and GC with flame-ionization detection, but neither can be recommended because of their relatively poor selectivity.

Intercomparison exercises have demonstrated a serious lack of comparability between specific hydrocarbon concentrations measured in different laboratories and using both analytical approaches described above (Farrington *et al.,* 1986). An interlaboratory performance study has been carried out within the QUASIMEME laboratory testing scheme in order to assess the level of comparability among laboratories conducting PAH analyses and to identify improvements in methodology (Law and Klungsøyr, 1996; Law *et al.,* 1998, QUASIMEME). Limits of determination within the range of 0.2 to 10 μg kg-1 wet weight for individual PAH compounds should be achievable by both GC-MS and HPLC-UVF techniques.

5.8 HPLC

Reversed-phase columns (e.g., octadecylsilane (RP-18)) 15–30 cm in length are used almost exclusively, in conjunction with gradient elution using mixtures of acetonitrile/water or methanol/water. A typical gradient may start as a 50 % mixture, changing to 100 % acetonitrile or methanol in 40 minutes. This flow is maintained for 20 minutes, followed by a return to the original conditions in 5 minutes and 5–10 minutes' equilibration before the next injection. The use of an automatic injector is strongly recommended. Also, the column should be maintained in a column oven heated to 10–30°C. The systems yielding the best sensitivity and selectivity utilize fluorescence detection. As different PAH compounds yield their maximum fluorescence at different wavelengths, for optimum detection of PAHs the wavelengths of the detector should be programmed so that the excitation/emission wavelengths detected are changed at pre-set times during the analytical determination. For closely eluting peaks, it may be necessary to use two detectors in series utilizing different wavelength pairs, or to affect a compromise in the selected wavelengths if a single detector is used. As the fluorescence signals of some PAHs (e.g., pyrene) are quenched by oxygen, the eluents must be degassed thoroughly. This is usually achieved by continuously bubbling a gentle stream of helium through the eluent reservoirs, but a vacuum degasser can also be used. Polytetrafluorethylene (PTFE) tubing must not then be used downstream of the reservoirs as this material is permeable to oxygen; stainless steel or polyether-etherketone (PEEK) tubing is preferred.

5.9 GC-MS

The two injection modes commonly used are splitless and on-column injection. Automatic sample injection should be used wherever possible to improve the reproducibility of injection and the precision of the overall method. If splitless injection is used, the liner should be of sufficient capacity to contain the injected solvent volume after evaporation. For PAH analysis, the cleanliness of the liner is also very important if adsorption effects and discrimination are to be avoided, and the analytical column should not contain active sites to which PAHs can be adsorbed. Helium is the preferred carrier gas, and only capillary columns should be used. Because of the wide boiling range of the PAHs to be determined and the surface-active properties of the higher PAHs, the preferred column length is 25–30 m, with an internal diameter of 0.15 mm to 0.3 mm. Film thicknesses of 0.3 μm to 1 μm are generally used; this choice has little impact on critical resolution, but thicker films are often used when one-ring aromatic compounds are to be determined alongside PAHs, or where a high sample loading is

needed. No stationary phase has been found on which all PAH isomers can be resolved; the most commonly used stationary phase for PAH analysis is 5 % phenyl methylsilicone (DB-5 or equivalent). This will not, however, resolve critical isomers such as benzo[*b*], [*j*] and [*k*]fluoranthenes, or chrysene from triphenylene. These separations can be made on other columns, if necessary. For PAHs there is no sensitivity gain from the use of chemical ionization (either positive or negative ion), so analyses are usually conducted in electron-impact mode at 70 eV. The choice of full-scan or multiple-ion detection is usually made in terms of sensitivity. Some instruments such as ion-trap mass spectrometers exhibit the same sensitivity in both modes, so full-scan spectra are collected, whereas for quadrupole instruments greater sensitivity is obtained if the number of ions scanned is limited. In that case, the masses to be detected are programmed to change during the analysis as different PAHs elute from the capillary column.

6. CALIBRATION AND QUANTIFICATION

6.1 Standards

A range of fully deuterated parent PAHs is available for use as standards in PAH analysis. The availability of pure PAH compounds is limited (Annex B-7). Although most of the parent compounds can be purchased as pure compounds, the range of possible alkyl-substituted PAHs is vast and only a limited selection of them can be obtained. In HPLC, where the resolving power of the columns is limited and the selectivity less than that which can be obtained using MS detection, only a single internal standard is normally used (e.g., phenanthrene-d10), although fluoranthene-d10 and 6-methyl chrysene, among others, have also been used. If GC-MS is used, then a wider range of deuterated PAHs can be utilized, both because of the wide boiling range of PAHs present and because that allows the use of both recovery and quantification standards. Suitable standards could range from naphthalene-d8 to perylene-d10. It is always recommended to use at least two and preferably three internal standards of hydrocarbons of small, medium, and high molecular weight (e.g., naphthalene-d8, phenanthrene-d10, perylene-d12. Crystalline PAHs of known purity should be used for the preparation of calibration standards. If the quality of the standard materials is not guaranteed by the producer or supplier (as for certified reference materials), then it should be checked by GC-MS analysis. Solid standards should be weighed to a precision of 10-5 grams. Calibration standards should be stored in the dark because some PAHs are photosensitive, and ideally solutions to be stored should be sealed in amber glass ampoules. Otherwise, they can be stored in a refrigerator in stoppered measuring cylinders or flasks that are gas tight to avoid evaporation of the solvent during storage.

6.2 Calibration

Multilevel calibration with at least five calibration levels is preferred to adequately define the calibration curve. In general, GC-MS calibration is linear over a considerable concentration range but exhibits non-linear behaviour when the mass of a compound injected is low due to adsorption. Quantification should be conducted in the linear region of the calibration curve, or the non-linear region must be well characterized during the calibration procedure. For HPLC-UVF, the linear range of the detection system should be large, and quantification should be made within the linear range. External standardization is often used with HPLC due to the relatively limited resolution obtainable with this technique as generally employed.

6.3 Recovery

The recovery of analytes should be checked and reported. Given the wide boiling range of the PAHs to be determined, the recovery may vary with compound group, from the volatile PAHs of low molecular weight to the larger compounds. For GC-MS analysis, deuterated standards can

be added in two groups: those to be used for quantification are added at the start of the analytical procedure, whilst those from which the absolute recovery will be assessed are added prior to GC-MS injection. This ensures that the calculated PAH concentrations are corrected for the recovery obtained in each case. In the case of HPLC, where only a single deuterated PAH standard is used, it is more common to assess recovery periodically by carrying a standard solution through the whole analytical procedure, then assessing recovery by reference to an external standard. This technique does not, however, correct for matrix effects, and so may be used in conjunction with the spiking of real samples.

7. ANALYTICAL QUALITY CONTROL

Planners of monitoring programmes must decide on the accuracy, precision, repeatability, and limits of detection and determination which they consider acceptable. Achievable limits of determination for each individual component are as follows:

- for GC-MS measurements: 0.2 μg kg-1 ww;
- for HPLC measurements: 0.5–10 μg kg-1 ww.

Further information on analytical quality control procedures for PAHs can be found elsewhere (Law and de Boer, 1995). A procedural blank should be measured with each sample batch, and should be prepared simultaneously using the same chemical reagents and solvents as for the samples. Its purpose is to indicate sample contamination by interfering compounds, which will result in errors in quantification. The procedural blank is also very important in the calculation of limits of detection and limits of quantification for the analytical method. In addition, a laboratory reference material (LRM) should be analysed within each sample batch. Test materials from the former runs of QUASIMEME Laboratory Proficiency Testing can be used as Laboratory Reference Material. The LRM must be homogeneous and well characterized for the determinands of interest within the analytical laboratory. Ideally, stability tests should have been undertaken to show that the LRM yields consistent results over time. The LRM should be of the same matrix type (e.g., liver, muscle, mussel tissue) as the samples, and the determinand concentrations should be in the same range as those in the samples. Realistically, and given the wide range of PAH concentrations encountered, particularly in oil spill investigations, this is bound to involve some compromise. The data produced for the LRM in successive sample batches should be used to prepare control charts. It is also useful to analyse the LRM in duplicate from time to time to check within-batch analytical variability. The analysis of an LRM is primarily intended as a check that the analytical method is under control and yields acceptable precision, but a certified reference material (CRM) of a similar matrix should be analysed periodically in order to check the method bias. The availability of biota CRMs certified for PAHs is very limited (Annex B-7; QUASIMEME), and in all cases the number of PAHs for which certified values are provided is small. At regular intervals, the laboratory should participate in an intercomparison or proficiency exercise in order to provide an independent check on the performance.

8. DATA REPORTING

The calculation of results and the reporting of data can represent major sources of error, as has been shown in intercomparison studies for PAHs. Control procedures should be established in order to ensure that data are correct and to obviate transcription errors. Data stored in databases should be checked and validated, and checks are also necessary when data are transferred between databases. Data should be reported in accordance with the latest ICES reporting formats.

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Annex XIII:

OSPAR COMMISSION

CEMP Guidelines for Monitoring Contaminants in Biota
(OSPAR Agreement 1999-02)
Technical Annex 3: Determination of parent and alkylated PAHs in biological materials

CEMP Guidelines for Monitoring Contaminants in Biota

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Technical Annex 3: Determination of parent and alkylated PAHs in biological materials

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) consist of a variable number of fused benzene rings. By definition, PAHs contain at least two fused rings. PAHs arise from incomplete combustion processes and from both natural and anthropogenic sources, although the latter generally predominate. PAHs are also found in oil and oil products, and these include a wide range of alkylated PAHs formed as a result of diagenetic processes, whereas PAHs from combustion sources comprise mainly parent (non-alkylated) compounds. PAHs are of concern in the marine environment for two main reasons: firstly, low molecular weight (MW) PAHs can cause tainting of fish and shellfish and render them unfit for sale; secondly, metabolites of some of the high MW PAHs are potent animal and human carcinogens — benzo[*a*]pyrene is the prime example. Carcinogenic activity is closely related to structure. Benzo[*e*]pyrene and the four benzofluoranthene isomers all have a molecular weight of 252 Da; however, they are much less potent than benzo[*a*]pyrene. Less is known about toxicity of alkylated PAHs. However, one study has demonstrated that alkylated PAHs may have increased toxicity compared to the parent compound (Marvanova *et al.,* 2008).

PAHs are readily taken up by marine animals both across gill surfaces (lower MW PAHs) and from their diet. They may bioaccumulate, particularly in shellfish. Filter-feeding organisms such as bivalve molluscs can accumulate high concentrations of PAHs, both from chronic discharges to the sea (e.g., of sewage) and following oil spills. Fish are exposed to PAHs both via uptake across gill surfaces and from their diet, but do not generally accumulate high concentrations of PAHs as they possess an effective mixed-function oxygenase (MFO) system which allows them to metabolise PAHs and to excrete them in bile. Other marine vertebrate and marine mammals also metabolise PAHs efficiently. An assessment of the exposure of fish to PAHs therefore requires the determination of PAH metabolite concentrations in bile, as turnover times can be extremely rapid.

There are marked differences in the behaviour of PAHs in the aquatic environment between the low MW compounds (such as naphthalene; 128 Da) and the high MW compounds (such as benzo[*ghi*]perylene; 276 Da) as a consequence of their differing physico-chemical properties. The low MW compounds are appreciably water soluble (e.g. naphthalene) and can be bioaccumulated from the dissolved phase by transfer across gill surfaces, whereas the high MW compounds are relatively insoluble and hydrophobic, and can attach to both organic and inorganic particulates within the water column. PAHs derived from combustion sources may actually be deposited to the sea already

adsorbed to atmospheric particulates, such as soot particles. The sediment will act as a sink for PAHs in the marine environment.

2. Appropriate species for analysis of parent and alkylated PAHs

2.1 Benthic fish and shellfish

Guidance on the selection of appropriate species for contaminant monitoring is given in the OSPAR Joint Assessment and Monitoring Programme guidelines. All teleost fish have the capacity for rapid metabolism of PAHs, thereby limiting their usefulness for monitoring temporal or spatial trends of PAHs. Shellfish (particularly molluscs) generally have a lesser metabolic capacity towards PAHs, and so they are preferred because PAH concentrations are generally higher in their tissues. The blue mussel (*Mytilus edulis*) occurs in shallow waters along almost all coasts of the Northeast Atlantic. It is therefore suitable for monitoring in near shore waters. No distinction is made between *M. edulis* and *M. galloprovincialis* because the latter species, which may occur along Spanish and Portuguese coasts, fills a similar ecological niche. A sampling size range of 30–70 mm shell length is specified to ensure availability throughout the whole maritime area. In some areas (e.g., the Barents Sea), other species may be considered. Recent monitoring studies have indicated a seasonal cycle in PAH concentrations (particularly for combustion-derived PAHs) in mussels, with maximum concentrations in the winter prior to spawning and minimum concentrations in the summer. It is particularly important, therefore, that samples selected for trend monitoring and spatial comparisons are collected at the same time of year, and preferably in the first months of the year prior to spawning.

For the purposes of temporal trend monitoring, it is essential that long time-series with either a single species or a limited number of species be obtained. Care should be taken that the sample is representative of the population and that it can be sampled annually. There are advantages in the use of molluscs for this purpose as they are sessile, and so reflect the degree of contamination in the local area to a greater degree than fish which are mobile and metabolise PAHs relatively efficiently. The analysis of fish tissues is often undertaken in conjunction with biomarker and disease studies, and associations have been shown between the incidence of some diseases (e.g., liver neoplasia) in flatfish and the concentrations of PAHs in the sediments over which they live and feed (Malins *et al*., 1988; Vethaak and Rheinallt, 1992). The exposure of fish to PAHs can be assessed by the analysis of PAH metabolites in bile, and by measuring the induction of mixed-function oxygenase enzymes which catalyse the formation of these metabolites.

3. Transportation

Live biota should be transported in closed containers at temperatures between 5°C and 10°C. For live animals it is important that the transport time is short and controlled (e.g., maximum of 24 hours). If biomarker determinations are to be made, then it will be necessary to store tissue samples at lower temperatures, for example, in liquid nitrogen at -196°C.

4. Pre-treatment and storage

4.1 Contamination

Sample contamination may occur during sampling, sample handling, pre-treatment, and analysis, due to the environment, the containers or packing materials used, the instruments used during sample preparation, and from the solvents and reagents used during the analytical procedures. Controlled

conditions are therefore required for all procedures. In the case of PAHs, particular care must be taken to avoid contamination at sea. On ships there are multiple sources of PAHs, such as the oils used for fuel and lubrication, and the exhaust from the ship's engines. It is important that the likely sources of contamination are identified and steps taken to preclude sample handling in areas where contamination can occur. A ship is a working vessel and there can always be procedures occurring as a result of the day-to-day operations (deck cleaning, automatic overboard bilge discharges, etc.) which could affect the sampling process. One way of minimizing the risk is to conduct dissection in a clean area, such as within a laminar-flow hood away from the deck areas of the vessel. It is also advisable to collect samples of the ship's fuel, bilge water, and oils and greases used on winches, etc., which can be used as fingerprinting samples at a later date, if there are suspicions of contamination in particular instances.

Freeze-drying of tissue samples may be a source of contamination due to the back-streaming of oil vapours from the rotary vacuum pumps. Furthermore, drying the samples may result in losses of the lower molecular weight and more volatile PAHs through evaporation (Law and Biscaya, 1994).

4.2 Shellfish

4.2.1 Depuration

Depending upon the situation, it may be desirable to depurate shellfish so as to void the gut contents and any associated contaminants before freezing or sample preparation. This is usually applied close to point sources, where the gut contents may contain significant quantities of PAHs associated with food and sediment particles which are not truly assimilated into the tissues of the mussels. Depuration should be undertaken in controlled conditions and in clean seawater; depuration over a period of 24 hours is usually sufficient. The aquarium should be aerated and the temperature and salinity of the water should be similar to that from which the animals were removed.

4.2.2 Dissection and storage

Mussels should be shucked live and opened with minimal tissue damage by detaching the adductor muscles from the interior of at least one valve. The soft tissues should be removed and homogenised as soon as possible, and frozen in glass jars or aluminium cans at –20°C until analysis. Plastic materials must not be used for sampling and storage owing to possible adsorption of the PAHs onto the container material. As PAHs are sensitive to photo-degradation, exposure to direct sunlight or other strong light must be avoided during storage of the samples as well as during all steps of sample preparation, including extraction and storage of the extracts (Law and Biscaya, 1994). The use of amber glassware is strongly recommended.

When samples are processed, both at sea and onshore, the dissection must be undertaken by trained personnel on a clean bench wearing clean gloves and using PAH-free stainless steel knives and scalpels. Stainless steel tweezers are recommended for holding tissues during dissection. After each sample has been prepared, all tools and equipment (such as homogenisers) should be cleaned by wiping with tissue and rinsing with solvent.

5. Analysis

5.1 Preparation of materials

Solvents and adsorptive materials must all be checked for the presence of PAHs and other interfering compounds. If found then the solvents, reagents, and adsorptive materials must be purified or cleaned using appropriate methods. Absorptive materials should be cleaned by solvent extraction and/or by heating in a muffle oven as appropriate. Glass fibre materials (e.g. Soxhlet thimbles and filter papers used in pressurised liquid extraction (PLE)) should be cleaned by solvent extraction or pre-baked at 450°C overnight. It should be borne in mind that clean materials can be re-contaminated by exposure to laboratory air, particularly in urban locations, and so the method of storage after cleaning is of critical importance. Ideally, materials should be prepared immediately before use, but if they are to be stored, then the conditions should be considered critically. All containers which come into contact with the sample should be made of glass or aluminium, and should be pre-cleaned before use. Appropriate cleaning methods would include washing with detergents, rinsing with water of known quality, and finally solvent rinsing immediately before use.

5.2 Lipid determination

Although PAH data are not usually expressed on a lipid basis, the determination of the lipid content of tissues can be of use in characterising the samples. This will enable reporting concentrations on a wet weight or lipid weight basis. The lipid content should be determined on a separate subsample of the tissue homogenate, as some of the extraction techniques used routinely for PAHs determination (e.g., PLE with fat retainers, alkaline saponification) destroy or remove lipid materials. The total lipid content of fish or shellfish should be determined using the method of Bligh and Dyer (1959) as modified by Hanson and Olley (1963) or an equivalent method such as Smedes (1999). Extractable lipid may be used, particularly if the sample size is small and lipid content is high. It has been shown that if the lipid content is high (>5%) then extractable lipid will be comparable to the total lipid.

5.3 Extraction

PAHs are lipophilic and so are concentrated in the lipids of an organism, and a number of methods have been described for PAH extraction (Ehrhardt *et al*., 1991). These methods generally utilise either Soxhlet extraction, or alkaline digestion followed by liquid-liquid extraction with an organic solvent. In the case of Soxhlet extraction, the wet tissue must be dried by mixing with a chemical drying agent (e.g., anhydrous sodium sulphate), in which case a time period of several hours is required between mixing and extraction in order to allow complete binding of the water in the sample. Samples are spiked with recovery standard and should be left overnight to equilibrate. Alkaline digestion is conducted on wet tissue samples, so this procedure is unnecessary.

Apolar solvents alone will not effectively extract all the PAHs from tissues when using Soxhlet extraction, and mixtures such as hexane/dichloromethane may be effective in place of solvents such as benzene and toluene, used historically for this purpose. Alkaline digestion has been extensively used in the determination of PAHs and hydrocarbons and is well documented. It is usually conducted in alcohol (methanol or ethanol), which should contain at least 10% water, and combines disruption of the cellular matrix, lipid extraction and saponification within a single procedure, thereby reducing sample handling and treatment. Solvents used for liquid-liquid extraction of the homogenate are usually apolar, such as pentane or hexane, and they will effectively extract all PAHs.

Alternatively extraction of wet or dry samples of biota may be carried out by pressurised liquid extraction (PLE). This is a more recent method, requiring less solvent and time for the extraction process. The wet biota sample is dried by mixing with sufficient anhydrous sodium sulphate to form a free flowing mixture and is packed into stainless steel extraction cells containing a glass fibre filter and sodium sulphate or glass powder to fill the cell. To ensure a better recovery samples may be extracted twice and extractions are performed at elevated temperatures and pressure.

5.4 Clean-up

Tissue extracts will always contain many compounds other than PAHs, and a suitable clean-up is necessary to remove those compounds which may interfere with the subsequent analysis. Different techniques may be used, either singly or in combination, and the choice will be influenced by the selectivity and sensitivity of the final measurement technique and also by the extraction method employed. If Soxhlet extraction was used, then there is a much greater quantity of residual lipid to be removed before the analytical determination can be made than in the case of alkaline digestion. An additional clean-up stage may therefore be necessary. The most commonly used clean-up methods involve the use of deactivated alumina or silica adsorption chromatography. When applying fractionation, the elution pattern has to be checked frequently. This should be carried out in the presence of sample matrix, as that can partially deactivate the clean-up column, resulting in earlier elution of the PAHs than in a standard solution.

Gel permeation chromatography (GPC) and high performance liquid chromatography (HPLC) based methods are also employed (Nondek *et al.,* 1993; Nyman *et al.,* 1993; Perfetti *et al.*, 1992). The major advantages of using HPLC-based clean-up methods are their ease of automation and reproducibility.

Isocratic HPLC fractionation of the extract can be used to give separate aliphatic and aromatic fractions (Webster *et al*., 2002). A metal-free silica column is used for the clean-up/fractionation as dibenzothiophene (DBT) can be retained on ordinary silica columns. The split time is determined by injection of a solution containing representative aliphatic and PAH standards. The silica column is regenerated by a cleaning cycle after a set number of samples. If PAHs are to be analysed by HPLC and there are significant amounts of alkylated PAHs present then the removal of the alkylated PAHs may be difficult.

5.5 Pre-concentration

In the methods suggested above, all result in an extract in which non-polar solvents are dominant. The sample volume should be 2 ml or greater to avoid errors when transferring solvents during the clean-up stages. Syncore parallel evaporators can be used with careful optimisation of the evaporation parameters. Evaporation of solvents using a rotary-film evaporator should be performed at low temperature (water bath temperature of 30°C or lower) and under controlled pressure conditions, in order to prevent losses of the more volatile PAHs such as naphthalenes. For the same reasons, evaporation to dryness must be avoided. When reducing the sample to final volume, solvents can be removed by a stream of clean nitrogen gas. Suitable solvents for injection into the GC-MS include pentane, hexane, heptane, *iso*-hexane and *iso*-octane.

5.6 Selection of PAHs to be determined

The choice of PAHs to be analysed is not straightforward, both because of differences in the range of PAH compounds resulting from combustion processes and from oil and oil products, and also because the aims of specific monitoring programmes can require the analysis of different representative

groups of compounds. PAHs arising from combustion processes are predominantly parent (unsubstituted) compounds, whereas oil and its products contain a much wider range of alkylated compounds in addition to the parent PAHs. This has implications for the analytical determination, as both HPLC-based and GC-based techniques are adequate for the determination of a limited range of parent PAHs in samples influenced by combustion processes, whereas in areas of significant oil contamination and following oil spills only GC-MS has sufficient selectivity to determine the full range of PAHs present. The availability of pure individual PAHs for the preparation of standards is problematic and limits both the choice of determinands and, to some degree, the quantification procedures which can be used. The availability of reference materials certified for PAHs is also rather limited. A list of target parent and alkylated PAHs suitable for environmental monitoring is given in Table A1.1. This differs both from the list previously developed within ICES specifically for intercomparison purposes, and the historic list of Borneff. In both cases, the lists were concentrated on a subset of parent (predominantly combustion-derived) PAHs due to analytical limitations. This approach completely neglects the determination of alkylated PAHs, which allows the interpretation of PAH accumulation from multiple sources including those due to oil inputs. It will not be necessary for all of these PAH compounds and groups to be analysed in all cases, but an appropriate selection can be made from this list depending on the specific aims of the monitoring programme to be undertaken.

Table A1.1: Compounds of interest for environmental monitoring for which the guidelines apply. For compounds in italics standards are not available for any isomers in this group.

Compound	MW	Compound	MW
Naphthalene	128	2, 3d-benzonapthothiophene	234
C_1-Naphthalenes	142	C_1-234	248
C_2-Naphthalenes	156	C_2-Fluoranthenes/Pyrenes	230
C_3-Naphthalenes	170	Benz[*a*]anthracene	228
C_4-Naphthalenes	184	Chrysene	228
Acenaphthylene	152	2,3-Benzanthracene	228
Acenaphthene	154	C_1- Benz[*a*]anthracene/ Chrysene	242
Biphenyl	154	C_2- Benz[*a*]anthracene/ Chrysene	256
Fluorene	166	C_3- Benz[*a*]anthracene/ Chrysene	270
C_1-Fluorenes	180	Benzo[*a*]fluoranthene	252
C_2-Fluorenes	*194*	Benzo[*b*]fluoranthene	252
C_3-Fluorenes	*208*	Benzo[*j*]fluoranthene	252
Dibenzothiophene	184	Benzo[*k*]fluoranthene	252
C_1-Dibenzothiophenes	198	Benzo[*e*]pyrene	252
C_2-Dibenzothiophenes	212	Benzo[*a*]pyrene	252
C_3-Dibenzothiophenes	226	Perylene	252
Phenanthrene	178	Indeno[1,2,3-*cd*]pyrene	276
Anthracene	178	Benzo[*ghi*]perylene	276
C_1-Phenanthrenes/Anthracenes	192	Dibenz[*a,h*]anthracene	278
C_2-Phenanthrenes/Anthracenes	206	Benzo[*k*]fluoranthene	252
C_3-Phenanthrenes/Anthracenes	220	Cyclopenta[*cd*]pyrene	226
Fluoranthene	202	Naphtho[2,1-*a*]pyrene	302
Pyrene	202	Dibenz[*a,e*]pyrene	302

C_1-Fluoranthenes/Pyrenes	216	Dibenz[*a,i*]pyrene	302
2, 1d-benzonapthothiophene	234	Dibenz[*a,l*]pyrene	302
1,2d-benzonapthothiophene	234	Dibenz[*a,h*]pyrene	302

5.7 Instrumental determination of PAHs

The greatest sensitivity and selectivity in routine analysis for parent PAH is achieved by combining HPLC with fluorescence detection (HPLC-UVF) or capillary gas chromatography with mass spectrometry (GC-MS). However, for the analysis of parent and alkylated PAHs GC-MS is the method of choice. In terms of flexibility, GC-MS is the most capable technique, as in principle it does not limit the selection of determinands in any way, while HPLC is suited only to the analysis of parent PAHs. In the past, analyses have also been conducted using HPLC with UV-absorption detection and GC with flame-ionisation detection, but neither can be recommended for alkylated PAHs because of their relatively poor selectivity. Both in terms of the initial capital cost of the instrumentation, and the cost per sample analysed, HPLC-UVF is cheaper than GC-MS. With the advent of high-sensitivity benchtop GC-MS systems, however, this cost advantage is now not as marked as in the past, and the additional information regarding sources available makes GC-MS the method of choice.

Limits of determination within the range of 0.05 to 0.5 µg kg^{-1} wet weight for individual PAH compounds should be achievable by GC-MS. However this limit can be lowered in routine analysis.

5.7.1 GC-MS

The three injection modes commonly used are splitless, on-column and PTV (programmed temperature vaporiser). Automatic sample injection should be used wherever possible to improve the reproducibility of injection and the precision of the overall method. If splitless injection is used, the liner should be of sufficient capacity to contain the injected solvent volume after evaporation. For PAH analysis, the cleanliness of the liner is also very important if adsorption effects and discrimination are to be avoided, and the analytical column should not contain active sites to which PAHs can be adsorbed. Helium is the preferred carrier gas, and only capillary columns should be used. Because of the wide boiling range of the PAHs to be determined and the surface-active properties of the higher PAHs, the preferred column length is 25–50 m, with an internal diameter of 0.15 mm to 0.3 mm. Film thicknesses of 0.2 µm to 1 µm are generally used; this choice has little impact on critical resolution, but thicker films are often used when one-ring aromatic compounds are to be determined alongside PAHs, or where a high sample loading is needed. No stationary phase has been found on which all PAH isomers can be resolved; the most commonly used stationary phase for PAH analysis is 5% phenyl methylsilicone (DB-5 or equivalent). This will not, however, resolve critical isomers such as benzo[*b*], [*j*] and [*k*]fluoranthenes, or chrysene from triphenylene. Chrysene and triphenylene can be separated on other columns, if necessary such as a 60 m non polar column such a DB5MS. For PAHs there is no sensitivity gain from the use of chemical ionisation (either positive or negative ion), so analyses are usually conducted in electron-impact mode at 70eV. Quadrupole instruments are used in single ion monitoring to achieve greater sensitivity. The masses to be detected are programmed to change during the analysis as different PAHs elute from the capillary column. In SIM the molecular ion is used for quantification. Qualifier ions can be used to confirm identification but they are limited for PAHs. Triple quadropole mass spectrometry can also be used and will give greater sensitivity. Some instruments such as ion-trap and time of flight mass spectrometers exhibit the same sensitivity in both modes, so full scan spectra can be used for quantification.

An example of mass spectrometer operating conditions in SIM mode is given in Table A1.2. The ions are grouped and screened within GC time windows of the compounds. In general the number of ions should not be greater than 20. The dwell time is important parameter and should be close for each ion. For GC capillary column analysis a dwell time should not be shorter than 20 ms, while a sum of a dwell in each retention time windows should not be greater than 500 ms. An example of conditions that can be used along with dwell times are shown in Table A1.2.

Table A.1.2: Example of operational conditions for the GC-MS analysis of parent and alkylated PAHs.

Group N°	Retention time (min)	Dwell time (ms)	Ions in group (AMU)					
1	8.00	100	128	136	142			
2	21.00	100	152	156	160			
3	23.70	100	154	164	168	170		
4	26.80	80	166	176	180	182	184	
5	31.60	80	178	184	188	194	196	198
6	35.30	100	192	198				
7	36.60	100	206	212				
8	39.40	80	202	206	212	216	220	226
9	44.65	100	216	220				
10	45.30	100	226	228	230	234	240	
11	48.58	90	242	248				
12	52.00	100	252	256	264	266		
13	59.00	100	266	276	278	288		

Alkylated homologues of PAHs (C1–C4), mainly associated with petrogenic sources, contain a number of different isomers that can give very complex but distinct distribution profiles when analysed by GC-MS. Integration of each isomer separately is difficult for most alkylated PAHs. 1- and 2-Methyl naphthalene give well resolved peaks that can be quantified separately. C1-Phenanthrene/anthracene gives five distinct peaks corresponding to 3-methyl phenanthrene, 2-methyl phenanthrene, 2-methyl anthracene, 4- and 9-methyl phenanthrene and 1-methyl phenanthrene. These may be integrated as a group or as separate isomers. For all other alkylated PAHs the area for all isomers may be summed and quantified against a single representative isomer. This method will lead, however, to an overestimation of the concentration as may include non-alkylated PAHs. Examples of integrations of both parent and alkylated PAHs are shown in Appendix 1.

6. Calibration and quantification

6.1 Standards

The availability of pure PAH compounds are limited. Although most of the parent compounds can be purchased as pure compounds, the range of possible alkyl-substituted PAHs is vast and only a limited selection of them can be obtained. PAH standards are available for at least one isomer of most alkyl group listed in Table A1.1. A range of deuterated PAHs (normally 5 to 7) should be used as internal standards to cover the range of PAHs being analysed in samples. A range of fully-deuterated parent PAHs is available for use as standards in PAH analysis. Suitable standards could range from d_8-naphthalene to d_{14}-dibenz[*a,h*]anthracene. Crystalline PAHs of known purity should be used for the preparation of calibration standards. If the quality of the standard materials is not guaranteed by the producer or supplier (as for certified reference materials), then it should be checked by GC-MS analysis. Solid standards should be weighed to a precision of 10^{-5} grams. Calibration standards should

be stored in the dark because some PAHs are photosensitive, and ideally solutions to be stored should be sealed in amber glass ampoules or sealed GC vials. Otherwise, they can be stored in a refrigerator in stoppered measuring cylinders or flasks that are gas tight to avoid evaporation of the solvent during storage.

6.2 Calibration

Multilevel calibration with at least five calibration levels is preferred to adequately define the calibration curve. In general, GC-MS calibration is linear over a considerable concentration range but may exhibit a change of slope at very low concentrations. Quantification should be conducted in the linear region of the calibration curve. A separate calibration curve may be used where sample concentrations are very low. An internal standard method should be employed, using a range of deuterated PAHs as internal standards.

6.3 Recovery

The recovery of analytes should be checked and reported. Given the wide boiling range of the PAHs to be determined, the recovery may vary with compound group, from the volatile PAHs of low molecular weight to the larger compounds. Deuterated standards can be added in two groups: those to be used for quantification are added at the start of the analytical procedure, whilst those from which the absolute recovery will be assessed are added prior to GC-MS injection. This allows the recovery to be calculated.

7. Analytical Quality Control

Planners of monitoring programmes must decide on the accuracy, precision, repeatability, and limits of detection and determination which they consider acceptable. Achievable limits of determination for each individual component are as follows:

- for GC-MS measurements: 0.05 µg kg^{-1} ww;

- Further information on analytical quality control procedures for PAHs can be found elsewhere (Law and de Boer, 1995). A procedural blank should be measured with each sample batch, and should be prepared simultaneously using the same chemical reagents and solvents as for the samples. Its purpose is to indicate sample contamination by interfering compounds, which will result in errors in quantification. The procedural blank is also very important in the calculation of limits of detection and limits of quantification for the analytical method. In addition, a laboratory reference material (LRM) should be analysed within each sample batch. The LRM must be homogeneous and well-characterised for the determinands of interest within the analytical laboratory. Ideally, stability tests should have been undertaken to show that the LRM yields consistent results over time. The LRM should be of the same matrix type (e.g. mussels) as the samples, and the determinand concentrations should be in the same range as those in the samples. Realistically, and given the wide range of PAH concentrations encountered, particularly in oil spill investigations, this is bound to involve some compromise. The data produced for the LRM in successive sample batches should be used to prepare control charts. It is also useful to analyse the LRM in duplicate from time to time to check within-batch analytical variability. The analysis of an LRM is primarily intended as a check that the analytical method is under control and yields acceptable precision, but a certified reference material (CRM) of a similar matrix should be analysed periodically in order to check the method bias. The availability of biota CRMs certified for PAHs is very limited, and in all cases the number of PAHs for which certified values are

provided is small. At present, only NIST 1974a (a frozen wet mussel tissue) and NIST 2974 (a freeze-dried mussel tissue) are available. At regular intervals, the laboratory should participate in an intercomparison or proficiency exercise in which samples are circulated without knowledge of the determinand concentrations, in order to provide an independent check on performance.

8. Data reporting

The calculation of results and the reporting of data can represent major sources of error, as has been shown in intercomparison studies for PAHs. Control procedures should be established in order to ensure that data are correct and to obviate transcription errors. Data stored on databases should be checked and validated, and checks are also necessary when data are transferred between databases. Data should be reported in accordance with the latest ICES reporting formats.

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Annex XIV:

European Commission Guidance Document No. 32. On Biota Monitoring

Technical Report - 2014 - 083
(The implementation of EQSBIOTA) under the Water Framework Directive

ANNEX A.7 Normalisation of measured data with respect to lipid and dry weight content

European Commission Guidance Document No. 32. On Biota Monitoring

Technical Report - 2014 - 083

(The implementation of EQSBIOTA) under the Water Framework Directive

ANNEX A.7 Normalisation of measured data with respect to lipid and dry weight content

The appropriate metric to use for normalisation of contaminant concentrations in biota will usually follow from the normalisation used in the bioaccumulation studies used to derive the biota EQS.

For substances that accumulate through hydrophobic partitioning into the lipids of organisms, measured concentrations in fish should be normalised to fish with a lipid content of 5% (EC 2011). The energy content for mussels of 19.3 kJ/g dw (Smit 2005; EFSA 2009) corresponds to a lipid content of approximately 1% for freshwater and marine bivalves (Bruner et al. 1994; Lazzara et al. 2012; Pleissner et al. 2012), and measured concentrations in bivalves should therefore be normalised to bivalves with a lipid content of 1%. The rationale behind this lipid normalisation is that the whole body biota concentration is linearly correlated with the lipid content of the species for those substances.

For a substance that does not accumulate by hydrophobic partitioning into lipids, but via another mechanism of accumulation, normalisation against another parameter, such as dry weight (e.g. for mercury), may be appropriate. The default dry weight content for fish is approximately 26% (Smit 2005; EFSA 2009). For mussels, EFSA has suggested a default dry weight content of 8.3% (Smit 2005; EFSA 2009).

Based on the above, contaminant concentrations should be normalised to lipid contents of 5% in fish and 1% in bivalves, or to dry weight contents of 26% in fish and 8.3% in bivalves, on the basis of the measured lipid content or dry weight, or on the basis of generic values for lipid content or dry weight for the relevant species obtained from FishBase, for example.

To calculate the normalised concentrations $conc_{norm,\ lipid}$ or $conc_{norm,\ dry\ weight}$ from measured concentrations $conc_{meas}$ for a fish species x, the following equations can be used (lipid content and dry weight content expressed as mass fractions):

$$conc_{norm,\ lipid} = conc_{meas} \cdot 0.05/\text{lipid content}_x$$

or

$$conc_{norm,\ dry\ weight} = conc_{meas} \cdot 0.26/\text{dry weight}_x$$

Similarly, to calculate the normalised concentrations $conc_{norm,\ lipid}$ or $conc_{norm,\ dry\ weight}$ from measured concentrations $conc_{meas}$ for a bivalve species x, the following equations can be used (lipid content and dry weight content expressed as mass fractions):

$$conc_{norm,\ lipid} = conc_{meas} \cdot 0.01/\text{lipid content}_x$$

or

$$conc_{norm,\ dry\ weight} = conc_{meas} \cdot 0.083/\text{dry weight}_x$$

Using the exact lipid or dry weight content of the biota samples is always preferred over generic values for the species (such as those available from FishBase).

It is acknowledged that for the organic priority substances, e.g. dioxins, covered by both the WFD and food legislation, lipid normalisation may result in different conclusions under the MSFD for descriptors 8 and 9, even when human health is the protection goal in both cases. The discrepancy will depend upon whether the actual lipid content is greater or less than the 5% benchmark. The results should therefore be interpreted with appropriate qualification.

Annex XV:

Background Assessment Criteria recommended to be used to assess concentrations in Mediterranean sediments, mussel (Mytilus galloprovincialis) and fish (Mullus barbatus)

(UNEP(DEPI)/MED WG.444/12, 6th Meeting of the Ecosystem Approach Coordination Group 2017)

Annex XV: Background Assessment Criteria recommended to be used to assess concentrations in Mediterranean sediments, mussel (*Mytilus galloprovincialis*) and fish (*Mullus barbatus*)
(UNEP(DEPI)/MED WG.444/12, 6th Meeting of the Ecosystem Approach Coordination Group 2017)

Background Assessment Criteria recommended to be used to assess concentrations in Mediterranean sediments, mussel (*Mytilus galloprovincialis*) and fish (*Mullus barbatus*)
UNEP(DEPI)/MED WG.444/12, 6th Meeting of the Ecosystem Approach Coordination Group 2017

Table of the proposed assessment criteria for trace metals (TMs)

Table A.1.1. Mediterranean Sea: Background Concentrations (Med BCs), Med BACs and EACs; Calculation =>BC = 50th (median); BAC=1.5 x BC (mussel, sediment); BAC=2.0 x BC (fish)

Trace metal	Mussel (MG) μg kg^{-1} d.w.			Fish (MB) μg kg^{-1} f.w.			Sediment μg kg^{-1} d.w.		
	BC	Med BAC	EC*	BC	Med BAC	EC*	BC	Med BAC	ERL**
Cd	730.0	1095.0	5000	(3.7)[a]	(16.0)[b]	50	85.0	127.5	1200
Hg	115.5	173.2	2500	50.6	101.2	1000	53.0	79.5	150
Pb	1542	2313	7500	(31)[a]	(40)[b]	300	16950	25425	46700

[a]Cd value is below the detection limit (<BDL) and Pb presents a majority of non-detected values in monitoring datasets.
[b]estimated BACs from reliable limits of detection (BAC=1.5 x LOD) using analytical data and certified reference material information (DORM-2) (see also text). However, liver tissue matrix should be recommended in fish for Cd and Pb as within OSPAR Convention.
*EC/EU 1881/2006 and 629/2008 Directives for maximum levels for certain contaminants in foodstuffs
** Long et al. 1995 (idem OSPAR adopted values)

Table of the proposed assessment criteria for polycyclic aromatic hydrocarbons (PAHs)

Table A.2.1. Mediterranean Sea Background Concentrations (BCs), Med BACs and EACs; Calculation =>BC = 50th (median); BAC=2.5 x BC (mussel); no data for sediment available

PAH compound	Mussel (MG) μg kg^{-1} d.w.			Sediment μg kg^{-1} d.w.		
	Med BC	Med BAC	[a]OSPAR EAC	[a]OSPAR BC	[a]OSPAR BAC	[c]ERL
F	1.0	2.5	-	-	-	-
P	7.1	17.8	1700	4.0	7.3	240
A	0.5	1.2	290	1.0	1.8	85
FL	3.0	7.4	110	7.5	14.4	600
PY	2.0	5.0	100	6.0	11.3	665
BaA	0.8	1.9	80	3.5	7.1	261
C	1.0	2.4	-	4.0	8.0	384
BkF	0.6	1.4	260	-	-	-
BaP	0.5	1.2	600	4.0	8.2	430
GHI	0.9	2.3	110	3.5	6.9	85
DA	0.5	1.3	-	-	-	-
ID	1.2	2.9	-	4.0	8.3	240

[*]Naphthalene, Acenaphtylene, Acenaphthene, Benz(e)pyrene and Benzo(b)fluoranthene are below detection limits (BDLs) or have limited monitoring datasets, and therefore their BACs are preliminary estimations.
[a]OSPAR Commission, CEMP: 2008/2009 Assessment of trends and concentrations of selected hazardous substances in sediments and biota (OSPAR PAHs sediment datasets from Spain, not TOC corrected;
[c]ERL: Effect Range Low

Table of the proposed assessment criteria for organochlorinated compounds (OCs)

(*Summary of OSPAR values to be used in the Mediterranean Sea*) Table A.3.1. OSPAR Region (Background Concentrations (BCs), BACsandEACs)[1]

OCs compound	Mussel $\mu g\ kg^{-1}$ d.w.			Fish $\mu g\ kg^{-1}$ w.w.			[d]Sediment $\mu g\ kg^{-1}$ d.w.		
	BC/LC[c]	BAC	EAC	BC/LC[c]	BAC	EAC (lipid w.)	BC/LC[c]	BAC	EAC/ERL
CB28[a]	0.25	0.75	3.2	0.05	0.10	64	0.05	0.22	1.7
CB52[a]	0.25	0.75	5.4	0.05	0.08	108	0.05	0.12	2.7
CB101[a]	0.25	0.70	6.0	0.05	0.08	120	0.05	0.14	3.0
CB105[a]	0.25	0.75	-	0.05	0.08	-	0.05	-	-
CB118[a]	0.25	0.60	1.2	0.05	0.10	24	0.05	0.17	0.6
CB138[a]	0.25	0.60	15.8	0.05	0.09	316	0.05	0.15	7.9
CB153[a]	0.25	0.60	80	0.05	0.10	1600	0.05	0.19	40
CB156[a]	0.25	0.60	-	0.05	0.08	-	0.05	-	-
CB180[a]	0.25	0.60	24	0.05	0.11	480	0.05	0.10	12
Σ7CBs ICES[b]	-	-	-	-	-	-	0.20	0.46	11.5*
Lindane[a]	0.25	0.97	1.45		-	11**	0.05	0.13[+]	3.0*
α-HCH[a]	0.25	0.64	-	-	-	-	-	-	-
pp'DDE[a]	0.25	0.63	5-50***	0.05	0.10	-	0.05	0.09[+]	2.2*
HCB[a]	0.25	0.63	-	0.05	0.09	-	0.05	0.16[+]	20.0*
Dieldrin[a]	-	-	5-50***	-	-	-	0.05	0.19[+]	2.0*

[1]OSPAR Commission, 2013.
[a]OSPAR Commission, CEMP: 2008/2009 Assessment of trends and concentrations of selected hazardous substances in sediments and biota, Monitoring and Assessment Series
[b]OSPAR Commission, Background document on CEMP assessment criteria for the QSR 2010, Monitoring and Assessment Series
[c]LC: Low concentrations calculated from QUASIMEME; However, BC values should be considered as zero for OCs
[d]Total organic carbon (TOC) corrected values; [+]LC from Spain (OSPAR, 2013)

*ERLs values instead EACs: Effect Range Low (Long et al. 1995); ERL for ICES Σ7CB is total CB concentration/2
**EAC for fish liver derived by applying a conversion factor of 10 on EAC for whole fish (CEMP 2008/2009)
***Ecotoxicological assessment criteria (earlier data from the QSR2000 Report-Chapter 4)

Annex XVI:

References

Annex XVI: References

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