Marine Environmental Quality Committee

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REPORT OF THE

ICES/HELCOM STEERING GROUP ON QUALITY ASSURANCE OF CHEMICAL MEASUREMENTS IN THE BALTIC SEA

ICES Headquarters
17–20 February 1997

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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1 OPENING OF THE MEETING

The meeting of the ICES/HELCOM Steering Group on Quality Assurance of Chemical Measurements in the Baltic Sea (SGQAC) was held from 17–20 February 1997 at ICES Headquarters in Copenhagen, Denmark.

The meeting was opened by the Chairman of the Steering Group, Mr Mikael Krysell, who expressed his gratitude to ICES for all arrangements involved in hosting the meeting.

The meeting was attended by representatives of Denmark, Estonia, Finland, Germany, Latvia, Lithuania, Poland and Sweden. Ms Janet Pawlak and Mr Jan Larsen represented ICES at the meeting. A list of meeting participants is attached as Annex 1 to this report.

2 TERMS OF REFERENCE

Concerning the tasks of the meeting, the Chairman referred to ICES C.Res. 1996/3:7, which is appended as Annex 2.

3 ADOPTION OF THE AGENDA AND APPOINTMENT OF A RAPPORTEUR

The provisional agenda was adopted and is attached as Annex 3 to this report. Ms Elzbieta Lysiak-Pastuszak was appointed as rapporteur for the meeting.

4 GUIDELINES ON QUALITY ASSURANCE OF CHEMICAL MEASUREMENTS IN THE BALTIC SEA

The Steering Group reviewed version 1 of the *Guidelines* as outlined at last year's meeting and as presented to the MEQC. Some minor corrections were made to the text, but the general structure of the document was accepted. The major change to the document concerns the amendments to Annex G (*Technical notes on the determination of contaminants in fish*). The new version takes into account the guidelines on quality assurance of the Oslo and Paris Commissions (OSPAR); these documents are now well harmonized. The discussion on Annex G and the new Annex H (*Technical notes on the determination of metals in sea water*) was conducted under Agenda Item 7.

The missing chapter on analytical problems in anoxic water (new Annex I) was discussed under Agenda Item 8.

It is anticipated that the new version of *Guidelines on quality assurance of chemical measurements in the Baltic Sea* (attached as Appendix 1 of this report) will be incorporated into the revised *Guidelines for marine monitoring in the COMBINE programme of HELCOM.* SGQAC suggested that the QA guidelines should be updated at least on the same basis as the general HELCOM COMBINE guidelines.

Because the document is not official yet, we have seen little response to it from the scientific community. ICES offered to help with the broader distribution of the final version of the QA guidelines as an official ICES document, after review by the Advisory Committee on the Marine Environment (ACME).

5 DISCUSSION ON THE INCLUSION OF OTHER COMPOUND GROUPS INTO THE GUIDELINES

A letter from Marianne Cleemann (Denmark) concerning method choice for petroleum hydrocarbon determination was circulated (Annex 4), together with a translation of a conference abstract by Arne Lund Kvernheim (Norway) regarding possible alternative methods that are chlorofluorocarbon-free. As regards petroleum hydrocarbons and PAHs, it was felt that more information is needed on the methods applied in this field. It was proposed to postpone this task for the next meeting of SGQAC after consulting QUASIMEME and the ICES Marine Chemistry Working Group.

SGQAC discussed the problem of including measurements of other parameters and compound groups in the guidelines. It was concluded that the present content of the *Guidelines* is satisfactory, though Contracting Parties are encouraged to continue analyses on such compounds as dioxins, organotins, and organohalogens (other than Cl) to provide relevant information in future.

Considering the lack of guidelines for the analysis of organohalogens (PCBs, DDT and its derivatives, HCH, HCB, etc.) in water, it was proposed that Ms Grazyna Sapota (Inst. of Meteorology and Water Management, Maritime Branch, Gdynia, Poland) be approached to prepare draft guidelines to be considered at the next meeting of SGQAC.

Regarding such variables as salinity, temperature, pH and oxygen, the Steering Group expressed the opinion that the state-of-the-art of quality assurance in measuring these parameters is acceptable and there is no need to develop special QA guidelines.

6 JOINT SESSION WITH THE SGQAB

For the joint session with the ICES/HELCOM Steering Group on Quality Assurance of Biological Measurements in the Baltic Sea (SGQAB), a paper containing suggested discussion points had been drafted by the Chairmen of the two Steering Groups (Annex 5).

The session was also attended by members of the ICES/OSPAR Steering Group on Quality Assurance of

Biological Measurements Related to Eutrophication Effects (SGQAE).

In the course of discussion, the main border parameters were recognized, e.g., chlorophyll *a*, primary production, and suspended particulate matter (SPM). SGQAB requested that the QA guidelines for biological determinands should be reviewed by the experts from SGQAC.

It was recognized that the implementation of the HELCOM COMBINE would bring in the measurement of additional border or supporting parameters (cofactors) necessary for the interpretation of results. It was suggested that the HELCOM Environment Committee (EC) Working Group on Monitoring and Assessment (EC MON) should be given the responsibility of indicating the priority parameters to be dealt with by the QA Steering Groups.

The first step in reviewing the analytical performance in the HELCOM Baltic Monitoring Programme (BMP) was established by the recommendation from the HELCOM EC that national annual data (hydrochemical) reports should be supplemented by additional statistical information containing some QA aspects (participation in intercomparison exercises).

The ICES representative reported that QA review is already well established for data on chemical contaminants. ICES is working on a new biological data reporting format for OSPAR with integral QA information; it is anticipated that HELCOM will join in this project so the reporting format will serve both programmes.

It was concluded that a QA review of the analytical performance of Baltic laboratories should start well before the assessment work commences.

Addressing the issue of fund raising for training courses, workshops, and 'hands-on' advice for individual laboratories on QA matters, the growing demand for such activities was stressed. At the same time, the overwhelming difficulties in obtaining finances for all QA activities were indicated, while QA should in fact be an integral part of the monitoring programme and therefore should be included in the overall monitoring costs. It was suggested that, in association with the approval of the new COMBINE programme (expected in autumn 1998), the approval of QA activities should take the form of a recommendation from HELCOM indicating that an increase of approximately 25 % in monitoring costs will be required for implementation of QA procedures.

In the discussion on sampling procedures, Mr M. Krysell gave a short presentation of the EU-funded QUASH project (Quality Assurance of Sampling and sample Handling in marine environmental measurements).

The session was closed by the Chairman of SGQAB who thanked all the participants for the informative and fruitful discussion.

7 TECHNICAL NOTES ON THE DETERMINATION OF CONTAMINANTS IN BIOTA AND SEA WATER

Draft papers on these topics had been prepared by Mr Uwe Harms and Ms Christa Pohl.

The papers were thoroughly reviewed and corrections were incorporated into the documents. The revised versions are now Annexes G and H, respectively, in the *Guidelines* in Appendix 1.

SGQAC noted that the general guidelines for the COMBINE programme contain the strategy for biota sampling (Table D-1 in "Outcome of the second workshop on the revision of the Baltic Monitoring Programme", BMP WS 2/96, 14/1), but the strategy for water sampling for analyses of trace contaminants has not been included.

8 PROBLEMS RELATED TO CHEMICAL ANALYSIS OF ANOXIC WATERS

A paper prepared by the Chairman was distributed and discussed. SGQAC agreed to include the revised paper as Annex I in the *Guidelines on quality assurance of chemical measurements.*

9 LEVEL OF PERFORMANCE REQUIRED FOR THE FUTURE BMP IN TERMS OF PRECISION AND BIAS FOR EACH DETERMINAND/MATRIX COMBINATION

For this agenda point a draft paper had been prepared by the Chairman as a background for the discussion on how to proceed with this delicate matter. The discussion revealed a need for detailed information from the Baltic laboratories on the state-of-the-art of analytical performance. The information should be collected intersessionally for review at the next meeting. The actual analytical performance should be related to sampling frequency and the geographical location of sampling stations and EC MON should specify their needs in this respect.

Mr Jan Larsen (ICES) gave a short introduction to the ICES Environmental Data Bank and the quality assurance information included in the data reporting formats.

A short discussion followed on a new field in quality assurance matters—uncertainty of measurements.

10 FUTURE WORK PROGRAMME AND RECOMMENDATIONS

SGQAC considered its future work programme and concluded that it should:

- prepare technical notes on quality assurance of organochlorine compound determinations in sea water (G. Sapota to be approached);
- collect information on PAH analysis in sea water and biota for subsequent preparation of technical notes (Chairman);
- prepare technical notes on mercury determination in sea water and biological tissue for monitoring purposes (C. Pohl and U. Harms);
- collect information on state-of-the-art levels of performance characteristics of the Baltic laboratories (Chairman);
- disseminate information on QA procedures for measurement of cofactors from sources like the QUASIMEME project for future incorporation into the QA *Guidelines* (Chairman);
- prepare a paper on 'Measurement uncertainty' for possible future inclusion in the QA *Guidelines* (U. Harms).

Recommendations from SGQAC are:

a) to organize a HELCOM-sponsored workshop in 1998 on 'In-house QA procedures and internal QA audits' for all laboratories reporting data to the HELCOM BMP;

b) to encourage EC MON to recognize that QA activities form an integral part of any monitoring programme and have to be accounted for in the overall costs;

c) to hold joint sessions with the SGQAB when issues of common concern arise and to cooperate intersessionally;

d) that EC MON should specify their needs with respect to new QA aspects in terms of the analytical performance required in the future BMP;

e) that the *Guidelines on quality assurance of chemical measurements in the Baltic Sea* should be updated periodically by SGQAC.

11 DATE AND VENUE OF THE NEXT MEETING

Ms Christa Pohl of the Baltic Sea Research Institute in Warnemünde (IOW), Germany, kindly offered that the next meeting can be hosted by the IOW. The Steering Group proposed that the meeting should be held from 16–19 February 1998. The full recommendation for this meeting is contained in Annex 6.

12 ANY OTHER BUSINESS

The membership list of SGQAC was updated.

The Steering Group has, up to now, been represented by its former Chairman, Uwe Harms, at the meetings of the QUASIMEME Advisory Board. The Steering Group recommended that the new Chairman (M. Krysell) represent SGQAC at future meetings of the QUASIMEME Advisory Board.

13 REPORT OF THE MEETING

The draft report was presented for consideration. After incorporation of amendments and corrections, it was approved by the Steering Group at the end of the meeting.

14 CLOSING OF THE MEETING

The Chairman thanked ICES for hosting the meeting and the participants for their valuable contributions. The meeting was closed at 12.00 hours on 20 February 1997.

ANNEX 1

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ANNEX 1 (continued)

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ANNEX 2

TERMS OF REFERENCE

ICES C.Res. 1996/3:7

The ICES/HELCOM Steering Group on Quality Assurance of Chemical Measurements in the Baltic Sea [SGQAC] (Chairman: Dr M. Krysell, Sweden) will meet at ICES Headquarters from 17-20 February 1997 to:

a) finalize the *Guidelines on Quality assurance of Chemical Measurements in the Baltic Sea* [HELCOM 1996/3];

b) begin review of the level of performance required for the future Baltic Marine monitoring Programme in terms of precision and bias for each determinand-matrix combination;

c) hold a joint session with the SGQAB to discuss issues of common concern.

The Steering Group will report to ACME before 1 June 1997 meeting and to the MEQC at the 1997 Annual Science Conference

ANNEX 3

AGENDA

1) Opening of the Meeting.
2) Adoption of the Agenda.
3) Appointment of a Rapporteur.
4) Current status of the *Guidelines on Quality assurance of Chemical Measurements in the Baltic Sea.*
5) Discussion on the inclusion of other compound groups into the guidelines.
6) Joint session with SGQAB.
7) Discussion and review of draft technical notes on the determination of contaminants in biota and sea water.
8) Discussion and review of a draft paper on problems related to chemical analysis of anoxic waters.
9) Discuss a draft paper on the level of performance required for the future Baltic Monitoring Programme in terms of precision and bias for each determinand-matrix combination.
10) Future work programme.
11) Date and venue of the next meeting.
12) Any other business.
13) Report of the meeting.
14) Closing of the meeting.

ANNEX 3 (continued)

ANNOTATIONS TO THE AGENDA

1) Opening of the meeting.
The meeting will start on Monday, 17 February at 10:00 hours.

2) Adoption of the agenda.
The steering group will be invited to adopt the agenda as provisionally outlined.

3) Appointment of a rapporteur.
The steering group will appoint its own rapporteur for drafting the report of the meeting.

4) Current status of the *Guidelines on Quality Assurance of Chemical Measurements in the Baltic Sea.*
How has the paper been received? Can we already now identify what is lacking and what can be improved? How is future updating organized?

5) Discussion on the inclusion of other compound groups into the guidelines.
This point is a result of a letter from Marianne Cleemann where the issue of method choice for petroleum hydrocarbon determination was raised (see enclosed copy).

6) Joint session with the SGQAB.
A joint session to discuss issues of common concern, expected to last for a maximum of one day, will be held. Discussion topics will be drafted by the two chairmen.

7) Discussion and review of draft technical notes on the determination of contaminants in biota and sea water.
Drafts made available by the German delegation will be discussed and finalized.

8) Discussion and review of a draft paper on problems related to chemical analysis of anoxic waters.
A draft drawn by the chairman will be circulated, discussed and finalized.

9) Discuss a draft paper on the level of performance required for the future Baltic Monitoring Programme in terms of precision and bias for each determinand-matrix combination.
A discussion paper on this topic will be drafted by the chairman and discussed at the meeting. This paper is not expected to be finalized at the meeting.

10) Future work programme.
Suggestions for the future work programme, partly as an outcome of the discussions held under agenda points 4 and 5, will be considered by the Steering Group.

11) Date and venue of the next meeting.
The Contracting Parties to the HELSINKI CONVENTION are encouraged to consider their possibilities to host the next meeting of the steering group and to make proposals. The steering group is further invited to propose a date for its next meeting.

12) Any other business.
Participants at the meeting may wish to discuss other matters related to the objectives of the Steering Group.

13) Report of the meeting.
A draft report will be presented for consideration and approval by the group at the end of the meeting.

14) Closing of the meeting.
The meeting is expected to terminate on Thursday, 20 February at 17:00 hours.

ANNEX 4

METHOD CHOICE FOR PETROLEUM HYDROCARBON DETERMINATION

MILJØ & ENERGI
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J.nr.133/1-0004
Ref. MC

22. October 1996

Petroleum hydrocarbons

Dear Uwe Harms,

I have been asked by a private Danish laboratory whether the analytical method recommended by HELCOM for analysing petroleum hydrocarbons in the future will still be the UV-spectrofluorometric method mentioned in the Guidelines for the Baltic Monitoring Programme for the third Stage, part B: Physical and Chemical Determinands in Sea Water, 1988.

In the Guidelines from 1988 it is recommended that improved techniques for determination of petroleum hydrocarbons should be developed. Thus, I wonder whether the ICES/HELCOM Steering Group on Quality Assurance of Chemical Measurements in the Baltic Sea will take care of this analytical method as well as the methods for nutrients, heavy metals and organics? If this is the case I would be grateful to get some informations on the plans. Otherwise I would be glad if you are able to tell me to whom I should adress the question of the recommended method for petroleum hydrocarbons.

Are there any plans of performing analyses of PAHs instead of petroleum hydrocarbons, and are biota samples as well as water samples considered?

Thank you in beforehand.

Best regards

M. Cleemann
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ANNEX 5

JOINT SESSION ICES/HELCOM SGQAC/SGQAB

Suggested discussion points:

1) Joint efforts when it comes to parameters where both chemical and biological expertise is of utmost importance (chlorophyll *a*, primary production, etc.).

   Intersessional cooperation between the two groups might be very fruitful. Are any parameters 'lost in space' between the two groups?

2) Recommended sampling procedures where both biological and chemical samples are drawn from the same sampler.

   It is important to identify possible cases where QA procedures and advice given by the two groups contradict each other or confuse the laboratories.

3) The road to reviewing the need for analytical performance in the future BMP.

   We should identify the goal and the general lay-out of such a review. Would cooperation between the groups give any obvious advantages?

4) How will training courses, workshops, etc., on the subject of QA be funded in the future?

   In the present situation the Steering Groups find themselves between ICES and HELCOM (and perhaps even OSPAR). Who is responsible for what? Who should we approach to clarify this matter?

5) Training of and 'hands-on' advice to individual laboratories. Who will perform it, who will pay?

   Some laboratories have very limited resources when it comes to staff, time, budget, etc. There may be a need for more individual support for some units. The benefit would be more reliable BMP data. Do we have any ideas?

ANNEX 6

RECOMMENDATIONS

SGQAC recommends that:

the ICES/HELCOM Steering Group on Quality Assurance of Chemical Measurements in the Baltic Sea (Chairman: Dr M. Krysell, Sweden) should meet 16–19 February 1998 in Warnemünde, Germany to:

a) prepare technical notes on the determination of mercury in biological tissue and sea water for monitoring purposes;

b) prepare technical notes on quality assurance aspects in the determination of organochlorine compounds in sea water;

c) prepare technical notes on quality assurance aspects in the determination of PAH compounds in sea water and biota;

d) prepare technical notes on quality assurance procedures for cofactors;

e) collate and evaluate information on state-of-the-art levels of performance characteristics of Baltic laboratories;

f) prepare a paper on measurement uncertainty, for possible inclusion in the *QA Guidelines*;

g) review additional needs in respect to QA aspects of analytical performance required for the future BMP and agree on activities to respond to these needs.

Justifications:

a) Mercury is included in the new Baltic Monitoring Programme (BMP) and must be dealt with separately from the other heavy metals due to the special problems associated with mercury analyses;

b) organochlorine compounds in sea water are included in the new BMP but the current guidelines do not contain QA information concerning their determination;

c) PAHs are included in the new BMP so QA procedures must be prepared concerning their determination;

d) cofactors are often neglected, but are a very important problem, where up-to-date information on procedures and current uncertainty must be compiled from external sources;

e) information on the performance of Baltic laboratories is needed when the group evaluates the required analytical performance regarding the future BMP (request from HELCOM EC MON);

f) there is a need for some kind of unification around the Baltic Sea (within the BMP) when it comes to expressing and calculating analytical performance. Such unified information is very important for future evaluation of the programme and the data;

g) HELCOM (EC MON) has requested a review of QA aspects of analytical performance.

APPENDIX 1

Working Document of the
ICES/HELCOM Steering Group on Quality Assurance of Chemical Measurements in the Baltic Sea

GUIDELINES ON QUALITY ASSURANCE OF CHEMICAL MEASUREMENTS IN THE BALTIC SEA

FOREWORD

This document provides an introduction to quality issues, in general, and quality assurance in Baltic marine monitoring laboratories, in particular. The guidelines are intended to assist laboratories in starting up and operating their quality assurance systems. For laboratories with existing quality systems, the guidelines may give inspiration for issues that can be improved. The guidelines contain information for all levels of staff in the marine laboratory.

Sections 1, 2, 3 and 6 together with Annexes A (Quality manual) and D (Quality audit) give guidance on organizational technical quality assurance principles that are relevant to *administrative managers*.

Sections 1, 2, 5 and 6 with Annexes A (Quality manual), B (Reference materials), and D (Quality audit), regarding the implementation and operation of a quality system, are the main sections of relevance for *quality managers*.

For *technical managers* all sections in the main part of the document are relevant. The guidelines provide technical managers with a description of the principles concerning how to introduce and maintain the technical aspects of quality assurance.

It is belived that *analysts* will find all of the guidelines and annexes relevant regarding optimization of their analytical work. The applicability of Annexes E (Sampling), F (Technical notes on nutrients) and G (Technical notes on contaminants) will, however, depend on the specific job description of each analyst.

It is the intention of the guidelines that other members of the *laboratory staff* can find use for specific parts of the guidelines, e.g., Annex E (Sampling) which contains principles in relation to sampling procedures and documentation.

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1 INTRODUCTION

1.1 Need for Quality Assurance of Chemical Analytical Procedures in Marine Monitoring

It has been seen that, although there has been considerable improvement in analytical chemistry over the past two decades, there was a large number of European laboratories which still had difficulties in providing reliable data in routine work (Topping, 1992). Topping based his conclusion on the results of a series of external quality assessments of analysis (generally referred to as intercomparison exercises), organized over the last twenty years by the International Council for the Exploration of the Sea (ICES), and which have shown that there are large interlaboratory differences in the measurements of contaminants in marine samples.

As a consequence of inproperly applied measures to assure the quality of analytical data, information about variations of contaminant levels both in space and time is often uncertain or misleading, and the effects of political measures to improve the quality of the marine environment cannot be adequately assessed. Therefore, the acquisition of relevant and reliable data is an essential component of any research and monitoring programme associated with marine environmental protection. To obtain such data, the whole analytical process must proceed under a well-established Quality Assurance (QA) programme. Consequently, the HELCOM Environment Committee (EC) at its fifth meeting (HELCOM, 1994a) recommended that: 'all institutes reporting data to BMP/CMP shall introduce in-house quality assurance procedures'.

In addition, the following principles of a quality assurance policy were formulated:

QUALITY ASSURANCE POLICY OF THE HELSINKI COMMISSION (HELCOM, 1994b)

1. Contracting Parties acknowledge that only reliable information can provide the basis for effective and economic environmental policy and management regarding the Convention area;

2. Contracting Parties acknowledge that environmental information is the product of a chain of activities, constituting programme design, execution, evaluation and reporting, and that each activity has to meet certain quality requirements;

3. Contracting Parties agree that quality assurance requirements be set for each of these activities;

4. Contracting Parties agree to make sure that suitable resources are available nationally (e.g., ships, laboratories) in order to achieve this goal;

5. Contracting Parties fully commit themselves to following the guidelines, protocols, etc., adopted by the Commission and its Committees in accordance with this procedure of quality assurance.

1.2 Objective

The objective of the guidelines outlined here is to support laboratories working in marine monitoring to produce analytical data of the required quality. The guidelines may also help to establish or improve quality assurance management in the laboratories concerned. The technical part of the guidelines provides advice on more practical matters. The guidelines will not focus on sampling in detail, since this will be dealt with at a later stage.

1.3 Topics of Quality Assurance

In practice, **Quality Assurance** applies to all aspects of analytical investigation, and includes the following principal elements:

- A knowledge of the purpose of the investigation is essential to establish the required data quality.

- Provision and optimization of appropriate laboratory facilities and analytical equipment.

- Selection and training of staff for the analytical task in question.

- Establishment of definitive directions for appropriate collection, preservation, storage and transport procedures to maintain the integrity of samples prior to analysis.

- Use of suitable pre-treatment procedures prior to analysis of samples, to prevent uncontrolled contamination and loss of the determinand in the samples.

- Validation of appropriate analytical methods to ensure that measurements are of the required quality to meet the needs of the investigations.

- Conduct of regular intralaboratory checks on the accuracy of routine measurements, by the analysis of appropriate reference materials, to assess whether the analytical methods are remaining under control, and the documentation and interpretation of the results on control charts.

- Participation in interlaboratory quality assessments (proficiency testing schemes) to provide an independent assessment of the laboratory's capability of producing reliable measurements.

- The preparation and use of written instructions, laboratory protocols, laboratory journals, etc., so that specific analytical data can be traced to the relevant samples and *vice versa*.

2 THE QUALITY SYSTEM

2.1 General

'Quality system' is a term used to describe measures which ensure that a laboratory fulfills the requirements for its analytical tasks on a continuing basis. A laboratory should establish and operate a Quality System adequate for the range of activities, i.e., for the type and and extent of investigations, for which it has been employed.

The Quality System must be formalized in a Quality Manual which must be maintained and up-to-date. A suggested outline of a Quality Manual is given in Annex A. Some comments and explanations are given in this section.

The person responsible for authorization and compilation of the Quality Manual must be identified, and an identification of holders of controlled copies should be listed in the manual.

The Quality System must contain a statement of the intentions of the laboratory top management in relation to quality in all aspects of its work (statement on Quality Policy).

General requirements applicable to all types of chemical measurements (all types of objective testing) are laid down in the European Standard 'General Criteria for the Operation of Testing Laboratories' (EN 45001), and in the 'General Requirements for the Technical Competence of Calibration and Testing Laboratories' (ISO Guide 25).

Guidance on the interpretation of EN 45001 and ISO Guide 25 was given by a joint international EURACHEM/WELAC Working Group (EURACHEM/WELAC, 1992). Specific guidance to Analytical Quality Control for Water Analysis was elaborated by a CEN Working Group (CEN/TC 230, 1995). Both publications have been taken into consideration when drafting these guidelines. References, which deal with specific aspects of quality assurance of chemical measurements, are cited in the text.

2.2 Scope

The laboratory's scope should be formulated in terms of:

- the range of products, materials or sample types tested or analysed;

- the types of tests or analyses carried out;

- the specification of method/equipment/technique used;

- the concentration range and accuracy of each test and analysis.

2.3 Organization, Management and Staff

2.3.1 Organization

The Quality System should provide general information on the identity and legal status of the laboratory and should include a statement of the technical role of the laboratory (e.g., employed in marine environmental monitoring).

The following information must be included in an organizational chart:

- Technical Manager, Quality Manager, and any deputies;
- general lines of responsibility within the laboratory (including the relationship between management, technical operations, quality control and support services);
- the lines of responsibility within individual sections of the laboratory;
- the relationship between the laboratory and any parent or sister organizations.

The appropriate chart should show that, for matters related to quality, the Quality Manager has direct access to the highest level of management at which decisions are taken on laboratory policy and resources, and to the Technical Manager.

2.3.2 Management

Job descriptions, qualifications, training and experience are necessary for:

- Technical Manager,
- Quality Manager,
- other key laboratory managerial and technical posts.

Job descriptions should include:

- title of job and brief summary of function,
- person or functions to whom jobholder reports,
- person or functions that report to jobholder,
- key tasks that jobholder performs in the laboratory,
- limits of authority and responsibility.

The Technical Manager. The Quality System should include a statement that the post-holder has overall responsibility for the technical operation of the laboratory and for ensuring that the Quality System requirements are met.

The Quality Manager. The Quality System should include a statement that the post-holder has responsibility for ensuring that the requirements for the Quality System are met continuously and that the post-holder has direct access to the highest level of management at which decisions are taken on laboratory policy or resources, and to the Technical Manager.

The Quality System should state explicitly the Quality Manager's duties in relation to control and maintenance of documentation, including the Quality Manual, and of specific procedures for the control, distribution, amendment, updating, retrieval, review and approval of all documentation relating to the calibration and testing work of the laboratory.

2.3.3 Staff

The laboratory management should define the minimum levels of qualification and experience necessary for engagement of staff and their assignment to respective duties.

Members of staff authorized to use equipment or perform specific calibrations and tests should be identified.

The laboratory should ensure that all staff receive training adequate to the competent performance of the tests/methods and operation of equipment. A record should be maintained which provides evidence that individual members of staff have been adequately trained and their competence to carry out specific tests/methods or techniques has been assessed. Laboratory managers should be aware that a change of staff might jeopardize the continuation of quality.

2.4 Documentation

Necessary documentation in connection with analysis includes:

- a clear description of the analytical methods;
- a strict keeping of laboratory journals;
- instrument journals;
- laboratory protocols for sample identification;
- clear labelling of samples, reference materials, chemicals, reagents, volumetric equipment, stating date, calibration status, concentration or content as appropriate and signature of the person responsible.

2.5 Laboratory Testing Environment

Samples, reagents and standards should be stored and labelled so as to ensure their integrity. The laboratory should guard against deterioration, contamination and loss of identity.

The laboratory should provide appropriate environmental conditions and special areas for particular investigations.

Staff should be aware of:

- the intended use of particular areas,
- the restrictions imposed on working within such areas,
- the reasons for imposing such restrictions.

2.6 Equipment

As part of its quality system, a laboratory is required to operate a programme for the necessary maintenance and calibration of equipment used in the laboratory to ensure against bias of results.

General service equipment (e.g., sample containers, hot plates, stirrers, non-volumetric glassware) should be maintained by appropriate cleaning and operational checks where necessary. Calibrations will be necessary where the equipment can significantly affect the analytical result.

The correct use of volumetric equipment, analytical balances, thermometers, barometers, etc., is critical to analytical measurements and this equipment must be maintained, calibrated and used in a manner consistent with the accuracy required of data. In certain situations, analysts should consider that measurements can often be made by mass rather than by volume.

Particularly for trace analyses, contamination through desorption of impurities from, or uncontrolled determinand losses through sorption on, surfaces of volumetric flasks can be significant. Therefore, special attention should be paid to the selection of appropriate types of material (quartz, PTFE, etc.) used for volumetric equipment and its proper cleaning and conditioning prior to analysis.

Periodic performance checks should be carried out at specific intervals on measuring instruments (e.g., for response, stability and linearity of sources, sensors and detectors, the separating efficiency of chromatographic systems).

The frequency of such performance checks will be determined by experience and based on the need, type and previous performance of the equipment. Intervals between checks should be shorter than the time the equipment has been found to take to drift outside acceptable limits and should be given in the equipment list.

2.7 Quality Audit

Quality audits are carried out in order to ensure that the laboratory's policies and procedures, as formulated in the Quality Manual, are being followed.
The quality audit is the periodic check that a laboratory makes on its own Quality System to guarantee that it is effective, implemented, and adhered to. It is recommended to use an external assessor on a regular basis. Arrangements for implementing an audit may be based upon a check list developed by the EURACHEM/WELAC Working Group (EURACHEM/WELAC, 1992), which is attached as Annex D to these Guidelines.

3 SPECIFYING ANALYTICAL REQUIREMENTS

3.1 General

The objective of analytical investigations is to obtain chemical information about materials or systems concerning their specific qualitative and quantitative composition and structure (Danzer, 1992).

Before the analyst starts an analytical investigation, the intended use of the data must be explicitly stated. That is, the minimum quality requirement the data must meet to make it useful for a given purpose should be established for every measurement situation. Careful specification of analytical requirements and critical consideration of data quality objectives are vital when designing analytical programmes.

Environmental analytical measurements are developed for a variety of purposes, such as the determination of the fate of a component in the context of biogeochemical studies, or the determination of the environmental concentration of a component for use in environmental risk assessment.

The broad range of applications of analytical data requires different analytical strategies, and the accuracy of the data obtained must be adequate for each use. A failure to pay proper attention to this topic can endanger the validity of an analytical programme, since the analytical results obtained may be inadequately accurate and lead to false conclusions.

Based on these considerations, the following parameters should be discussed and evaluated before an investigation is carried out:

- the determinand of interest,
- the type and nature of the sample,
- the concentration range of interest,
- the permissible tolerances in analytical error.

3.2 Determinand of Interest

Frequently, a single method may be used for analysis of a determinand in a wide variety of matrices. However, one has to recognize that many determinands exist in different matrices in a variety of chemico-physical forms (species), and most analytical methods provide a different response to the various forms. Therefore, particular care must be exercised that the determinand of interest is clearly defined and the experimental conditions selected allow its unambiguous measurement.

3.3 Type and Nature of the Sample and its Environment

A precise description of the type and nature of the sample is essential before the analytical method can be selected. Suitable measures and precautions can only be taken during sampling, sample storage, sample pretreatment and analysis, if sufficient knowledge about the basic properties of the sample is available. There may be other, non-analytical factors to consider, including the nature of the area under investigation.

3.4 Concentration Range of Interest

It is important that samples of a definite type and nature have been characterized by the concentration range of the determinand. If such information is not given, needless analytical effort may be expended or, vice versa, insufficient effort may jeopardize the validity of the analytical information gained.

3.5 Permissible Tolerances in Analytical Error

Taylor (1981) pointed out that 'the tolerance limits for the property to be measured are the first condition to be determined. These are based upon considered judgement of the end user of the data and present the best estimate of the limits within which the measured property must be known, to be useful for its intended purpose'...'Once one has determined the tolerance limits for the measured property, the permissible tolerances in measurement error may be established'.

In the whole analytical chain, there are systematic errors (biases) and random errors, as indicated by the standard deviation. The bounds representing the sum of both must be less than the tolerance limits defined for the property to be measured, if the analytical data are to be useful.

4 VALIDATION OF ANALYTICAL METHODS

4.1 General

On the basis of the specifications developed in the items under Section 3, the method must now be examined to determine whether it actually can produce the degree of specificity and confidence required. Accordingly, the objective of the validation process is to identify the performance of the analytical method and to demonstrate that the analytical system is operating in a state of statistical control.

When analytical measurements are 'in a state of statistical control', it means that all causes of errors remain the same and have been characterized statistically.

4.2 Validation

Validation of an analytical method is the procedure that 'establishes, by laboratory studies, that the performance characteristics of the method meet the specifications related to the intended use of the analytical results' (Wilson, 1970; EURACHEM/WELAC, 1992).

Performance characteristics include:

- selectivity,
- sensitivity,
- range,
- limit of detection,
- accuracy (precision, bias).

These parameters should be clearly stated in the documented method description so that the suitability of the method for a particular application can be assessed.

In the following, a brief explanation and, where appropriate, guidance on the estimation of these parameters is given.

4.2.1 Selectivity

Selectivity refers to the extent to which a particular component in a material can be determined without interference from the other components in the material. A method which is indisputably selective for a determinand is regarded as specific.

Few analytical methods are completely specific for a particular determinand. This is because both the determinand and other substances contribute to the analytical signal and cannot be differentiated. The effect of this interference on the signal may be positive or negative depending upon the type of interaction between determinand and interfering substances.

The applicability of the method should be investigated using various materials, ranging from pure standards to mixtures with complex matrices.

- Each substance suspected to interfere should be tested separately at a concentration approximately twice the maximum expected in the sample (use Student's t-test to evaluate).

- Knowledge of the physical and chemical mechanisms of interference operative in the particular method will often help to decide for which substances tests should be made.

Interference effects causing restrictions in the applicability of the analytical method should be documented.

4.2.2 Sensitivity

Sensitivity is the difference in determinand concentration corresponding to the smallest difference in the response by the method that can be detected at a certain probability level. It can be calculated from the slope of the calibration curve.

Most analytical methods require the establishment of a calibration curve for the determination of the (unknown) determinand concentration. Such a curve is obtained by plotting the instrumental response, y, versus the determinand concentration, x. The relationship between y and x can be formulated by performing a linear regression analysis on the data. The analytical calibration function can be expressed by the equation $y = a + bx$, where b is the slope or response and a is the intercept on the y-axis.

As long as the calibration curve is within the linear response range of the method, the more points obtained to construct the calibration curve the better defined the b value will be. A factor especially important in defining the slope is that the measurement matrix must physically and chemically be identical both for samples to be analysed and standards used to establish the calibration curve.

4.2.3 Detection limit

The detection limit of an analytical method is the smallest concentration (the smallest amount) that the analyst can expect to detect with a given degree of confidence.

The IUPAC (1978) has recommended that the limit of detection, defined in terms of either concentration (c_L) or amount (q_L), be related to the smallest measure of response (x_L) that can be detected with reasonable certainty in a given analytical method.

According to this definition, the detection limit is given by

$$c_L(\text{or } q_L) = k \cdot s_B/s,$$

where s is the sensitivity of the method.

A value of $k = 3$ is strongly recommended by IUPAC (based on the confidence interval).

This concept is further clarified by Long and Winefordner (1983) and the ACS Committee on Environmental Improvements (ACS, 1983). The Analytical Methods Committee (1987) stressed that estimation of the limit of detection must be based on measurements of a 'field blank' (effectively a hypothetical sample containing zero determinand concentration). This implies that the matrices of the samples and the corresponding field blank are identical so that unique interference effects for individual samples can be excluded.

4.2.4 Range

The range of the method is defined by the smallest and greatest determinand concentrations for which experimental tests have actually achieved the degree of accuracy required.

The concentrations of the calibration standards must bracket the expected concentration of the determinand in the samples.

It is recommended to locate the lower limit of the useful range at $x_B + 10s_B$, where x_B is the measured value for the blank, and s_B is the standard deviation for this measurement.

The range extends from this lower limit to an upper value (upper limit) where the response/determinand concentration relationship is no longer linear.

4.2.5 Accuracy

The term 'accuracy' is used to describe the difference between the expected or true value and the actual value obtained. Generally, accuracy represents the sum of random error and systematic error or bias (Taylor, 1981).

Random errors arise from uncontrolled and unpredictable variations in the conditions of the analytical system during different analyses. Fluctuations in instrumental conditions, variations of the physical and chemical properties of sample or reagent taken on different occasions, and analyst-dependent variations in reading scales are typical sources causing random errors.

The term 'precision' should be used when speaking generally of the degree of agreement among repeated analyses. For numerical definition of this degree of agreement, the parameter standard deviation or relative standard deviation should be used.

Systematic errors or biases originate from the following sources:

a) instability of samples between sample collection and analysis

Effective sample storage, sample stabilization and sample preservation, respectively, are essential to ensure that no losses or changes of the physical and chemical properties of the determinand occur prior to analysis. Effective sample stabilization methods exist for many determinands and matrices, but they must be compatible with the analytical system being employed, and with the particular sample type being analysed.

b) deficiencies in the ability to determine all relevant forms of the determinand

Many determinands exist in different matrices in a variety of physical and/or chemical forms ('species'). The inability of the analytical system to determine some of the forms of interest will give rise to systematic negative deviations from the true value, if those forms are present in the sample.

c) biased calibration

Most instrumental methods require the use of a calibration function to convert the primary analytical signal (response) to the corresponding determinand concentration. Generally, calibration means the establishment of a function by mathematically modelling the relationship between the concentrations of a determinand and the corresponding experimentally measured values.

An essential prerequisite when establishing a calibration function is that the sample and calibration standards have similar matrices and are subject to the same operational steps of the analytical method, and that identical concentrations of the determinand in standards and sample give the same analytical response.

d) incorrect estimation of the blank

It is common practice to correct quantitative analytical results for a constant systematic offset, denoted the 'blank'. A definite answer must be found to what the true blank in an analysis is, in order to make correction for the blank satisfactory.

A good review of several kinds of 'blank' and their use in quantitative chemical analysis was given by Cardone (1986a, 1986b).

Principally, it is important to realize that a 'blank' is the response from a solution containing all constituents of the sample, except the determinand, processed through all procedural steps of the method under study. The analyst must know that the size of the blank and its influence on the analytical result can only be assessed if the sample matrix has been adequately approximated and the whole analytical process has been considered.

4.2.5.1 Estimating random errors

The within-batch standard deviation, s_w, represents the best precision achievable with the given experimental conditions, and is of interest when the analyst is concerned with the smallest concentration difference detectable between two samples.

The between-batch standard deviation, s_b, is a measure of the mutual approximation of analytical results obtained from sequentially performed investigations of the same material in the same laboratory.

The total standard deviation, s_t, is calculated from the formula $\sqrt{} s_w^2 + s_b^2$. It is of interest to analysts concerned with the regular analysis of samples of a particular type in order to detect changes in concentration.

A realistic approach to estimate s_w and s_b is to perform n determinations on a representative group of control samples in each of m consecutive batches of analysis.

The experimental design recommended to estimate s_w, s_b and s_t is to make n replicate analyses per batch in a series of m different batches. The design should be modified according to practical experience gained from the analytical method tested. In particular, when s_w is assumed to be dominant, n=4 to 6 could be chosen. The product $n{\cdot}m$ should not be less than 10 and should preferably be 20 or more.

Analysis of variance (ANOVA) allows identification of the different sources of variation and calculation of the total standard deviation s_t. A general scheme of ANOVA is given in the following paragraphs.

General scheme of Analysis of Variance (after Doerffel, 1989):

Source of variability	Sum of squares	Degrees of freedom	Estimated variances
Between batches	$Qs_b=n\,\Sigma(x_j-x)^2$	$f_b=m-1$	$s_b^2=Qs_b/m-1$
Within batches (analytical error)	$Qs_w=\Sigma\Sigma\,(x_{ij}-x_j)^2$	$f_w=mn-m$	$s_w^2=Qs_w/mn-m$

m = number of batches of analysis;
n = number of replicate analyses per batch;
x_j = mean of each batch;
x = overall mean;
x_{ij} = jth replicate analytical value in ith batch.

Carry out F-test to see if s_b is significantly larger than s_w.

If the testing value $s_b^2/s_w^2 < F(f_b, f_w, 95\ \%)$, one can conclude that s_b is only randomly larger than s_w. In this case $s_t = s_w$.

If the testing value $s_b^2/s_w^2 > F(f_b, f_w, 95\ \%)$, one can conclude that s_b significantly influences the total standard deviation.

Accordingly, the estimate of the total variance of a single determination is $s_t^2 = s_b^2 + s_w^2$.

For routine analysis, it is recommended that s_b does not exceed the value of s_w by more than a factor of two.

A step-wise approach to scrutinize experimental design and to optimize analytical performance may be necessary. This process might be repeated iteratively until target values of s_w, s_b and s_t, respectively, are attained.

4.2.5.2 Estimating systematic errors (biases)

a) Using an independent analytical method

The analyst can test for systematic errors in the analytical procedure under investigation by using a second, independent analytical method (Stoeppler, 1991). A t-test can be carried out to check for differences in the measured values obtained (on condition that the precision of both methods applied is comparable). A significant difference between the results obtained by both procedures indicates that one of them contains a systematic error. Without further information, however, it is not possible to say which one.

b) Using Certified Reference Material (CRM)

An analytical procedure should be capable of producing results for a certified reference material (CRM) that do not differ from the certified value more than can be accounted for by within-laboratory statistical fluctuations.

In practice, when performing tests on CRM, one should ensure that the material to be analysed and the certified reference material selected have a similar macrocomposition (a similar matrix) and approximately similar determinand concentrations.

c) Participation in intercomparison exercises

In an intercomparison exercise, the bias of the participating laboratory's analytical method is estimated with respect to the assigned value X for the concentration of the determinand in the sample which was distributed to participants. The assigned value X is an estimate of the true value and is predetermined by some 'expert' laboratories. In some instances, X is a consensus value established by the coordinator after critical evaluation of the results returned by the participants. The bias is equal to the difference between the determinand concentration x reported by the participant and the determinand concentration X assigned by the coordinator.

If a target standard deviation s representing the maximum allowed variation consistent with valid data can be estimated, the quotient $z = (x - X)/s$ is a valuable tool for appropriate data interpretation. If z exceeds the value of 2, there is only a 5 percent probability that the participating laboratory can produce accurate data (Berman, 1992).

5 ROUTINE QUALITY CONTROL (WITHIN-LABORATORY QUALITY CONTROL)

5.1 General

The objective of a quality assurance programme for chemical measurements is to reduce analytical errors to required limits and to assure that the results have a high probability of being of acceptable quality.

Having developed an analytical system suitable for producing analytical results of the required accuracy, it is of eminent importance to establish a continuous control over the system and to show that all causes of errors remain the same in routine analyses (i.e., that the results are meaningful). In other words, continuous quantitative experimental evidence must be provided in order to demonstrate that the stated performance characteristics of the method chosen remain constant.

According to international standards (ISO 9000, EN 29000 and EN 45000 series), a defined analytical quality must be achieved, maintained, and proven by documentation. The establishment of a system of control charts is a basic principle applied in this context.

5.2 X-charts

In marine chemistry the X-chart is applicable for stable samples, e.g., certified reference materials for trace elements, nutrients and organics, stabilized biota for trace elements, or laboratory preparations of synthetic quality control materials, such as nutrient or trace metal solutions with a stable and preferably known theoretical content.

A simple X-chart is constructed in the following way:

- Select an appropriate laboratory reference material (LRM) which, if possible, has been checked against a relevant certified reference material (CRM). This LRM is to be analysed later on a regular basis with environmental samples. See also Annex B.
- Analyse the LRM at least ten times for the given determinand. The analyses should be done on different days spread over a period of time to ensure that the full range of random errors (for within- and between-batch analyses) are covered. This enables a calculation of the total standard deviation (s_t).
- Calculate the mean value (x), the standard deviation (s_t) and the following values: $x + 2s_t$, $x - 2s_t$, $x + 3s_t$, $x - 3s_t$. Use these data to produce the plot.

If the data for the LRM follow a Normal distribution, 95 % of them should fall within $x \pm 2s_t$ (between the Upper Warning Limit and Lower Warning Limit) and 99.7 % should fall within $x \pm 3s_t$ (between the Upper Control Limit (UCL) and Lower Control Limit (LCL)).

The X-chart as described here is relevant for checking that the chemical data produced are within statistical control for precision, expressed as the total standard deviation. The X-chart data can be used for periodic calculations of this statistical parameter.

5.3 Control Charts for Spiked Sample Recovery

In marine chemistry the control charts for spiked sample recovery are especially useful when the sample matrix can be suspected of causing interferences that have an influence on the analytical response. They are useful in trace metal analysis and in nutrient analysis where the sample matrix can affect the chemical reaction of the signal response.

The control chart for spiked sample recovery can be constructed as follows:

- Use the same spike concentration in all series of the same determinand, concentration range and matrix.
- Select and analyse a natural sample in each analytical series.
- Spike by adding to the sample a known concentration of the analyte to be determined, and re-analyse. If possible, use a CRM concentrate.
- Calculate the measured difference in concentration by subtraction and correction for dilution from spiking.
- Plot the spike concentration in a chart of concentration *versus* time.
- The average recovery (R) and the total standard deviation (s_t) can be calculated on the basis of at least ten analytical series. Calculate the following values: R + 2s_t, R – 2s_t, R + 3s_t, R – 3s_t. Use these data to produce the plot.

With the presumption that the measured recoveries are Normally distributed, the data should be distributed within the same limits as described for the X-charts. The chart is relevant for checking that the chemical data produced are within statistical control for recovery of spikes and for precision, expressed as the total standard deviation. The chart data can be used for periodic calculations of these statistical parameters.

5.4 Cusum Charts

From the Cusum plots, the relative recovery and relative standard deviation may be calculated. The advantage of a Cusum chart is the possibility of combined control of two concentration levels, low and high, in one plot. This is useful when the concentration range of the samples varies from batch to batch, where selection of reference samples with different concentrations is relevant. Typical samples that can be applied are CRMs, natural laboratory reference materials that have been checked against a CRM, or synthetic laboratory reference materials with known theoretical concentrations.

A Cusum chart enables the control of analyses done on a routine basis or sporadically. In this case, the control samples are prepared on the day of analysis. Control samples, containing constant or variable concentrations of a measured compound, are analysed in sequence with environmental samples. The number of control samples depends on the assumed level of quality assurance in the laboratory, e.g., 5 % means one control sample per twenty environmental samples. The 'cusum' (cumulative summation of differences – positive and negative – between the result of the analysis of the reference material/control sample (x_i) and the expected/true value (r_i) value is calculated from the equation:

$$\text{cusum} = \sum_{i=1}^{n} \frac{x_i - r_i}{r_i} \times 100\%$$

where i = number of control samples.

The results of analyses are presented in the form of a table and a chart.

5.5 Blank Control Chart

The blank control chart represents a special application of the X-chart (mean control chart). The following (constant) systematic error sources may be identified by the blank control chart:

- contamination of container for sampling, sample storage and sample pretreatment;
- contamination of reagents, reaction vessels or laboratory equipment used during analysis.

Generally, the simultaneous determination of the blank value would be required for each analysis. Since this requirement can seldom be met due to the considerable effort, it appears reasonable to determine a minimum of two blank values during the series of analyses (at the beginning and at the end of each batch of samples).

5.6 Interpretation of Control Charts

The results of analyses of reference material analysed with each batch of environmental samples indicate whether the errors fall within acceptable limits. The results are satisfactory if they fall within the warning limits, i.e., between $\pm 2s_t$. If one result falls outside the 'warning limits', there is no reason for alarm, providing that the next result falls within the warning limits. The results of control analyses should not fall between the warning and control limits ($+2s_t/+3s_t$ and $-2s_t/-3s_t$) more frequently than once in twenty determinations. If the results fall outside the warning limits too frequently, particularly if the same warning limit has been crossed more than once on consecutive results, then the analyst needs to assess the source of this systematic error. If the results on more than ten successive occasions fall on the same side of the x line (above or below), then the analyst must check the analytical procedure to determine the source of this systematic error. If a result falls outside the UCL or LCL limits, the analyst should stop the analysis, then check the analytical procedure to determine the source of error. The lines $+3s_t$ and $-3s_t$ are regarded as the permissible limits; the results should not cross these limits more often than once in 100 analyses. If any of the limits is crossed, the results of the analysis of this particular batch of environmental samples should be rejected. The analyst should not continue until the source(s) of the errors have been identified and the analysis is again under control. Excepted from this are analyses that cannot be deterred or delayed (e.g., due to lack of an appropriate preservation method for ammonia), where the analyst can choose to perform the analyses with a suboptimal quality. In this situation, data should not be reported unless a valid retrospective correction of data can be performed.

Control charts are ideal for daily routine analyses. When the batches of analyses are done at different times, e.g., on cruises, the analyst should recommence the analysis of environmental samples only when the procedure is still under control, i.e., replicate analyses of LRM must be done before the routine work recommences. Only when the results fall within the acceptable limits on the previously constructed control chart can the routine work be restarted.

It has to be noted that the accuracy of the method can be checked with a CRM or a well-characterized LRM. But if the analyst only uses reference materials without documented accuracy, he/she is controlling only the precision of the measurements.

6 EXTERNAL QUALITY ASSESSMENT

For marine environmental monitoring programmes, it is essential that the data provided by the laboratories involved are comparable. Therefore, participation in an external quality assessment scheme by the laboratories concerned should be considered indispensable.

While the use of a validated analytical method and routine quality control (see above) will ensure accurate results within a laboratory, participation in an external quality assessment or proficiency testing scheme provides an independent and continuous means of detecting and guarding against undiscovered sources of errors and acting as a demonstration that the analytical quality control of the laboratory is effective.

Generally, proficiency testing is useful to obtain information about the comparability of results, and ensures that each of the participating laboratories achieves an acceptable level of analytical accuracy.

Details of the development and operation of proficiency testing schemes are outlined in ISO Guide 43. An overview of the structure and an assessment of the objectives of proficiency testing have been given by the Analytical Methods Committee (1992).

An approach known as the paired sample technique, which has been described by Youden and Steiner (1975), provides a valuable means of summarizing and interpreting in graphical form the results of interlaboratory comparison exercises.

Most proficiency testing schemes are based on the distribution of identical sub-samples (test materials) from a uniform bulk material to the participating laboratories. The test material must be homogeneous and stable for the duration of the testing period. Amounts of the material should be submitted that are sufficient for the respective determinations.

The samples are analysed by the different laboratories independently of one another, each under repeatable conditions. Participants are free to select the validated method of their choice. It is important that the test material is not treated in any way different from the treatment of samples ordinarily analysed in the laboratory. In this way, the performance established by the proficiency testing results will reflect the actual performance of the laboratory.

Analytical results obtained in the respective laboratories are returned to the organizer where the data are collated, analysed statistically, and reports issued to the participants.

7 DEFINITIONS

In the following, a summary of the technical/scientific terms used in this document is given. Sections are mentioned when the terms have been explained in the text. Definitions are provided for terms not explained in the text.

Accuracy. See Section 4.2.5.

Analytical method. The set of written instructions completely defining the procedure to be adopted by the analyst in order to obtain the required analytical result (Wilson, 1970).

An **analytical system** comprises all components involved in producing results from the analysis of samples, i.e., the sampling technique, the 'method', the analyst, the laboratory facilities, the instrumental equipment, the nature (matrix, origin) of the sample, and the calibration procedure used.

HELCOM BMP. Baltic Monitoring Programme.

Blank control chart. See Section 5.5.

Calibration is the set of operations which establish, under specified conditions, the relationship between values indicated by a measuring instrument or measuring system, or values represented by a material measure, and the corresponding known values of a measurand.

HELCOM CMP. Coastal Monitoring Programme.

CRM (Certified Reference Material) is a material one or more of whose property values are certified by a technically valid procedure, accompanied by or traceable to a certificate or other documentation which is issued by a certifying body.

Cusum Charts. See Section 5.4.

Detection limit. See Section 4.2.3.

Errors. See Sections 4.2.5, 4.2.5.1 and 4.2.5.2.

External quality assessment. See Section 6.

LCL. Lower control limit.

LRM. Laboratory Reference Material.

Matrix. The totality of all components of a material including their chemical, physical and biological properties.

Performance characteristics of an analytical method used under given experimental conditions are a set of quantitative and experimentally determined values for parameters of fundamental importance in assessing the suitability of the method for any given purpose (Wilson, 1970).

Proficiency testing is the determination of the laboratory calibration or testing performance by means of interlaboratory comparisons.

Quality. Characteristic features and properties of an analytical method/analytical system in relation to their suitability to fulfill specific requirements.

The term **Quality Assurance** involves two concepts: **Quality control** and **Quality assessment.**

- **Quality control** is 'the mechanism established to control errors', and **quality assessment** is 'the system used to verify that the analytical process is operating within acceptable limits' (ACS Committee, 1983; Taylor, 1981).
- **Quality assessments** of analyses, generally referred to as intercomparison exercises, have been organized over the last twenty years by the International Council for the Exploration of the Sea (ICES).

Quality audits are carried out in order to ensure that the laboratory's policies and procedures, as formulated in the Quality Manual, are being followed.

Quality Manual is a document stating the quality policy and describing the quality system of an organization.

Quality policy forms one element of the corporate policy and is authorized by top management.

Quality system is a term used to describe measures which ensure that a laboratory fulfills the requirements for its analytical tasks on a continuing basis.

Range. See Section 4.2.4.

Selectivity. See Section 4.2.1.

Quality Manager. The Quality System should include a statement that the post-holder has responsibility for ensuring that the requirements for the Quality System are met continuously and that the post-holder has direct access to the highest level of management at which decisions are taken on laboratory policy or resources, and to the Technical Manager.

Technical Manager. The Quality System should include a statement that the post-holder has overall responsibility for the technical operation of the laboratory and for ensuring that the Quality System requirements are met.

Traceability. Results obtained from an analytical investigation can only be accurate if they are traceable. Traceability of a measurement is achieved by an unbroken chain of calibrations connecting the measurement process to the fundamental units. In most instances, when analyses are carried out, the chain is broken because due to the sample pretreatment and preparation the original material is destroyed. In order to approach full traceability, it is necessary to demonstrate that no loss or contamination has occurred during the analytical procedure.

Traceability to national or international standards can be achieved by comparison with certified reference standards or certified reference materials, respectively, the composition of which must simulate to a high degree the sample to be analysed. Consequently, if analytical results for a certified reference material are in agreement with the certified values, it should be realized that owing to discrepancies in composition between certified reference material and sample, there is still a risk that the results on real samples may be wrong.

UCL. Upper control limit.

Validation of an analytical method is the procedure that 'establishes, by laboratory studies, that the performance characteristics of the method meet the specifications related to the intended use of the analytical results' (EURACHEM/WELAC, 1992).

X-charts. See Section 5.2.

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ANNEX A

PRINCIPAL COMPONENTS OF A QUALITY MANUAL

The quality system should be formalized in a quality manual which must be maintained and kept up-to-date.

The person responsible for authorization and compilation of the quality manual should be identified. A distribution list of the quality manual and identification of holders of controlled copies of the quality manual should be included.

The quality manual should contain, for example, the following items or their equivalent:

1) Scope.
2) References.
3) Definitions.
4) Statement of quality policy.
5) Organization and management.
6) Quality system audit and review.
7) Personnel.
8) Accommodation and environment.
9) Equipment and reference material.
10) Measurement, traceability and calibration.
11) Calibration and test methods.
12) Handling of calibration and test items.
13) Records.
14) Certificates and reports.
15) Sub-contracting of calibration or testing.
16) Outside support services and supplies.
17) Complaints.

ANNEX B

EXAMPLES OF REFERENCE MATERIALS FOR INTERNAL QUALITY CONTROL

In order to check the individual standards, the analyst needs reference materials (RMs) and certified reference materials (CRMs). Both RM and CRM should have a similar matrix and concentration of the determinand as the sample(s) to be analysed.

The effect of matrix (salt effect) in the Baltic Sea with strong salinity gradients has to be determined experimentally and the analytical results corrected correspondingly, especially for silicate and ammonia.

The supply of CRMs is at present limited to a certain extent by the available quantities and sometimes by prices. In some areas, e.g., for some organic determinands, no certified materials with relevant matrix, concentration and determinand are available.

For nutrient analyses in the Baltic Sea area, examples of CRMs and RMs available in 1997 are:

QC TYPE SW1 (NO_3 and PO_4) from the Kattegat (from the Water Quality Institute (VKI), DK);

QC TYPE SW2 (NO_3 and PO_4) from the Baltic Sea (from VKI, DK);

QC TYPE RW1 (NO_3 and PO_4) for addition to artificial or natural sea waters (from VKI, DK);

QC TYPE RW2 (TN and TP) for addition to artificial or natural sea waters (from VKI, DK);

Sagami standards (NO_3 and PO_4) in NaCl solution.

For inorganic and organic determinands in biota, the following CRMs are available in 1997:

Material	Matrix	Determinands certified	Source
DORM-2	Dogfish muscle	12 trace metals	NRCC
DOLT-2	Dogfish liver	12 trace metals	NRCC
LUTS-1	Non-defatted lobster hepatopancreas	17 trace metals	NRCC
TORT-1	Partially defatted lobster hepatopancreas	16 trace metals	NRCC
CRM 278	Mussel tissue	10 trace metals	BCR
CRM 422	Cod muscle	10 trace metals	BCR
CRM 349	Cod liver oil	CBs (IUPAC Nos. 28, 52, 101, 118, 153, 180)	BCR
CRM 350	Mackerel oil	CBs (IUPAC Nos. 28, 52, 101, 118, 153, 180)	BCR
QOR01CA	solution	CBs (IUPAC Nos. 28, 52, 101, 105, 118, 138, 153, 156, 180)	QUASIMEME
QOR02CA	solution	31 CBs	QUASIMEME
QOR03CA	solution	HCB, α-HCH, β-HCH, γ-HCH, dieldrin, p,p′-DDE p,p′-DDD, p,p′-DDT, *trans*-nonachlor	QUASIMEME

NRCC = National Research Council, Canada.

BCR = Community Bureau of Reference, Brussels (now the Standards, Measurement and Testing Programme of the European Commission).

For sea water, the use of CRMs may be supplemented by the preparation and use of well-characterized laboratory reference materials (LRMs). If no other certified reference material is available, a combined mixture of standards with appropriate concentrations prepared in artificial sea water (after Grasshoff) can be adopted as a laboratory control/reference sample. Whenever possible, an alternative product to the material used for calibration should be chosen for the LRM. For example, a KNO_3 solution may be used as an LRM if the calibrant is $NaNO_3$. For LRMs with limited stability, a stock solution can be prepared.

Aminot and Kérouel (1991) have developed the following method to prepare LRMs for inorganic nutrients. They aged natural sea water in polyethylene carboys, filtered it through a Whatman GF/C membrane, lowered its pH to 7.5 by the addition of hydrochloric acid, and sterilized it by autoclaving (heating to 120 °C, steady temperature for 20 minutes and accelerated cooling). Such water was found to be stable for 14 months and could serve as an LRM. Special care should be taken to assure the quality of the bottles with regard to adsorption/desorption of nutrients.

The preservation and storage conditions of LRMs and corresponding stock solutions should be documented. Whenever LRMs can be checked against CRMs, the documentation of the accuracy is improved.

Model compounds for procedural checks in total phorphorus determinations have been evaluated by Kérouel and Aminot (1996). For procedural checks of total nitrogen, EDTA is recommended.

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ANNEX C

VALIDATION OF AN ESTABLISHED ANALYTICAL METHOD

By means of the experimental design described in this Annex, data are obtained for calculation of the following:

- Range.
- Linearity of calibration on the day when N2 and N6 are analysed.
- Sensitivity.
- Verification of the precison at the limit of detection.
- Accuracy of synthetic samples at two concentration levels (N2 and N5) and of a spiked sample (N4 minus N3).
- Repeatability at four levels for synthetic and natural samples.
- Standard deviation between series for synthetic and natural samples at four concentration levels.

Start by performing six replicate measurements of a blank sample (or a sample with a concentration close to the expected detection limit), and calculate the detection limit (see Section 4.2.3, in body of report). Wherever possible, use natural samples with a known concentration. As these are rarely available, the experimental design could be as follows:

Concentration level	N1	N2	N3	N4	N5	N6
Measurements in each analytical series	2	2	2	2	2	2
Number of analytical series	6	1	6	6	6	1

Level N1: A synthetic sample, or if possible a natural sample with a concentration near the detection limit.

Level N2: A synthetic sample with concentration between N1 and N3.

Level N3: A natural sample with a concentration in the middle of the concentration range.

Level N4: The natural sample N3 with addition of property/determinand (a spiked sample).

Level N5: A synthetic sample with a concentration at the upper level of the range.

Level N6: A synthetic sample with a concentration approximately 20 % higher than N5.

In at least one of the analytical series, a calibration is performed with replicate measurements at all six concentration levels.

If the natural samples are not reference materials and for this reason the true concentrations are unknown, synthetic samples with approximately the same concentrations are also analysed. These samples are only measured in the same series as N2 and N6.

The analyses are performed in randomized order to obtain a realistic value for the standard deviation within a series.

All data are thus obtained by performing six determinations in one series, twelve determinations in another series and eight determinations in each of five further series, which is in total 58 determinations. This design gives sufficient data for determination of the range, the linearity, the selectivity, the detection limit and the accuracy. If a reference material has been applied as a natural sample, the data documentation is of an even higher quality.

If the type and concentration of the samples that have been chosen for the method validation have been chosen appropriately, they can be applied as the first data in the internal quality control charts. This is, of course, presuming that the method validation was satisfactory.

ANNEX D

QUALITY AUDIT

Areas of particular importance to a chemistry laboratory (drafted by the WELAC/EURACHEM WG, 1992).

D.1 Staff

Staff are properly trained and up-to-date training records are being maintained.
Tests are only carried out by authorized analysts.
The performance of staff carrying out analyses is observed.

D.2 Equipment

The equipment in use is suited to its purpose.
Major instruments are correctly maintained and records of this maintenance are kept.
Equipment, e.g., balances, thermometers, glassware, time pieces, pipettes, etc., is calibrated, and the appropriate calibration certificates demonstrating traceability to national or international standards are available.
Calibrated equipment is appropriately labelled or otherwise identified.
Instrument calibration procedures are documented and records of calibrations are satisfactorily maintained.
Appropriate instructions for use of equipment are available.
Instrument performance checks show that performance is within specifications.

D.3 Methods and Procedures

In-house methods are fully documented and appropriately validated.
Alterations to methods are appropriately authorized.
The most up-to-date version of the method is available to the analyst.
Analyses are following the methods specified.

D.4 Standards and Certified Reference Materials

The standards actually required for the tests are held.
The standards are certified or are the 'best' available.
The preparation of working standards is documented.
Standards and reference materials are properly labelled and correctly stored.
New batches of standards are compared against old batches before use.
The correct grade of materials is being used in the tests.
Where reference materials are certified, copies of the certificate are available for inspection.

D.5 Quality Control

There is an appropriate degree of calibration for each test.
Where control charts are used, performance has been maintained within acceptable criteria.
QC check samples are being tested by the defined procedures, at the required frequency, and there is an up-to-date record of the results and actions taken where results have exceeded action limits.
Results from the random re-analysis of samples show an acceptable measure of agreement with results from the original analyses.
Where appropriate, performance in proficiency testing schemes and/or interlaboratory comparisons is satisfactory and has not highlighted any problems or potential problems. Where performance has been unsatisfactory, corrective action has been taken.

D.6 Sample Management

There is an effective documented system for receiving samples, identifying samples against requests for analysis, and showing progress of analysis and fate of sample.
Samples are properly labelled and stored.

D.7 Records

Notebooks/worksheets include the date of test, analyst, analyte, sample details, test observations, all rough calculations, any relevant instrument traces, and relevant calibration data.

Notebooks/worksheets are completed in ink, mistakes are crossed out and not erased, and the records are signed by the analysts.

Where a mistake is corrected, the alteration is signed by the person making the correction.

The laboratory's procedures for checking data transfers and calculations are being complied with.

Vertical audits on random samples have not highlighted any problems (i.e., checks made on a sample, examining all procedures associated with its testing from receipt through to the issue of a report).

REFERENCE

EURACHEM/WELAC (Cooperation for Analytical Chemistry in Europe/Western European Legal Metrology Cooperation). 1992. Information Sheet No. 1 (Draft): Guidance on the Interpretation of the EN 45000 series of Standards and ISO Guide 25. 27 pp.

ANNEX E

GENERAL REMARKS ON SAMPLING

Sampling for the performance of analytical investigation has to be oriented towards the particular analytical task. Different aspects of sampling programmes are comprehensively dealt with in articles by Kratochvil and Taylor (1981), the ACS Committee on Environmental Improvement (ACS, 1983), and Garfield (1989).

Based on information provided by the above-mentioned authors, an acceptable sampling programme should include the following:

1) a predetermined sampling plan that takes into account the specific purpose of the investigations, including the contaminants to be determined, their expected concentration range, and the type of matrix to be analysed;

2) sample collection by personnel trained in the sampling techniques and procedures specified;

3) maintenance of the sample integrity by

   - using sampling devices that have been found to be suitable for the particular purpose,
   - avoiding contamination of samples from the use of unclean equipment,
   - using transportation procedures that ensure that the composition of the sample or the concentrations of the determinands are not altered;

4) instructions for labelling the sample specifying its identity;

5) a record that demonstrates an unbroken control over the sample from collection to its final disposition.

Detailed guidelines on sampling will be dealt with at a later time. Recommendations from other bodies or working groups will be taken into consideration when available.

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ANNEX F

TECHNICAL NOTES ON THE DETERMINATION OF NUTRIENTS

The commonly designated nutrients are inorganic nitrogen compounds (NO_3^-, NO_2^-, NH_4^+), phosphate (PO_4^{3-}) and silicate (SiO_4^{3-}). Total phosphorus (P_{tot}) and total nitrogen (N_{tot}) are also included because of their importance in relation to ecosystem analysis and budgets.

Nutrients in sea water are considered trace determinands and their analysis is liable to various sources of contamination. Sea water for nutrient analysis is usually collected from research vessels or ships of opportunity (e.g., ferry boats, fishing boats, coast guard or navy vessels). The reference method for measuring nutrients in the Baltic Sea (including storage and pretreatment) is Grasshoff (1976) 'Methods of Seawater Analysis'.

F.1 Sample Handling

Special attention must be paid to possible nutrient sample contamination generated by the ship. Wastewater discharged from wash basins, showers and toilets contains significant amounts of phosphorus and nitrogen compounds and, therefore, can contaminate surface waters to be sampled. For this reason, the water sampler must be deployed far from wastewater outlets, even if no sewage is discharged at the time of sampling. Although most modern ships are equipped with special sewage tanks, they are often emptied at sea owing to a lack of appropriate reception facilities in ports. In addition, there are potential problems with kitchen garbage.

Mixing by the ship's propeller can disturb the natural distribution of the determinands in the surface layer, particularly as regards oxygen. These problems, including the exact location of the ship, should be considered along with the natural variability.

Phosphorus and nitrogen compounds are secreted from human skin. However, touching of the sampler and the sample bottles by hands does not cause problems unless the sample comes into contact with the outer surface of the sampler or sample bottle. This is something that should never happen since the outer surfaces cannot be kept free of contamination on-board a ship. In view of the potential for contamination, the analyst should preferably supervise the collection of samples. The attaching of bottles to a hydrowire or the preparation of a rosette and the subsequent removal and transport of samples to the ship's laboratory should be done by trained personnel.

The written instructions for the collection of samples should include the precautions to be taken when a sub-sample is transferred to the storage container. The instructions must include the details of the essential record of the sample: station location, station code, depth of sampling, date, time, etc., and the identity of the person responsible for sampling.

F.2 Storage of Samples

The stability of nutrients in seawater samples depends strongly on the season and the location from which the samples were taken. Nutrients in seawater samples are generally unstable. Grasshoff (1976) recommends that ammonia and nitrite are measured no later than one hour after sampling. Samples for nitrate, phosphate and silicate should preferably be analysed within six hours after sampling, and no later than ten hours. If for practical reasons samples cannot be analysed within these time limits, the corresponding data should be flagged if stored in databases, unless the storage method has been validated

Samples should be stored protected from light and refrigerated. Plastic bottles must be used if silicate is measured. New sample bottles sometimes adsorb nutrients onto their walls. The new bottles, if necessary, should be cleaned with phosphate-free detergent, rinsed generously with distilled/deionized water and left filled with sea water containing nutrients for a few days. Then checks for adsorption of nutrients onto the walls or losses due to transformation to another chemical form should be carried out. Sample bottles should always be rinsed with the seawater sample from the sampler before they are filled. As regards ammonia determination, glassware for ammonia should always be cleaned with dilute hydrochloric acid.

If samples cannot be analysed within the above-mentioned time limits, the following methods of storage can be recommended.

Silicate	0–4 °C protected from light. Do not freeze (polymerization may occur).
Nitrite	Freezing or 0–4 °C protected from light. Do not acidify (rapid decomposition).
Ammonia	No known preservation methods are applicable.
Nitrate	Freezing.
Total nitrogen	Freezing or 0–4 °C protected from light. Do not acidify (enhanced risk of contamination).
Phosphate	Freezing or acidification.
Total phosphorus	Freezing or acidification with sulphuric acid with storage at 0–4 °C protected from light.

Addition of mercury or chloroform are alternative preservation methods for all nutrients except ammonia. These chemicals can affect the reaction kinetics, especially with automated methods, and this effect should be evaluated by the laboratory. The same chemical preservation of calibrants and quality controls can compensate for this effect. The use of mercury should be minimized and optimum disposal procedures should be ensured.

These preservation methods are all second choice to immediate analysis. They should, as mentioned, be validated by each laboratory, taking into account the concentration levels, storage time and environment, differences in sample matrices, and the analytical method of the laboratory.

Since no preservation method for nutrients can, at present, be recommended for general use, each laboratory must validate its storage methods for each nutrient before they are used routinely.

F.3 Sample Pretreatment

Sea water contains microorganisms and other suspended matter of different composition. In some cases, these particles bias the measurement of the determinand in the soluble phase. The suspended matter can be removed either by filtration or centrifugation. Unnecessary manipulation of the sample should be avoided, but in particle-rich waters (e.g., coastal waters, during plankton blooms) filtration or centrifugation may become necessary. It is important that the procedure used for filtration/centrifugation has been validated.

For removing algae from the water sample, a GF/C filter is adequate. For work in open oceans with low concentrations of suspended matter, GF/F filters are considered suitable for suspended matter separation from open sea water. Filtration in closed systems with a neutral gas is recommended. Centrifugation is especially advisable for samples destined for ammonia determination.

If a sample containing particles is not filtered, the turbidity causes light scattering which can bias a colorimetric measurement. In this case, a turbidity blank should be carried out by measuring light absorption of the sample before adding the colour-forming reagents.

F.4 Appropriate Chemical Analytical Methods

The choice of an analytical method should be based on the following criteria:

- the method should measure the desired constituent, i.e., be adequately specific, with accuracy sufficient to meet the data needs in the presence of interferences normally encountered in natural samples;

- the method should be sufficiently simple and rapid to permit routine use for the examination of large numbers of samples.

The reference methods used for manual nutrient measurements are described by Grasshoff (1976). Any changes to the reference methodology should be validated before use for routine work (see Annex C).

Apart from manual methods, various automated methods are in use, including different types of continuous flow analysis (CFA, steady state mode, and peak mode) or flow injection analysis (FIA or Reverse Flow Injection). The analyst has to be aware of the effects of the different analytical conditions in automated analysis which might affect accuracy.

F.5 Calibration and the Blank

Stock standard solutions should be prepared separately for each determinand using analytical grade reagents that can be pretreated to a precise stochiometric composition, e.g., by drying excess moisture. Reagents containing crystal water should be dried at a sufficiently low temperature in order not to remove the crystal water (the drying temperature is compound dependent). Stock standard solutions containing more than 1 mM are stable for long periods (up to one year refrigerated), but working calibration solutions must be prepared daily and used within hours of preparation.

Blank sea water may be prepared from a bulk sample of offshore surface sea water collected in summer, when the nutrients are at low or below-detection concentrations (Kirkwood, 1994). Blank sea water and reagents totally devoid of nutrients are, however, difficult to achieve, especially regarding the content of ammonia. Optimum handling precautions should be taken to minimize the content of nutrients to below approximately 10% of the measuring range. The concentrations of nutrients in the blank and reagents can be assessed by the standard addition method.

For ammonia analysis, the salinity of the samples affects the reaction kinetics, mainly due to the buffer effect of marine water, that results in a sub-optimum end pH. This effect can give biased results, especially with kinetically dependent automated methods. In the Baltic Sea, the salinity ranges from approximately 0 to 30, and therefore the size of this bias will be variable. This kinetic effect should be checked by standard addition, or by checking the pH of the reagent-sample mixture, which should be in the range between 10.5 and 11.

Whenever compensation for this bias is deemed necessary, one of the following methods is suggested:

a) If all samples have the same salinity, calibrate using the addition of calibrants to one of the samples. In some situations, low-nutrient sea water can be prepared by aging and filtering natural sea water (as mentioned above).

b) Empirical correction in accordance with the measured sample salinity or pH value.

For all photometric nutrient measurements differences in light refraction, caused by differences in the salt concentration, can give rise to shifts in blank/baseline values, especially in light-measuring cells with round windows. This can be compensated by using blanks and calibrants of the same salt concentration as the samples.

Particles can give rise to light-scattering effects that result in interferences in all photometric nutrient analyses. This bias can be avoided by measuring the sample before addition of the colour reagent, or by filtration or centrifugation where this does not cause contamination.

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ANNEX G

TECHNICAL NOTES ON THE ANALYSIS OF CHLORINATED BIPHENYLS, ORGANOCHLORINE PESTICIDES AND METALLIC TRACE ELEMENTS IN MARINE FISH

G.1 Sampling and Sample Handling of Fish Muscle and Liver Tissue

Muscle tissue or liver of fish have to be dissected while they are in good condition. If biological tissue deteriorates, uncontrollable losses of determinands or cross-contamination from other deteriorating tissues and organs may occur. To avoid this, individual fish specimens must be dissected at sea if adequate conditions prevail on board, or be frozen immediately after collection and transported frozen to the laboratory, where they are dissected later. Commercial catches can be used if fish transport to the laboratory does not take longer than 24 hours. The fish must be transported on ice. The dissection then takes place at the laboratory.

If the option chosen is dissection on board the ship, two criteria must be met:

1) The work must be carried out by personnel capable of identifying and removing the desired organs according to the requirements of the investigations; and

2) There must be no risk of contamination from working surfaces or other equipment.

Crushed pieces of glass or quartz knives, and scalpels made of stainless steel or titanium are suitable dissection instruments.

Colourless polyethylene tweezers are recommended as tools for holding tissues during the dissecton of biological tissue for metallic trace element analysis. Stainless steel tweezers are recommended if biological tissue is dissected for analysis of chlorinated biphenyls (CBs) and organochlorine pesticides (OCPs).

After each sample has been prepared, including the samples of different organs from the same individual, the tools should be changed and cleaned.

The following procedures are recommended for cleaning tools used for preparing samples:

1) for analysis of metallic trace elements

   a) Wash in acetone or alcohol and high purity water.

   b) Wash in HNO_3 (p.a.) diluted (1+1) with high purity water. Tweezers and haemostates in diluted (1+6) acid.

   c) Rinse with high purity water.

2) for analysis of CBs and OCPs

   a) Wash in acetone or alcohol and rinse in high purity water.

The glass plate used during dissection should be cleaned in the same manner. The tools must be stored dust-free when not in use.

The dissection room should be kept clean and the air should be free from particles. If clean benches are not available on board ship, the dissection of fish should be carried out in the land-based laboratory under conditions of maximum protection against contamination.

For analysis of fish muscle, the epidermis and subcutaneous tissue should be carefully removed from the fish. Samples should be taken under the red muscle layer. In order to ensure uniformity of samples, the right side dorso-lateral muscle should be taken as the sample. If possible, the entire right dorsal lateral filet should be used as a uniform sample, from which subsamples can be taken after homogenizing for replicate dry weight and contaminant determinations. If, however, the amount of material obtained by this procedure is too large to handle in practice, a specific portion of the dorsal musculature should be chosen for the sample. It is recommended that the portion of the muscle lying directly under the first dorsal fin be utilized in this case. As both fat and water content vary significantly in the muscle tissue from the anterior to the caudal muscle of the fish (Oehlenschläger, 1994), it is important to obtain the same portion of the muscle tissue for each sample.

To sample liver tissue, the liver must be identified in the presence of other organs such as the digestive system or gonads. The appearance of the gonads will vary according to the sex of the fish and the season. After opening the body cavity with a scalpel, the connective tissue around the liver should be cut away and as much as possible of the liver is cut out in a single piece together with the gall bladder. The bile duct is then carefully clamped and the gall bladder dissected away from the liver.

When fish samples which have been frozen at sea are brought to the laboratory for analysis, they should be dissected as soon as the tissue has thawed sufficiently.

The dissection of fish is easiest when the material, at least the surface layers of the muscle tissue, is half frozen. For dissection of other organs, the thawing must proceed further, but it is an advantage if, for example, the liver is still frozen. It must be noted that any loss of liquid or fat due to improper cutting or handling of the tissue makes the determinations of dry weight and fat content, and consequently the reported concentrations of determinands, less accurate.

After muscle preparations, the liver should be completely and carefully removed while still partly frozen to avoid water and fat loss. Immediately after removing it from the fish, the liver should be returned to the freezer so that it will be completely frozen prior to further handling. This is particularly important for cod liver.

G.2 Storage of Fish Samples

Material from single fish specimens should be packaged and stored individually.

- Samples for analysis of metallic trace elements can be stored in polyethylene, polypropylene, polystyrene or glass containers.

- Samples for analysis of CBs and OCPs should be packaged in precleaned aluminium foil or in precleaned glass containers.

Liver tissue can deteriorate rather rapidly at room temperature. Consequently, samples should be frozen as soon as possible after packaging. They can be frozen rapidly by immersion in liquid nitrogen or blast freezing, but both these techniques need care. Whatever system is used, freezing a large bulk of closely packed material must be avoided. The samples in the centre will take longer to cool and will therefore deteriorate more than those in the outer layer.

Once frozen, samples can be stored in a deep freezer at temperatures of –20 °C or below.

Frozen liver tissue should not be stored longer than six months, while lean muscle tissue can be stored up to two years. Each sample should be carefully and permanently labelled. The label should contain at least the sample's identification number, the type of tissue, and the date and location of sampling.

G.3 Determination of Chlorinated Biphenyls and Organochlorine Pesticides

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, determinand 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.

G.3.1 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 °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 redistillation 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).

G.3.2 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' under Section G.3.4).

G.3.3 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.

G.3.4 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 indispensible 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 owing to 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 instrumental equipment) 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 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 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 coeluting 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 Geus *et al.*, 1996; de Boer *et al.*, 1995).

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 homologues of dichloroalkylbenzylether can be used. For GC with mass selective detection (GC-MSD), ^{13}C-labelled CBs must be used as internal standards. With GC/MS, ^{13}C-labelled CBs should preferably be used as internal standards.

G.4 Determination of Metallic Trace Elements

Metallic elements appear in different marine biological matrices in trace concentrations, ranging from the mg/kg through the µg/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.

G.4.1 Pretreatment of laboratory ware and reagents; contamination control

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 pretreatment 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 ft^3 or $3.6 \cdot 10^3$ per m^3 of 0.5 µm 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).

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), tetrafluorethyleneperfluorpropylene (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 Lindström (1977), Tschöpel *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 (Tschöpel *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 solutions of analytes 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 systems are described in detail by Matthinson (1972), Kuehner *et al.* (1972), Dabeka *et al.* (1976), Tschöpel *et al.* (1980), Mitchell (1982b), Moody and Beary (1982), Moody *et al.* (1989), and Paulsen *et al.* (1989). Acids of extreme high purity are produced by multiple batchwise distillation of reagent-grade acids in a silica apparatus, which is placed in a laminar-flow hood.

G.4.2 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).

G.4.3 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.

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; Würfels *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) may be suitable to prevent losses of volatile elements. The use of special materials (quartz, titanium, stainless steel) for the construction of sample containers may be helpful to minimize 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 volatilization 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 consequence that an enormous reduction in digestion time occurs. Furthermore, a reduction of acid volume (McCarthy and Ellis, 1991) and less contamination during the decomposition process were found (Dunemann, 1994; Sheppard *et al.*, 1994).

The admittance 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.

G.4.4 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.

Single or mixed working element 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 µg/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).

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ANNEX H

TECHNICAL NOTES ON THE DETERMINATION OF TRACE METALS (Cd, Pb, Cu, Co, Zn, Ni, Fe) IN SEA WATER

H.1 Clean Laboratory, Clean Benches

Particles are everywhere, including dust in the air or on clothes, hair or skin. Because of the clothes, the person who is working with the samples for trace metal analysis is the main source of contamination because this person is a particle producer. One of the most important concerns during sample pretreatment for trace metal analysis is to eliminate particles that can contaminate the samples or sample containers from the laboratory environment. The best way to eliminate most of this contamination is to work under a laminar flow box with a laminar horizontal flow (sample protection). Recommended conditions for a 'clean bench' or a 'clean lab' are class 100 (US Norm) which means that there are still about one hundred particles present per cubic foot or class 3 (DIN Norm), which equals 3000 particles per m^3 (corresponding to class 100 US Norm).

H.2 Preparations

In order to avoid contamination problems, all plastic ware, bottles and containers must be treated with acids (HCl or HNO_3) for several weeks, then rinsed with high-purity water and covered in plastic bags until used.

The following procedures (Patterson and Settle, 1976) are suggested:

Laboratory ware

Store in 2M HCl (high purity) for one week, rinse with high-purity water, store in high-purity water for one week and dry under dust-free conditions (clean bench).

Samplers and bottles

Sampling devices: Fill with 1 % HNO_3 (high purity), store at room temperature for three weeks, and rinse with high-purity water.

Teflon/quartz bottles: Store bagged in warm (40 °C ± 5 °C) 1:1 diluted HCl for one week, then rinse with high-purity water and store with 1M HNO_3 (high purity) until final use (a minimum of three weeks).

Filters

Polycarbonate filters (e.g., Nuclepore) (0.4 µm 47 mm diameter): Store in 2M HCl (high purity) for a minimum of three weeks. After rinsing with high purity water, store for one more week in high purity water.

If trace metals in suspended particulate matter (SPM) are to be determined, filters have to be put in precleaned plastic dishes, dried in a clean bench for two days, and then stored in a desiccator until they are weighed using an electronic microbalance with antistatic properties. Each filter has to be weighed daily for several days until the weight is constant. The same procedure for drying and weighing should be applied to the filters loaded with SPM (Pohl, 1997).

Chemicals

High-purity water (e.g., 'Milli-Q' water, 18 $M\Omega\,cm^{-1}$, freshly prepared).
A sub-boiling quartz still is recommended for the distillation of highly purified acids and solvents.
A teflon still is recommended for the distillation of HF.

H.3 Sampling and Sample Handling

The basis for the reliable measurement of extremely low concentrations of trace metals in sea water is a well-performed sampling to avoid contamination risk from the ship. Careful handling is recommended because copper and tin are still the main substances used in antifouling paints on ships and there is also a risk of contamination by zinc (anodes of the ship), iron, or lead.

In coastal and continental shelf waters, samples are collected using 30 l Teflon-coated GO-FLO (General Oceanics, close-open-close system) bottles with Teflon O-rings deployed on Kevlar or on a Hostalen-coated wire. Niskin bottles deployed on rosettes using standard stainless steel hydrowire are also acceptable. For surface waters, an all-Teflon MERCOS Sampler (Hydrobios) could be chosen.

PVC gloves should be worn during subsampling into the precleaned quartz or teflon bottles (teflon has an extra low content of trace metals). Subsampling should be carried out in a clean lab or a clean lab container, if available.

Pumping of samples using peristaltic or teflon piston pumps has to be carried out using precleaned silicon or teflon-lined tubes.

In the absence of clean-lab conditions, sampling and sample handling must be carried out in a closed system, or contamination cannot be avoided.

H.4 Filtration Procedure

In the environmental and geochemical scientific community concerned with water analysis, it has generally been accepted that the term 'dissolved' refers to that fraction of water and its constituents which have passed through a 0.45 µm membrane filter. This is an operationally defined fraction. Coastal and shelf water samples have to be filtered to eliminate particles from the water. A number of metal species pass through this filter pore size, including metals bound to colloids or clays or to humic, fulvic, amino, and fatty acids.

To prevent desorption of metal ions from particle surfaces or from biological degradation of SPM, separation between the dissolved phase and the particulate phase has to be done immediately after sampling by filtering the water through a 0.45 µm polycarbonate filter. This procedure should be carried out under clean conditions (clean benches are recommended on board the ship).

If metals in both the dissolved and particulate phases are to be analysed, pressure filtration with nitrogen is recommended. After filtration, the filter should be rinsed with high purity isotonic solution to remove sea salt residues. Only a few milliliters are necessary because a change of pH could cause desorption of metal ions from the particles. In pumping systems, on-line filtration is possible.

H.5 Storage of Samples

To avoid wall adsorption of metal ions, 1.5 ml HNO_3 or HCl (high purity) should be added per liter of seawater sample immediately after filtration for acidification to pH 1.0–1.6. The sample containers should be stored in plastic bags under controlled environmental conditions. The filters should be stored in plastic dishes at −18 °C or below. Under these conditions, both water samples and SPM on filters can be stored for at least one year.

H.6 Sample Pretreatment

Water samples

Depending on the expected concentration range (10^{-7}–10^{-9} g kg^{-1}) of trace metals (dissolved) in Baltic Sea water and because of the salt matrix interfering during the measurement process, preconcentration techniques and/or the elimination of sea salt has to be carried out prior to the analytical measurement. Detailed method information is available in the open literature (e.g., Danielsson *et al.*, 1978; Kremling *et al.*, 1983; and Pohl, 1994).

Filters

Different methods to analyse the material on the filter are described by Hovind and Skei (1992) and Loring and Rantala (1991). Pressure decomposition with an acid mixture (HCl, HNO_3, HF) is recommended. If the silica content is high due to diatoms, the HF concentration should be increased accordingly. If the organic content increases, it is advisable to work with perchloric acid.

Depending on the digestion system used (high pressure autoclave, microwave digestion, wet ashing in an open system, or dry ashing), the completeness of the digestion is a function of temperature, time, digestion material and pressure, and has to be tested and validated in pilot studies with (certified) reference materials (see the detailed remarks in Annex G, Section G.4.3).

H.7 Instrumentation

For the analytical measurements, several analytical techniques can be used, such as GFAAS (graphite furnace atomic absorption spectrometry), electrochemical methods, ICP-MS (inductively coupled plasma-mass spectrometry), ICP-AES (inductively coupled plasma-atomic emission spectrometry), or total-reflection X-ray fluorescence (TXRF).

H.8 Quality Control

Blank

Particularly in the case of trace metal analysis, with high contamination risks at each step of the analytical work, a satisfactory blank control is necessary. Therefore, it is important to control the blank daily, for reproducibility and constancy over a longer time. The blank should include all analytical pretreatment procedures, including the addition of the same quantities of chemical substances as for the sample.

Calibration

For calibration purposes, single element standard stock solutions at a concentration of 1000 mg l^{-1}, purchased from a qualified manufacturer, should be available. Preparation date and concentration should be marked on the bottle. From this stock solution, a multi-element working standard solution can be prepared using dilute HCl or HNO_3, as required (normally 1M acid is used). Traceability can be ensured by the use of CRMs or participation in intercomparison exercises.

The working standard should be prepared from the stock standard solution for every batch of samples and kept no longer than two weeks. Precleaned teflon containers are preferable for storage.

To evaluate effects from the matrix, the method of standard addition can be used, particularly in connection with the analytical method of voltammetric stripping. For other techniques, the method of standard addition should generally be used with care (Cardone, 1986a, 1986b).

Reference materials

Owing to problems in defining the blank, the use of a low-concentration CRM is important. Regular participation in intercomparison exercises should be considered mandatory.

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ANNEX I

CHEMICAL ANALYSIS OF ANOXIC WATERS

I.1 Introduction

Anoxic sea water is generally found in enclosed areas with restricted water exchange. In most cases, a physical barrier (sill) as well as a pronounced density stratification will prevent oxygen from reaching the deeper parts of the sea area. Anoxic conditions will occur if the rate of oxidation of organic matter by bacteria is greater than the supply of oxygen. Anoxic waters are a natural phenomenon (Richards, 1965; Sarmiento *et al.*, 1988), and anoxic waters have occurred during the geological history of the Baltic Sea (Jerbo, 1972; Hallberg, 1974). Recently, there have been some indications that eutrophication has increased the extent of the anoxic areas in, e.g., the Baltic Sea. Primary factors promoting anoxic conditions are stagnant conditions and density stratification (Gerlach, 1994).

Anoxic conditions result from several factors, for example, stagnation periods, inputs of organic material, and strong thermoclines. The bacterial production of sulphide starts in the sediments, where the bacteria find suitable substrates, and then expands into the water column.

When oxygen is depleted in a basin, bacteria first turn to the second-best electron acceptor, which in sea water is nitrate. Denitrification occurs, and the nitrate will be consumed rather rapidly. After reducing some other minor elements, the bacteria will turn to sulphate. The reduction of sulphate occurs according to the reaction:

$$(CH_2O)_{106}(NH_3)_{16}H_3PO_4 + 53\ SO_4^{2-} \rightarrow 53\ CO_2 + 53\ HCO_3^- + 53\ HS^- + 16\ NH_3 + 53\ H_2O + H_3PO_4$$

If anoxic sea water becomes reoxygenized, sulphides will be oxidized to sulphate according to:

$$HS^- + 2\ O_2 \rightarrow HSO_4^-$$

I.2 Experimental Problems Encountered

I.2.1 Hydrogen sulphide

No ideal method for the determination of hydrogen sulphide in sea water exists today. The presently most widespread method, which is based on the formation of methylene blue and spectrophotometric measurement, although robust and simple to perform in the field, suffers from several weaknesses. The calibration of the reagents is an elaborate procedure requiring, among other things, the availability of oxygen-free water. Another obstacle is that Na_2S, which is used as the sulphide source in the calibration, is not available as a water-free compound of pro analysi quality. Furthermore, the stock and working solutions of sulphide made up for the calibration are extremely unstable, and the working solution will change concentration substantially in a short time (1–2 hours). Sulphides that are commercially available in the pure form generally suffer from extremely low water solubility, and thus are not suitable for this kind of work.

Sampling is carried out using the same technique as for oxygen, and thus is not a general problem for the trained marine scientist. If the samples will not be measured within acceptable time limits, they are generally preserved with zinc acetate (to form zinc sulphide) prior to analysis. The relatively poor precision of the method, often 5–10 %, could probably be attributed to the combined effects of all steps in the sampling and sample pretreatment procedure.

Validation of results is very difficult, since there are no certified reference materials (CRMs) available for sulphide in sea water. The parameter is very rarely included in interlaboratory comparison excercises, mainly due to problems in withdrawing multiple samples with the same sulphide concentration from one sample container.

Very high concentrations of sulphide in certain unusually stagnant areas will cause problems. In some cases, the absorption of the sample will lie outside the working range of the spectrophotometer. Dilution of the sample is possible, but will undoubtedly introduce more uncertainty into the measurement.

I.2.2 Oxygen

In cases where sensors are used for measuring the oxygen content of the water column, anoxic layers will poison the sond and quickly deteriorate its performance. The best way to avoid this is not to lower the sond into any anoxic water layers, which will make it rather impractical in many areas of the Baltic Sea.

I.2.3 Salinity

The combined effect of mineralization of organic matter and accumulation of nutrients may cause a shift in the salinity measurements by conductivity by no more than 0.02 PSU (Grasshoff, 1975). This difference is caused by differences in ionic composition between the sample and the standard sea water used for calibrating the salinometer. Practical problems may occur, possibly due to particles in the water, causing a certain instability in the conductivity reading. After running a series of anoxic samples, the salinometer has to be rinsed carefully with deionized water and ethanol.

I.2.4 Nutrients

Of the inorganic nutrients, phosphate is the compound giving rise to special problems in anoxic waters. Both the natural turbidity of anoxic samples and the influence of the sulphide present on the colour reaction may cause biased results or results of low precision. The reduction of the phosphomolybdenum complex to the blue complex is catalysed by antimony. Sulphide could react with the antimony ions to form a yellow-greenish turbidity, which disturbs the photometric measurement (Nehring, 1994). In addition, colloidal sulphur may be formed when the acid molybdate reagent is added (Grasshoff *et al*, 1983). These problems can be overcome by removing the sulphide by oxidation with bromine or degassing under a stream of nitrogen. The wavelength at maximum absorbance of the colour complex also coincides with strong absorption caused by turbidity. The safety limit of interferences has previously been reported to be 2 mg/l of sulphide for phosphorus and ammonia.

In anoxic waters, nitrate will be reduced to ammonia, disappearing rapidly as the oxygen disappears. The presence of small amounts of nitrate in anoxic waters is possible, but only in layers influenced by rapid mixing with overlying water masses (Grasshoff, 1975). In order to find measurable quantities of nitrate in these waters, the speed of mixing has to be higher than the speed of denitrification of the nitrate. The presence of nitrate in anoxic waters should otherwise be treated with care, since it is probably a result of oxidation of ammonia in the sample upon contact with the atmosphere when sampling.

Nitrite is normally not present in detectable amounts in anoxic waters, as it has been reduced to ammonia. However, nitrite has been observed in the presence of large quantities of ammonia in anoxic waters, possibly as a result of rapid oxidation upon contact with the atmosphere. Sulphide has been reported to interfere with the nitrite measurements, and should (if possible) be removed from the sample.

Ammonia accumulates in the anoxic water and remains fairly stable. The oxidizer for the development of the indophenol blue, hypochlorite, is partly consumed by the oxidation of sulphide. It may thus be necessary to increase the amount of hypochlorite added to the sample in strongly sulphidic waters (Nehring, 1994). In all particle-rich waters, including anoxic waters, it is necessary to measure and subtract the seawater blank.

Silicate accumulates in stagnant waters, and the high concentrations make the determination less sensitive to interferences. Sulphide concentrations up to approximately 150 µmol/l will not affect the formation of the colour complex for the determination of silicate, even if the silicomolybdic acid may partly be reduced. At higher concentrations, it may be advisable to remove the sulphide or to dilute the sample.

I.2.5 Total Phosphorus and Nitrogen

The hydrogen sulphide is oxidized to elemental sulphur or sulphate by the oxidation reagent used in the analysis of total phosphorus and nitrogen and thus does not interfere directly. In extreme cases, with extraordinarily high sulphide concentrations, all of the sulphide may not be oxidized and may possibly create a problem (see Section I.2.4, above). A high particle content may, as for inorganic phosphorus, give rise to blank problems. In the analysis of total phosphorus, the oxidation and hydrolysis of phosphorus compounds may not be complete, especially when both nitrogen and phosphorus compounds are combusted simultaneously in alkaline media (cf. Koroleff, 1983). It has furthermore been demonstrated that the oxidation of organic phosphorus compounds using potassium peroxodisulphate ($K_2S_2O_8$) is an unsuitable method in the presence of dissolved iron, possibly due to the formation of iron(III) phosphate during the oxidation process (Ichinose *et al.*, 1984).

I.2.6 Organic Carbon and Nitrogen

The most modern technique for determining the levels of particulate organic carbon or nitrogen (POC/PON) in sea water starts with filtration of the water through 0.45 µm filters. The filters, with their content of particulate matter, are combusted in an oxygen-rich atmosphere to produce gaseous CO_2 and NO_2. The analysis is very straightforward and robust, and there seem to be no problems (theoretical or practical) involved in the analysis of samples originating from anoxic waters. The samples are characterized by high levels of POC/PON, since anoxic waters are rich in particles, detritus, and other non-living organic material.

I.2.7 Halogenated Organic Contaminants

The methods used for the determination of halogenated organic contaminants in water are based on extraction of the contaminants from the sea water matrix followed by gas chromatographic separation and some kind of detection. For work in the open sea, the electron capture detector (ECD) is the preferred choice due to its selectivity and sensitivity. The electron capture detector is very selective towards elements with large electron-capturing capability, for example, the halogens. However, the detector also has a certain response towards oxygen and sulphur, and will thus be disturbed by the occurrence of compounds containing these elements. This may give rise to great difficulties in detecting and quantifying, in particular, volatile halogenated compounds in anoxic waters (Krysell *et al.*, 1994).

Anoxic environments will cause a breakdown of many halogenated compounds, complicating the distribution patterns and lowering their concentrations. Chlorophenolic compounds have been shown to dehalogenate in anoxic sediments (Abrahamsson and Klick, 1989) and the breakdown of carbon tetrachloride has been observed in anoxic waters (Krysell *et al.*, 1994; Tanhua *et al.*, 1996).

I.2.8 Metallic Trace Elements

The concentrations of certain metal ions, most importantly copper (Cu), lead (Pb) and zinc (Zn), and to some extent cadmium (Cd), decrease rapidly in anoxic waters due to the low solubility of their corresponding sulphides. The relatively lower concentrations that follow cause problems mainly when it comes to the correction for blanks, since the blanks become disproportionately high.

In basins with very high sulphide concentrations, elemental sulphur may under some circumstances cause problems in the analysis, since it will be extracted into the same fraction as the metals.

Methods involving ion exchangers for sample work-up and concentration may give a very low yield unless the strength of the ion exchange resin can match that of the strongly bound metal sulphides.

I.2.9 pH

Electrode deterioration may occur, because in sulphidic waters sulphide will react with the Ag/AgCl electrode, considerably shortening the lifetime of the electrode.

I.2.10 Alkalinity

There are no experimental problems, but anoxic waters contain an organic fraction which contributes to the alkalinity. The nature of this organic fraction is still under discussion; it has been suggested that it consists of amino acids or humic substances. Since the true nature of the organic fraction has not been determined, there are still doubts about how it fits into the definition of alkalinity and how the data should be treated and normalized. When determining alkalinity in sulphidic waters, it is more reliable to use a titration method with an indicator because sulphide will react with the Ag/AgCl electrode used in potentiometric titration.

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