ASEAN
MARINE WATER QUALITY
Management Guidelines and Monitoring Manual

Australian Government

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Foreword

This first edition of the *ASEAN Marine Water Quality: Management Guidelines and Monitoring Manual* was published in 2008 in recognition of the growing importance of effectively managing the marine waters within the ASEAN region.

In 2002 the ASEAN Environment Ministers endorsed the ASEAN Marine Water Quality Criteria (AMWQC). Seventeen parameters were unanimously agreed and adopted as common ASEAN marine water quality criteria for the protection of the coastal and marine environment and human health. These are to be used to guide concerted national level action to protect the shared marine waters of ASEAN.

Between 2004 and 2008 AusAID, through the ASEAN Australia Development Cooperation Program, funded two phases of a project titled *Capacity Building for Implementation of the ASEAN Marine Water Quality Criteria* to support the transition from policy to practice.

The project was designed and implemented as a partnership between the ASEAN Working Group on Coastal and Marine Environment (AWGCME), the ASEAN Secretariat and Australian Marine Science and Technology Ltd, an Australian consultancy, and involved the active participation of each ASEAN Member State (AMS).

In the broad sense, the project was intended to contribute materially to the goal of strengthening ASEAN capacity to address regional development challenges (in this instance, marine water quality) enabling greater integration of activities within the ASEAN region and enhanced participation in the global economy.

Phase I (2004-2005) was designed to initiate the implementation of the AMWQC adopted by ASEAN in accordance with ASEAN's Hanoi Plan of Action and the Vientiane Action Programme, by developing strategies for harmonisation of national monitoring frameworks and by building capacity in the design and implementation of marine water quality monitoring programs. This included how the AMWQC were defined and managed in each country, including identifying legal issues, regulatory frameworks and management approaches.

In brief, Phase I was designed to initiate operationalisation of the parameters adopted by ASEAN. Integral to this was the review and subsequent design and application of standardised and comparable monitoring programs. This took into account financial and human resource constraints with each ASEAN country.

While Phase I successfully built upon previous work to develop the AMWQC, the need for an effective delivery mechanism was identified. Delivery depends on inter-related/inter-dependent elements, such as the development of agreed policies and

management approaches (both within ASEAN and within each of the AMSs) and the attainment of these policies and management approaches through the implementation of agreed and complementary marine water quality monitoring programs.

The objective of Phase II (2007-2008) then, was to leverage and extend the success of the Phase I in achieving progress towards implementation of the AMWQC. This phase built upon the basic outcomes of the earlier work by developing agreed management concepts, regulatory approaches, monitoring approaches and methodologies, and reporting frameworks.

This publication is the culmination of the two phases of the project and is designed to provide ASEAN Member States with (i) guidelines to assist in the management of marine waters within the ASEAN region to the agreed parameters and (ii) approaches and methods for the development and implementation of marine water quality monitoring programs, to ensure this is achieved.

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In preparing the *ASEAN Marine Water Quality: Management Guidelines and Monitoring Manual* there are many who should be acknowledged for the their oversight and input. First and foremost Prof Dr Nguyen Chu Hoi, Chairman of the ASEAN Working Group on Coastal and Marine Environment and Dr Raman Letchumanan, Head of Environment and Disaster Management Unit in the ASEAN Secretariat deserve particular thanks for their leadership and support.

The *ASEAN Marine Water Quality: Management Guidelines and Monitoring Manual* would not be possible if it were not for the support and project funding provided by AusAID through their ASEAN Australia Development Cooperation Program Regional Partnership Scheme. Project direction and management for the Project (*Capacity Building for the Implementation of the ASEAN Marine Water Quality Criteria*) was provided by AMSAT Ltd staff, Mr. Jim Travers, Project Director and Mr. David Walter, Project Manager.

Dr Jon Brodie provided guidance and technical leadership as Chief Technical Advisor for the Project. Prof Chris Crossland led preparation of the Management Guidelines. Dr Soo Loong Tong and Dr Michelle Devlin assisted Dr Brodie in preparing the Monitoring Manual. Additional technical input and scientific editing was provided by Mrs Jan Marshall Crossland. Their significant involvement in bringing the publication to fruition is acknowledged.

Along with the Technical Advisors engaged for the development of the management guidelines and the monitoring manual, over 20 ASEAN officials, representing agencies responsible for developing policies and approaches to manage ASEAN marine waters or institutions involved in monitoring the agreed ASEAN marine water quality parameters, provided input throughout the publication's development. Their input has proved invaluable and they are (in alphabetical order):

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Introduction

1 Marine Water Quality: the ASEAN Context

Coastal and marine resources provide a wide range of essential ecological, economic and social benefits worldwide. This is particularly apparent within the region covered by the Association of Southeast Asian Nations (ASEAN). These marine waters provide livelihoods directly and indirectly to millions of people, provide food, serve as sinks for materials from land-based sources, maintain environmental cycles, regulate climatic conditions, and maintain the complex ecological balance of the array of marine and estuarine ecosystems that characterise the region. Any degradation of the key biological or physical processes and ecosystem resources can lead to long term deleterious and sometimes irreversible impacts on the socio-economic opportunities and environmental and human health of the region.

ASEAN has a coastline of 173,000 km with a total area of 4.5 million km^2 and, as of 2006, the ASEAN region has a population of about 560 million, a combined gross domestic product of around US$ 1,200 billion, and a total trade of about US$ 1,400 billion. As well it has 35% of the world's mangrove forests, and about 30% of the coral reefs. Therefore sustainably managing the coastal and marine resources as well as maintaining the water quality on which they depend has been a high priority for ASEAN over the last several decades.

Marine and estuarine water quality represents significant environmental issues within countries across the ASEAN region. Recognising the commonalities of the underpinning problems and solutions, and their management, member states have been making concerted effort to develop a harmonised framework of approach within ASEAN.

The Hanoi Plan of Action (1999-2004) (HPA) called for the development of a framework to improve regional coordination for the integrated protection and management of coastal zones, development of a regional action plan for the protection of the marine environment from land-based and sea-based activities, and promote regional coordination to protect Marine Heritage Parks and Reserves[1].

[1] Para 6.12 identifies the need to - "*Develop a Regional Action Plan for the Protection of the Marine Environment from Land-based and Sea-based Activities by the year 2004*". This objective was reiterated in the Vientiane Action Programme (VAP) in November 2004 (the Measure identified in 3.3.7.2 of Annex 3 is to - "*Further expand and implement the ASEAN Marine Water Quality Criteria*".

The Vientiane Action Programme (2004-2010) (VAP), adopted and endorsed by the ASEAN Leaders during the 10th ASEAN Summit in Vientiane, Lao PDR, in 2004, succeeded the Hanoi Plan of Action. One of the key strategic thrusts of the ASEAN Socio-Cultural Community (one of the three pillars outlined in the VAP) is to promote environmental sustainability through environmental and natural resource management.

Assistance programs (notably with Australia, Canada and the USA) supported ASEAN regional initiatives to address both marine and estuarine resources and water quality issues and management. Regional actions have achieved the primary purposes with a focus on mapping of marine resources, information and technological transfer and marine scientific and management capacity building. Importantly, there has developed a strong set of institutional and personal relationships from these collaborative actions, at national, regional and international levels which are sustained.

In recent years, the ASEAN community has identified the growing need for the development of a framework to: (i) improve regional coordination for the integrated protection and management of coastal zones, (ii) guide development of a regional action plan for the protection of the marine environment from land-based and sea-based activities, and (iii) promote regional coordination to protect Marine Heritage Parks and Reserves. Partnership arrangements are being pursued to progress these needs, building on the enhanced human capacities and institutional linkages from earlier actions within the region, and on institutional associations beyond ASEAN.

2 Past Progress in the Development of a Harmonised Framework

The ASEAN Marine Water Quality Criteria (AMWQC) were developed by ASEAN scientists during the period from 1992–1997, through a rigorous investigation of how to determine 'good' marine water quality (*sensu* the European Union Water Framework Directive), focusing on a range of known pollutants such as heavy metals (e.g., lead, arsenic, zinc and cadmium), suspended solids, chemicals (e.g. nitrogen and phosphorus) and bacteria[2].

As part of the deliberations within ASEAN, an ASEAN/ UNEP study (2002) and the ASEAN/ UNEP Workshop on Coastal and Marine Environment of Southeast Asia: Status and Opportunities for Regional Cooperation, held in Bangkok, Thailand from 11-13 March 2002, reviewed the AMWQC. This resulted in a recommendation to the ASEAN Committee on Science and Technology (COST) to accept the marine water quality criteria proposed for 17 out of the total of 19 parameters studied. The 17

[2] These studies were conducted with the support of the ASEAN Canada Cooperative Programme on Marine Science Phase II, funded by the Canadian International Development Agency.

proposed marine water quality criteria set scientifically derived limits under which marine life was considered safe.

In 2002 the ASEAN Environment Ministers endorsed the set of 17 ASEAN Marine Water Quality Criteria at the 7th Informal ASEAN Ministerial Meeting on the Environment on 20 November 2002 in Vientiane, Lao PDr The 17 parameters unanimously agreed and adopted as common marine water quality criteria for the protection of the coastal and marine environment in ASEAN included:

- sixteen parameters for aquatic life protection (ammonia, cadmium, hexavalent chromium, copper, lead, mercury, cyanide, total phenol, tributyltin, nitrate, nitrite, phosphate, temperature, dissolved oxygen, oil and grease, and total suspended solids); and
- one parameter for human health protection, (i.e., bacteria).

These 17 parameters are known as the AMWQC and set values to guide concerted national level action to protect the shared marine waters of ASEAN.

Following formal adoption of the AMWQC, ASEAN has set about systematically building the capacity for their effective implementation. In 2004 the ASEAN Working Group on Coastal and Marine Environment (AWGCME), the ASEAN Secretariat and an Australian consultancy, Australian Marine Science and Technology Limited (AMSAT) proposed an ASEAN-wide project "*Capacity Building for Implementation of the ASEAN Marine Water Quality Criteria*". The project was funded by the Australian Agency for International Development (AusAID) through the ASEAN Australia Development Cooperation Program - Regional Partnership Scheme (AADCP RPS). This Project, now identified as Phase I, began in 2004 and concluded in 2005. It was designed to advance the implementation of the AMWQC in accordance with the HPA and the VAP by developing strategies for harmonisation of national monitoring frameworks and by building capacity in the design and implementation of marine water quality monitoring programs. In brief, Phase I was designed to operationalise the limits adopted by ASEAN.

Integral to this was the review and subsequent design and application of monitoring programs with the objective of developing standardised or harmonised approaches throughout ASEAN, taking into account financial and human resource constraints within each AMS and providing guidance on sampling frequency and sampling techniques.

Phase I contributed materially to the goal of strengthening ASEAN capacity to address regional development challenges (such as marine water quality and in particular the AMWQC) enabling greater integration of activities within the ASEAN Region and enhanced participation in the global economy.

Specifically, it achieved the following:

- An analysis of national regional and international laws and regulations applicable to marine water quality management and preparation of a comprehensive report and recommendations on approaches to harmonising the regulatory frameworks in ASEAN.
- Enhanced capacity in ASEAN to select and apply standard analytical techniques to the monitoring of marine water quality.
- Enhanced understanding of the design and implementation of marine water quality monitoring programs with an emphasis on the monitoring and management objective rather than monitoring for its own sake.
- Enhanced understanding and direct experience in the conduct and benefits of laboratory inter-calibration to improve the performance and reliability of analyses conducted by national laboratories.
- Formation of a network of officials capable of further development of harmonised marine water quality standards.
- Development of clear recommendations for inclusion of arsenic and zinc criteria in the already agreed AMWQC.

While Phase I successfully built upon the earlier work to develop the AMWQC, conducted under the ASEAN Canada program, an effective mechanism to deliver these into mainstream ASEAN marine water quality management programmes was not apparent. The AWGCME determined that effective delivery depended on inter-related and inter-dependent elements, such as the development of agreed policies and management approaches relating to the maritime estate (both within ASEAN and within each AMS) and the attainment of these policies and management approaches through the implementation of agreed marine water quality monitoring programs that are complementary between each AMS.

3 Current Initiatives

Constraints to managing ASEAN marine water quality to AMWQC standards are significant and include existing impacts on marine water quality attributable to economic development, current and future levels of environmental degradation, increasing population pressures, climate change implications and variation in capacity (including technical infrastructure, financial support and human resource competencies) among the member states. While there are opportunities for harmonisation of marine water management and monitoring amongst member states, there is a major constraint, in that harmonisation must be considered in the context of respecting the sovereign right of each member state to implement mechanisms and standards appropriate to its particular circumstances.

Following completion of Phase I of the Project *Capacity Building for Implementation of the ASEAN Marine Water Quality Criteria* in 2005, the AWGCME and the ASEAN Senior Officials on the Environment (ASOEN) reviewed the outcomes of Phase I and determined that a second phase to the Project was necessary to fully realise the investment in the earlier Phase by making advances in the following areas:

- convert knowledge of standardised testing methodologies into a regionally acceptable manual of standard methodologies;
- convert communication/professional networks into vehicles for achieving actual agreement on standardised methodologies; and
- apply concepts and management approaches in a realistic setting to build acceptance of their relevance and practicality for general adoption.

This was supported by the ASEAN Secretariat and led to the consideration and funding by AusAID under the AADCP RPS, for an extension of the Project to address the opportunities identified. Phase II of the Project, titled *Capacity Building for the Implementation of the ASEAN Marine Water Quality Criteria: Phase II* commenced in May 2007.

The goal of Phase II of the Project was to leverage and extend the success of the Phase I in progressing implementation of the AMWQC by working to develop agreed management concepts, regulatory approaches, monitoring approaches and methodologies and reporting frameworks. The Project supports the ability of the AWGCME and its specialist networks (regulatory and technical) to adopt and implement agreed, documented and published approaches to managing and monitoring marine waters in accordance with the AMWQC.

The key objective of Phase II of the Project was to develop two outputs, namely the *ASEAN Marine Water Quality: Management Guidelines* and the *ASEAN Marine Water Quality: Monitoring Manual*. The Management Guidelines will provide the basis on which common or agreed policies/management approaches can be developed and the Monitoring Manual will provide the methodologies (describing a series of agreed procedures for each of the AMWQC standards) that can be applied to achieve these policies.

These two documents have been published together in this single volume.

ASEAN Marine Water Quality: Management Guidelines

To meet sustainable development objectives, there needs to be an integrated policy-setting, management and assessment approach to controlling impacts and monitoring the status of marine resources and marine waters within the member countries of

ASEAN. The integrated environmental management and assessment approach must address two main elements, viz.:

- marine and estuarine ecosystems and allied living resources, and
- marine and estuarine water quality.

The *ASEAN Marine Water Quality: Management Guidelines* have been developed to provide guidance on a set of common approaches and methodologies that address marine water quality issues within the ASEAN region. They provide a shared set of broad objectives across the ASEAN region and they provide information from which to derive flexible, alternative approaches that can be considered, as appropriate, by each member state to meet the differing needs for various bodies of water and to fit the differences in governance institutions and financial and human capacities that exist. The Guidelines are not intended to be proscriptive.

The *ASEAN Marine Water Quality: Management Guidelines* also recognise that a dynamic approach to environmental management and planning will be required to deal with diversity in the status of aquatic systems, especially in coastal and estuarine settings, both within each member state and across the ASEAN region. In all cases a set of robust, integrated water quality management approaches should ensure sustained environmental and socio-economic benefits.

ASEAN Marine Water Quality: Monitoring Manual

The *ASEAN Marine Water Quality: Monitoring Manual* has been developed as a guide, documenting recommended methods for the implementation of marine water quality monitoring programs. Methods for program design, sampling, data analysis and interpretation and reporting and information dissemination have been selected on the basis of their suitability for use in the ASEAN region.

The methods described focus on the 19 parameters (18 where nitrate and nitrite are counted as one) included in the initial AMWQC report even though only 17 of these were accepted by the ASEAN ministers in 2002 (noting that zinc and arsenic were originally excluded as needing further studies). One other parameter – chlorophyll – has been included in the methods as it is now realised that this is a critical indicator of nutrient enrichment.

Where possible, alternative methods are described. This recognises that each member state does not have access to the same analytical laboratory capacity. This Manual does not list all methods suitable for the AMWQC but attempts to include those believed most likely to be of use in the region.

Project design and data analysis are emphasized in the *ASEAN Marine Water Quality: Monitoring Manual*. Worldwide, many existing monitoring programs have inadequate design – i.e. they are either not set up to answer a clear question (objective) or, are designed such that the data collected is not adequate to answer the key question.

Long-term environmental monitoring is essential to determine baselines, measure change and assess overall ecosystem health. Effective monitoring programmes will improve the management and protection of marine resources and, ultimately, will better protect human health. The creation of an integrated monitoring network that encompasses estuarine, coastal and offshore waters allows documentation of status and change and informs management actions.

4 Future Directions and Priorities

Future directions for ASEAN in the management of the maritime estate within the ASEAN region can be identified in a number of areas. ASEAN has recognized the need for action on key subject areas (listed below) and all rely on sound management policies and effective monitoring and response programmes. They are:

- coral reef, sea grass and mangroves;
- tanker sludge and ballast water;
- solid, liquid and hazardous waste management;
- coastal erosion;
- eco-tourism;
- coastal wetlands, including protected marine areas; and
- clean technology.

Each of these priority areas requires the effective implementation of the AMWQC throughout ASEAN through responsible policy/management agencies and monitoring agencies using agreed monitoring methodologies and reporting systems.

In the future there will be the need for consideration and possible inclusion of more criteria (than the 17 already identified and agreed by the ASOEN and ASEAN Environment Ministers) including, for example, persistent organic pesticides , pH, hydrocarbons and endocrine disruptors. Also of particular relevance and growing significance is the environmental (and concomitant economic and social) implications attributable to climate change and increasing pollution. There will also be the need to consider the emerging paradigms of science relevant to environmental management such as carbon sequestration.

Another possible area for future attention is increasing cooperation between ASEAN nations in areas such as joint technical training, joint monitoring programs (such as, for example, similar programs to the joint monitoring of Straits of Johor by Malaysia and Singapore and monitoring conducted by the Mekong River Commission). This area includes the formalisation of routine inter-laboratory comparisons.

It is the hope of the sponsors editors and contributors that this volume will assist in the improvement of marine water quality in ASEAN and deliver lasting benefits to the people of the region.

Part 1

Management Guidelines

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Part 1

Management Guidelines

1 Introduction

Coastal and estuarine waters are increasingly impacted by pollutants and materials discharged from land-based activities. While this is a global problem, it is a visible and significant issue within the ASEAN region reflecting population expansion and increasing socio-economic development. Increased pollution of marine waters from oceanic and atmospheric transfer of materials is a lesser but significant element. The drivers of changes and impacts reflect, in particular, increasing shipping and transportation activities, as well as intensified urbanisation, industrialisation and agricultural (including mariculture) activities within and outside the ASEAN region. The resultant degradation of water quality impacts on the function and viability of marine ecosystems reduces the aesthetic quality of the environment and has ramifications for human health such that socio-economic opportunities and community well-being are diminished.

ASEAN has a long commitment to the development of regional action and enhanced capacity that will ensure the protection and sustainable use of coastal and marine resources throughout the region. This collective purpose results from recognition of both the geographical continuity of oceanic waters and the tropical marine ecosystems across the region and also a history of trade, cooperation and mutual dependency among member states. Over several decades, ASEAN has continued to build environmental assessment and management capacities within its member states, to initiate joint actions and to build governance frameworks seeking to remediate impacts on coastal marine resources. ASEAN concerns and initiatives have been broadened by engagement with international programs, more recently through establishment of the Partnerships in Environmental Management for the Seas of East Asia (PEMSEA) by the Global Environment Facility (GEF)/ United Nations Development Programme (UNDP)/ International Maritime Organisation (IMO) aiming to ensure the sustainable development of shared waters and coastal and marine resources in East Asian seas.

The ASEAN Socio-Cultural Community Plan of Action has a broad goal for ASEAN, viz.:

'*Nurturing human, cultural and natural resources for sustained development in a harmonious and people-centred ASEAN*".

To contribute towards meeting these sustainable development objectives, there is need for an integrated policy-setting, management and assessment approach for controlling the impacts and for monitoring the resultant changes and the status of the marine resources and marine waters within the member states of ASEAN. The integrated environmental management and assessment must address two main elements, i.e.:

(a) marine and estuarine ecosystems and allied living resources, and

(b) marine and estuarine water quality.

It is now clearly recognised that effective environmental management and assessment of marine resources requires a holistic, ecosystem approach, wherein the status of biological resources and that of associated water quality need be evaluated as a whole. For example, the finding of low dissolved inorganic nitrogen and phosphorus in the water column of a system does not necessarily imply a lack of pollution sources but rather that there may be a high uptake rate (and potentially enhanced levels) of phytoplankton or epiphytes on seagrasses within the system. In this case, appropriate spatial and temporal monitoring design should identify and clarify any nutrient pollution problems and provide insight into potential sources of pollutants that require management action.

Related to this holistic approach of environmental assessment and management has been the shift in management approaches from a focus on trying to manage individual physical and chemical water parameters to one that addresses issues of Beneficial Uses or Environmental Values relating to a designated marine/estuarine area. These terms are explained in Section 7.3.

ASEAN has made significant progress in capacity-building and in harmonising the approach towards evaluation of marine and estuarine ecosystems assessment across the region, supported in particular by the cooperative development of a *Survey Manual for Marine Tropical Resources* (English et al. 1994). This manual addresses approaches, methodologies and assessment of marine ecosystems in the ASEAN region and has been developed, promulgated and implemented across the region (and elsewhere). It provides a common methodological approach for the evaluation of tropical marine and estuarine ecosystems within the region.

Within ASEAN member states (AMS's) there is a range of water quality monitoring programs recently summarised in the Report from Phase I of this project (see AMSAT 2005). The Phase I project identified that different standards and methodologies were being applied by AMS's to marine water quality assessment and management. The need for a harmonised approach to marine water quality across the ASEAN region

provided the primary motivation for the current Phase II project, including its principal outputs – management guidelines and monitoring manual. These guidelines (this document, Part 2–*ASEAN Marine Water Quality: Management Guidelines)*, and the associated monitoring manual (Part 3–*ASEAN Marine Water Quality Monitoring Manual)* provide steps towards a set of common approaches and methodologies that address marine water quality issues within the ASEAN region. These build on and extend earlier cooperative development actions in the ASEAN region that yielded, *inter alia*, a set of 16 key physical and chemical parameters and one human health parameter (bacteria) as ASEAN Marine Water Quality Criteria (AMWQC) (see *ASEAN-Canadian Cooperative Programme on Marine Science*, *Phase II*; McPherson et al. 1999). The AMWQC, slightly modified, were adopted in 2002 by the ASEAN Environment Ministers (Table 1). These ambient water quality criteria provide a contributing benchmark for evaluation of marine and estuarine environmental and human health by managers and policy-makers across the region.

This suite of ambient marine water quality criteria forms a framework for the development of environmental management decisions and judgments from which to develop relevant guidelines, objectives and standards applicable to specific water bodies and water quality in geospatially bounded areas (see Table 2). This latter process is within the prerogative of individual ASEAN member states and represents a vital action in the evolution of a harmonised and an integrated environmental management approach to marine resource management.

It must be recognised that the AMWQC have been scientifically derived as *ambient* marine water quality criteria. However, while the criteria represent the best current scientific information, there remains scientific uncertainty in the knowledge base and understanding of the interactions and the effects of pollutants within ecosystems. For example, data on the effects of the various parameters on biological systems and individual species are not comprehensive, and how pollutants behave (e.g., their transformation, storage and release) in marine and estuarine systems often remains uncertain. Thus, these ambient marine water quality criteria should not be regarded as enforceable limits for water quality pollutants – they are **criteria** and not standards (Table 2). In this they reflect aggregated scientific information about pollutant values (or limiting concentrations) below which it could be expected that in the most sensitive marine environments a particular organism, an ecosystem or a use of the water could occur with a reasonable degree of safety i.e., there should be no biological (or human health) change in the system in response to the specified pollutant if it is present at levels below the prescribed concentration or value.

Table 1. ASEAN Marine Water Quality Criteria (from ASEAN website (http://aseansec.org).

Note: Metrics for two parameters (Arsenic and Zinc) have been subsequently developed by the ASEAN Working Group on Coastal and Marine Environment and, while generally agreed at the technical level, have yet to be formally adopted.

For Aquatic Life Protection		
Parameter	**Criteria Values**	**Note**
Ammonia (NH_3-N)	70 µg L^{-1}	
Cadmium	10 ug L^{-1}	
Chromium (VI)	50 µg L^{-1}	Criteria value proposed by CPMS-II is 48 µg L^{-1}. The meeting recommended adoption of 50 µg L^{-1}, following the existing national standards of member states.
Copper	8 µg L^{-1}	As the proposed value 2.9 µg L^{-1} is too stringent, the Meeting agreed to use rounded-up value of 7.7 µg L^{-1}, the product of the lowest LOEC from a chronic study 77 µg L^{-1} for reproduction for *Mysidopsis bahia* and a safety factor of 0.1.
Temperature	Increase not more than 2C° above the maximum ambient temperature.	
Cyanide	7 µg L^{-1}	
Dissolved oxygen	4 mg L^{-1}	
Lead	8.5 µg L^{-1}	
Mercury	0.16 ug L^{-1}	
Nitrate (NO_3-N)	60 µg L^{-1}	A single criteria value should be derived for nitrate and nitrite combined in future.
Nitrite (NO_2-N)	55 µg L^{-1}	
Oil and grease	0.14 mg/L	Other related parameters, e.g., PAH, should be included in future monitoring.
Total phenol	0.12 mg L^{-1}	
Phosphate (PO_4^{3-}-P)	15 µg L^{-1} (coastal) 45 µg L^{-1} (estuarine)	
Tributyltin	10 ng L^{-1}	
Total suspended solids	Permissible 10% maximum increase over seasonal average concentration.	

For Human Health Protection		
Parameter	**Criteria Values**	**Note**
Bacteria	100 faecal coliform 100 mL^{-1} 35 enterococci 100 mL^{-1}	Coastal water quality for recreational activities.

Importantly, these criteria are independent of environmental settings for the diversity of natural or human-influenced marine waters and are also independent of any notion of Beneficial Uses or Environmental Values. While it is possible that some locations in the ASEAN region may exhibit water quality characteristics that reflect this suite of ambient criteria (for example, some open ocean coral reef environments), these criteria are not water standards metrics for management. Indeed, to make such an operational application would not only be inappropriate but would exhibit ignorance of the importance of environmental settings and socio-economic contexts which are vital planks in water quality management approaches.

Table 2 Terminologies used to explain marine water quality (from AMSAT 2005).

Marine Water Quality Criteria	The scientific information relating to pollutants and the risk or magnitude of the effects caused by such exposure, upon which decisions or judgments may be made on whether a particular quality of water will support a particular environmental value (beneficial use) of the water. The criteria are derived and normally expressed in the form of limiting concentrations that, when not exceeded, will protect an organism, an ecosystem, or a prescribed water use or quality with an adequate degree of safety.
Marine Water Quality Guidelines	The numerical concentrations or narrative statements recommended to support and maintain designated uses of the marine environment. These will generally discuss socio-economic information in principle.
Marine Water Quality Objectives	Numerical concentrations or narrative statements that have been established to support and protect the designated uses of the marine environment *at a specific site*. These will take consideration of relevant socio-economic data for a specific geospatial area.
Marine Water Quality Standards	Marine environmental quality objectives that are recognised in enforceable environmental control laws of a level of government. These are auditable performance benchmarks.

This ASEAN-Australia project (*Capacity Building for the Implementation of the ASEAN Marine Water Quality Criteria – Phase I*: AMSAT 2005) provides the necessary complementary documentation and support information to assist in the development of fully integrated environmental management and assessment of the marine resources and waters in ASEAN.

2 Use of the Guidelines

The ASEAN Marine Water Quality Management Guidelines developed here should provide a shared set of broad objectives across the ASEAN region while allowing flexibility for member states to plan and manage their different circumstances at local and regional levels. The Guidelines provide information from which to derive flexible and alternative approaches that can be considered, as appropriate, by ASEAN member states to meet the different needs for various water bodies and to fit the differences in governance institutions, and financial and human capacities that exist within the ASEAN region.

The Guidelines also recognise that a dynamic approach to environmental management and planning will be required to meet the diversity in status that exists for aquatic systems, especially in coastal and estuarine settings, both within each member state and across the ASEAN region. The marine and estuarine water quality in some systems is highly degraded by pollutants and other outputs from human activities and requires major policy and management decisions regarding the potential for remediation and associated investment. In other marine and estuarine aquatic systems, human impacts are less marked and relatively simple, but in all cases a set of robust, integrated water quality management approaches should ensure sustained environmental and socio-economic benefits.

3 Guiding Principles

The guiding principles, encapsulated in these Guidelines, for the management of ASEAN marine water quality are:

- to achieve sustainable management of the ASEAN marine and estuarine ecosystems;
- to recognise the high variability and complexity inherent in natural water resources and take this into account when evaluating water quality or developing risk-based management strategies;
- to employ an integrated (coordinated and cooperative) approach to water quality management;

- to ensure wherever possible community involvement (including local and indigenous groups and the private sector) in water resource management, including the establishment of Beneficial Uses/ Environmental Values and development of management plans;
- to have AMS's' endorsement of the water quality policy objectives and management arrangements (within an appropriate legal framework); and
- to utilise collaborative and complementary approaches for monitoring marine water quality and in responses to adverse marine water quality events, including choosing appropriate indicators for the issues under consideration.

4 Resource assessment and issues

The marine and coastal environment is an important economic, social and cultural asset in the ASEAN region, providing a wide range of resources that underpins a diversity of socio-economic development and opportunities within member states. It is also of significant biological value representing a range of biodiverse tropical marine ecosystems and landscapes. The marine ecosystems that sustain these resources and Beneficial Uses are subject to existing and potential deleterious impacts, especially from land-based sources. Human interruptions to the hydrological cycle together with discharge of wastes from industrial, agricultural/maricultural and urban activities and infrastructure reflect outcomes from increased population and economic activities. Transport by water and, to a lesser extent, air is the primary mechanism for transfer of pollutants and other materials into the marine and coastal systems. Active management of water quality in river basins and coastal waters can obviate or reduce deleterious effects and yield enhanced opportunities for sustainable socio-economic advantage.

Generally, marine and coastal resource management approaches need to address the sources of impacting materials, considering land-based, atmospheric and oceanic pathways of pollutant materials and the allied human activities that constitute the drivers and pressures from which the materials originate. These include:

- Adjacent land-based sources which usually represent the primary inputs of deleterious materials to marine and coastal systems; these are usually more readily amenable to management actions (Crossland et al. 2005). Generally, the successful management of coastal resources and their waters is dependent on the effective management of associated river basins or catchments (Salomons et al. 1999; von Bodungen and Turner 2001).
- Impacts from atmospheric transport, which may be derived from local, national and often regional transboundary transfer of predominantly land-based materials in particulate and aerosol states with deposition often related to precipitation

events (Steffen et al. 2004). Materials include dust, smoke, industrial effluents and nutrients (nitrogen/nitrates and phosphorus/phosphates) from urban, industrial and rural land-use activities. The phenomenon of transboundary transfer of pollutants (e.g., Asian dust cloud) usually requires management at the multi-national level involving international and national governance, management and assessment for resolution. An example of this approach is the 1974 Helsinki Convention signed under the Helsinki Commission, or HELCOM, that addresses the protection of the Baltic Sea from all sources of pollution.

- Oceanic sources and transfer of deleterious materials in ASEAN seas represent a transboundary context that requires consideration in the development of marine and coastal resource management (PEMSEA 2006). For example, shipping and transportation contributes oil, contaminants, waste and debris, introduction of non-indigenous marine species and health pathogens in ballast waters. While these issues are the focus of international conventions and accords, especially in the IMO, they usually require additional regional and national initiatives for management, e.g., national oil-spill management plans and conditions for operation of oil and gas industries.

The issue of climate change is an emerging challenge for the management of water quality and wider marine and coastal resources. Potential changes in climate patterns and in the regional hydrological cycle (with probable changes in temperature regimes and rainfall patterns across time and space) can be expected to modify the existing distribution and flows of water within river basins and their discharge to coastal seas. Projections for shifts in land use, especially in rural lands, can be expected to yield modifications in the timing and intensity of pollutants carried in runoff and groundwater and thus the water quality of surface and submarine groundwater discharges to coastal seas. The broader issue of marine and coastal vulnerability (coastal geomorphology, integrity of ecosystems and ecosystem services, coastal infrastructure) in addition to water quality will need to be factored into related marine and coastal management approaches.

These issues require the development of policy and management plans to ensure sustainable development of marine and coastal resources – a process that is gaining momentum within the AMS's. These issues are not unique to the ASEAN region. Globally, there is a developing accord in approaches to marine and coastal management, including that:

- A holistic ecosystem approach should be adopted (Salomons et al. 1999);
- The continuum of a river basin or catchment and its adjacent coastal sea represents the coastal management unit (Crossland et al. 2005);
- River basin or catchment sizes (small catchments <10 000 km^2 or large catchments >10 000 km^2) may represent different sets of physical and biogeochemical

dynamics that need to be differentially recognised in management approaches (Smith et al 2005);

- Much of the deposition, sequestration and transformation of pollutants occurs within a relatively small distance of the shoreline and in estuaries (water residence time is a factor in biogeochemical transformations that affect assimilation capacity) (Smith et al. 2005); and
- Water quality is a vital component of overall coastal management and needs to be integrated into overall environmental management plans and processes.

Environmental management approaches for marine and coastal environments and water need to give consideration to the special geographical and climatic settings of the ASEAN region, including:

- Tropical systems and waters. Most examples of coastal management approaches (and underpinning scientific understanding) relate to temperate systems and waters; there are few models of tropical management systems that are analogous with the ASEAN settings.
- Monsoonal climate regimes. Monsoonal rainfall and its effect on the patterns of river system discharges to coastal seas (and/or continental shelves) need to be considered in the development of management approaches and have ramifications for monitoring and assessment designs. For example, residence times of water in estuaries will change markedly between monsoon and drier seasons, influencing the capacity of estuarine and coastal systems for sustaining chemical transformation processes and modifying the seasonal composition of water discharged to coastal seas.
- Sediment discharge. Sediments discharged from the continental landmass of the ASEAN region represent a high proportion of the global sediment load to the ocean. Sediment discharge is a common characteristic of many river systems in the tropics, representing both natural transport and flows exacerbated by human activities in river catchments (Figure 1). The potential for relatively high natural or "background" sediment discharges from mainland river systems in the region should be recognised in the development of management strategies.
- Status of coastal and estuarine waters. The long history of human pressures in the ASEAN coastal zone allied with extensive socio-economic development and relatively high human population has resulted in a number of degraded coastal systems. Management of marine and coastal systems needs to encapsulate a wide range of conditions from relatively pristine to degraded environments and water conditions. Costs for remediation of systems, where possible, can be expected to be far greater than those for the maintenance of existing condition of systems and their water conditions.

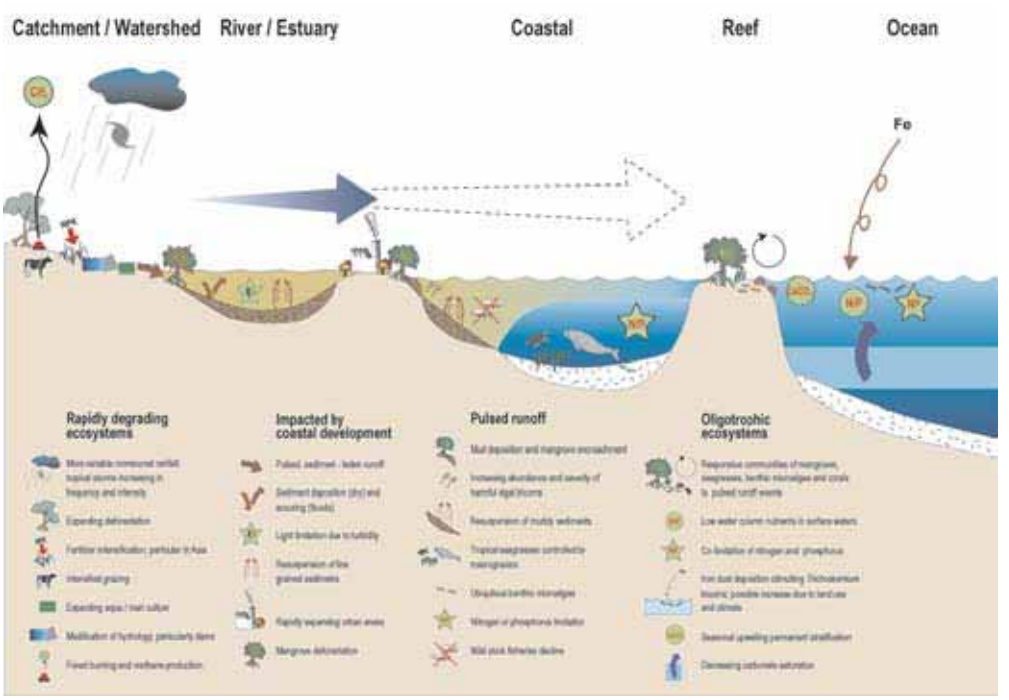

Figure 1. Representation of tropical coastal systems and human pressures (from Crossland et al. 2005).

5 Policy objective and values

The broad coastal environmental **policy objective** is founded in the four strategic thrusts supporting the goals of the ASEAN Socio-cultural Community Vientiane Actions Programme (2004):

1. building a community of caring societies;
2. managing the social impact of economic integration;
3. promoting environmental stability; and
4. promoting an ASEAN identity.

A specific measure aimed at *promoting environmental stability* and sustainable natural resource management that meets the current and future needs is to "*promote the sustainable use of ASEAN's coastal and marine environment through the implementation of the ASEAN criteria for marine waters, and marine heritage and protected areas*."

This is essentially a Sustainable Development approach to marine waters and their companion environmental resources, in essence seeking to manage coastal zone resources and to maintain or improve coastal and estuarine water quality. As a consequence, a number of principles that underpin management approaches for marine water quality could be included within the management and planning actions by member states of ASEAN.

The **management principles** include:

- strategic and integrative planning that encompasses environmental, economic and social elements across short-term and long-term scales;
- application of the precautionary principle where there is a presumed threat of significant environmental damage and lack of scientific certainty;
- promotion of economic developments that have opportunity to enhance environmental protection;
- adaptive and flexible policies and instruments for the management of industries and their discharges rather than a solely regulatory approach;
- awareness of the potential for global level environmental (see Beneficial Uses/ Environmental Values) impacts from policy decisions; and
- involvement of stakeholders (e.g., industry, community) in the planning and decision-making processes.

These principles and the AMWQC could be expected to underpin the evolution of a common approach in **management processes** for marine and estuarine water quality within the ASEAN member states. The management processes should incorporate:

- national consistency in methods for setting environmental goals and standards;
- clear and transparent administrative processes;
- explicit assignment of responsibilities for the various phases of administration and operation of the management actions;
- accountability for progress towards desired water quality and wider environmental goals based on monitoring and open reporting;
- matching of administrative and management structures to geospatially defined physical and socio-economic constraints (usually units for management are based on the geospatial boundaries of a river basin catchment/sub-catchments and adjoining coastal sea);
- administrative mechanisms that are responsive to change and development in both the natural milieu and socio-economic opportunities (including technological options); and

- involvement of relevant stakeholders in defining environmental and water quality goals, development of plans and implementation of strategies, regulatory and management actions.

Incorporation of these processes will lead to a consistent approach in the development and evolution of national strategies for marine and estuarine water quality management in member states across the ASEAN region, while also providing for flexibility to encompass different political and legislative frameworks, and socio-economic and environmental settings.

6 A Framework for Water Quality Management

Globally, a variety of environmental management approaches for marine and estuarine resources and water quality has been adopted and is being implemented, nationally and across regions (e.g., the European Union Water Directive: European Union 2000). Over the last few decades, these approaches have evolved towards a relatively common management framework of essential elements, in keeping with the increased understanding of the requirements to utilise an effective integrated coastal management approach.

6.1 Integrated Coastal Management

The application of an integrated coastal management (ICM) approach can ensure that sustainable environmental, social and economic advantages are obtained from regional marine and coastal resources. The maintenance or improvement of water quality is a fundamental element for management in such an approach. ICM provides a sound and dynamic context and set of process elements that constitute adaptive management to meet changes in the natural settings and the evolving demands of socio-economic development. An important attribute of ICM is that management actions are taken to achieve management goals and that these actions are monitored and assessed through time to evaluate the success or otherwise of the management policies and operational decisions (see cycle 1, Figure 2). As a consequence, the management actions and goals can be re-focused and modified in a transparent way, based on sound and practical information. This allows for continuous and improved effort to meet the stakeholder-informed management objectives for the area, as represented by the second (and any subsequent) cycle in Figure 2.

An important attribute of ICM is that it allows staged approaches to the management of marine and coastal resources and water quality, e.g., through the setting of initial management goals aimed at remediation of an ecosystem or water body and then the setting of subsequent and increasingly focussed goals and management actions for sustaining the quality of the ecosystem or water body. Similarly, an ICM management

approach can underpin a progressive and staged economic investment in ecosystem remediation or sustainable development.

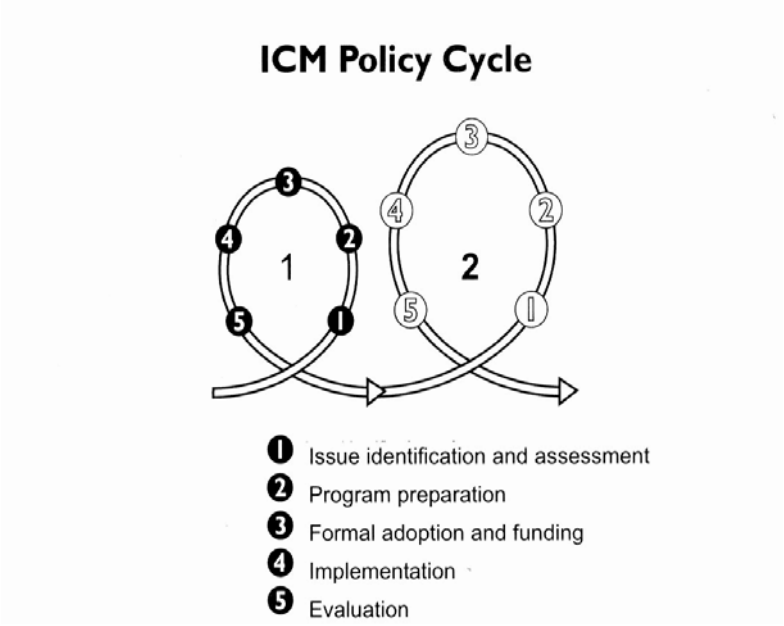

Figure 2. The steps in the integrated coastal management process and cycles (from GESAMP 1996, as adapted in Olsen et al. 1999).

6.2 Planning and sequence of approach

The development of a management approach and plan for marine and coastal waters can be readily achieved by application of ICM encapsulating the framework for marine and water quality earlier developed within ASEAN. A generic model for the management and planning of coastal and marine resources and water quality is shown in Figure 3.

The key elements encompass the development of site-related resource and water quality management goals reflective of environmental values, the setting of relevant water standards where required, implementation of appropriate environmental monitoring and assessment, and management responses and modified actions relating the field status to the management goals.

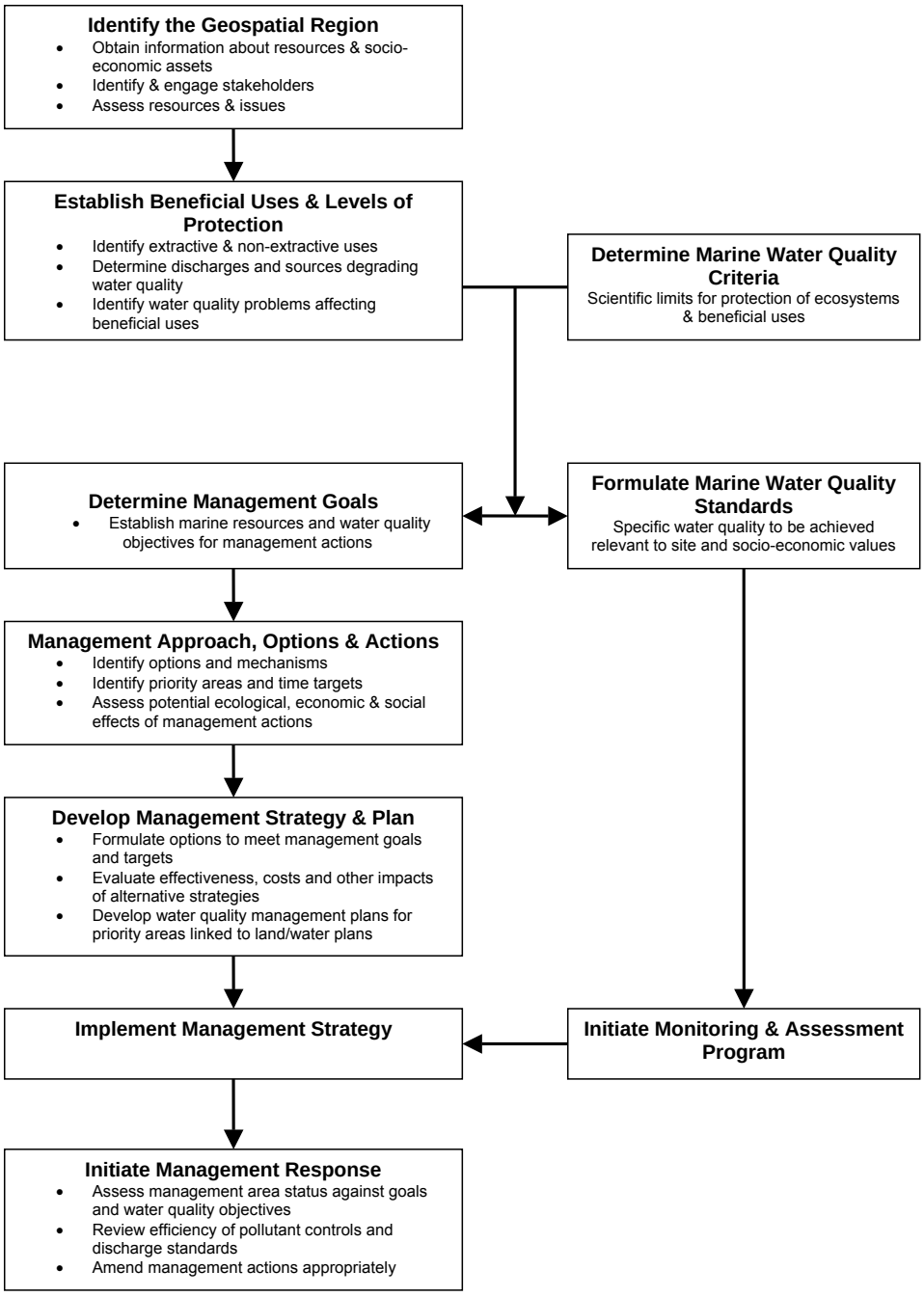

Figure 3. Model for management and planning of coastal and marine resources and water quality.

7 Management of Marine Water Quality

7.1 Processes

Achievement of the broad environmental policy objective for marine waters and resources adopted by ASEAN requires:

the implementation of management approaches that incorporate the generic management principles and processes (outlined above) and

recognition that water quality management is not separate from but is a vital part of the broader marine and coastal management imperative.

Generally, it can be justifiably argued that water quality monitoring and/or assessment *in the absence of associated environmental management goals and strategies* is a poor investment of time and resources i.e., water quality actions should be part of and inform a broader marine and coastal management approach.

In 2004, a workshop forum of the ASEAN Working Group on the Coastal and Marine Environment (AWGCME) and international experts in Kuala Lumpur, Malaysia identified a management framework for applying marine water quality criteria and developing water quality standards (Figure 4).

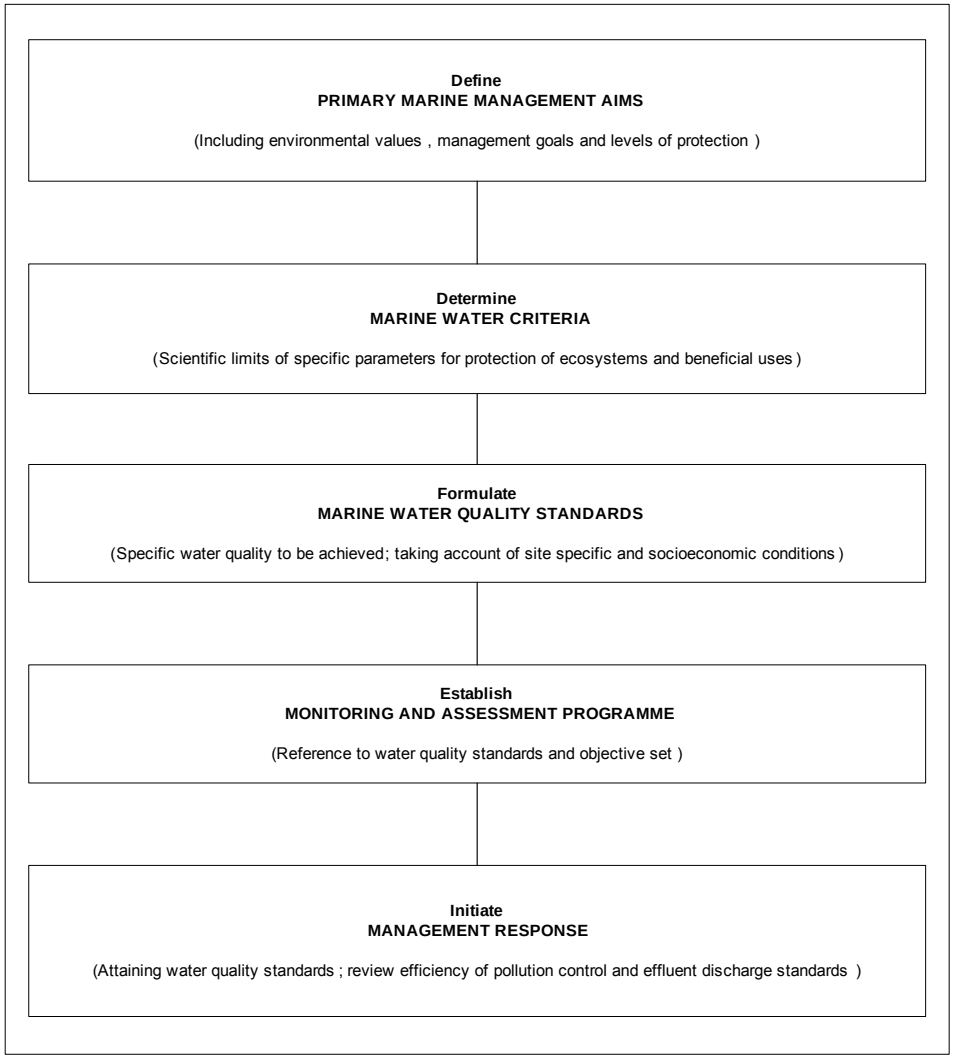

Figure 4. A management framework for marine and estuarine water quality in ASEAN as an element of marine environmental management (from Tong 2004).

This framework outlines a process for determining water quality standards and broadly relates water quality monitoring and assessment to the wider management approach (top and bottom box). However, while achieving its original purpose, the framework provides only a limited consideration of the key processes for integrating the socio-economic settings (Beneficial Uses) with water quality criteria and establishing the broader management goals and objectives that are fundamental to overall environmental management. These elements are considered below.

Management goals and water quality objectives are crucial elements of regional and site-specific coastal management plans. An integrated environmental management approach that includes specific marine water quality objectives reflective of the Beneficial Uses should provide an effective, flexible and adaptive framework for application by AMS's.

7.2 ASEAN Marine Water Quality Criteria

The ambient water quality criteria contained in the AMWQC (Table 1) are a regional benchmark from which to develop water quality management strategies and plans within the ASEAN region. The AMWQC, in conjunction with socio-economic or Beneficial Use considerations, provide a common reference point for derivation of water quality guidelines and objectives for integrated coastal management approaches in ASEAN member states. The AMWQC contain two different sets of metrics for protection of aquatic life and protection of human health, effectively foreshadowing the development of different classes of water type in management application. Indeed, most ASEAN member states in formulating water quality guidelines and standards are embracing this approach of identifying specify Beneficial Uses with a set of metrics for water quality parameters and concentrations i.e., Class Designators, defining different water types related to a management purpose for a defined area.

The set of AMWQC were developed in the mid-1990s and are based on the scientific knowledge available at that time. Further work within ASEAN can be expected to modify and expand this initial set of criteria as relevant scientific information and marine systems understanding continues to advance and lead towards diminution of our existing uncertainty about marine environment-toxicological relationships, and to meet the additional needs of the region. In particular, the understanding of the role of sediments as sinks and sources of pollutants is evolving such that the assessment of pollutants in marine sediments rather than in the water column may in future provide a more effective monitoring and assessment approach, especially for monitoring and evaluation of trace metal and organic contaminants. In addition, there is considerable global scientific effort being invested into the derivation of robust indicators of environmental change, especially in relation to pollutants and associated materials. Usually the targeted indicators comprise a naturally occurring biological entity or a readily measured biogeochemical process that integrates pollutant effects or other drivers of change in systems, in the way that chlorophyll concentration is used as an indicator of eutrophication.

7.3 Beneficial Uses (or Environmental Values)

Beneficial Uses (also referred to as Environmental Values) are particular uses or values of the environment that are conducive to public benefit, welfare, safety or health and which require protection from the effects of pollution, waste discharges and deposits (see ANZECC 1994). Environmental planners and managers tend to

refer to Beneficial Uses to describe a water body based on its intended use or function; scientific literature tends to refer to Environmental Values reflective of the parameters and criteria for the water body to perform a particular function or use. From an operational management viewpoint, the terms Beneficial Uses and Environmental Values are representative of two different approaches to the same issue within the context of ESD (e.g., wise management of a specified water body), but lead to a set of common outcomes (e.g., prescribed environmental criteria or standards required if the water body is to perform or maintain a particular use in an ecologically sustainable manner). Sustainable development and environmental and human well-being in coastal regions depend on the maintenance of a high standard of Beneficial Uses.

Beneficial Uses relevant to marine and estuarine waters include:

- marine and estuarine ecosystems;
- recreational and aesthetic values;
- human health;
- agricultural water*;
- industrial water*; and
- drinking water*.

[* usually relate to freshwater]

Agricultural, industrial and drinking water are Beneficial Uses that are usually associated with fresh water quality objectives; however, they may also be important Beneficial Uses in some coastal and estuarine settings.

The determination of Beneficial Uses for a specified area or water body constitutes a fundamental step in the management process and represents the uses that stakeholders (including the wider community) want to preserve. As part of the development of management goals, the identified Beneficial Uses are matched to scientifically-based marine water quality criteria to provide water quality objectives for management purposes, thus converting environmentally-related Beneficial Uses into quantitative water quality objectives that will support and protect the coastal and marine resources and environment of the defined management area.

Clearly, the management of marine water quality within ASEAN waters should be supported by the determination and recognition by ASEAN member states of a number of types or classes of water bodies and ecosystems within their jurisdiction ranging from the relatively pristine to relatively degraded. The Beneficial Uses ascribed to each water body and the human uses (current and potential) should provide a tapestry of water zones reflecting classes of water and ecosystem types (i.e., Class Designators: AMSAT 2005), each addressed by geospatial site-related management plans and approaches.

The objectives of management for each water body or managed area thus would be expected to reflect (but not exclusively):

- the requirement for sustaining the broad "health" of marine waters in the ASEAN region;
- the need for protecting and maintaining a diversity of marine and estuarine ecosystems (e.g., pelagic waters, coral reefs, mangroves and seagrass communities) that are within the national waters of ASEAN member states; and
- the necessity for continued sustainable socio-economic development that provides national and regional advantage from the Beneficial Uses (e.g., ports, fisheries, tourism) in marine and estuarine waters of AMS's.

The determination of Beneficial Uses for a locality or region would usually involve broad stakeholder consultation and often negotiations, and would particularly involve local community stakeholders to ensure ownership and acceptance of both the environmental attributes and the management goals.

7.4 Management Goals

Management goals for a coastal area describe what is to be protected and the level of protection. They need to be developed in a way that they can become key objectives for achievement through management plans and other regulatory tools and they should be related to environmental parameters that can be measured. Management goals need to reflect the specific problems and threats, the desired levels of protection for marine ecosystems and the attributes of the resources that are to be protected (the Beneficial Uses). Management goals often encompass a dynamic approach, recognising that a series of progressive targets across time may be required and that these would be addressed through implementation of strategies aimed to sequentially attain higher environmental quality targets and metrics for water quality.

The setting of management goals for a defined area requires consideration of the array of marine and coastal resources and water characteristics, biodiversity and biological processes, the socio-economic status and potential for the area, the desires of stakeholders and national resource and water quality policies. Determination of water quality issues and objectives are part of the overall process of determining management goals. This approach presumes the need for water quality monitoring as a vital part of assessing the performance and effectiveness of the overall management strategy and actions in the attainment of the management goals (which may including goals of amelioration or diminution of pollution as opposed to goals of maintenance of existing environmental and water quality status).

Generally, management goals for marine and coastal areas will reflect issues and the actions needed to be taken to address pressures on water quality from the associated river catchments, atmospheric and oceanic inputs of materials including pollutants (natural and human-influenced) and the extraction of water and resources. Transboundary issues will need to be recognised and considered in the development of both management goals and water quality objectives, in particular those associated with materials transport and fluxes within atmospheric and oceanic domains. Here, the movement of people (for example, tourist movements and tourism activities) may also be at issue.

7.5 Water Quality Objectives and Standards

The establishment of water quality objectives is a key part of any integrated coastal management approach that is developed and implemented to ensure judicious use of the marine resources and water quality of a coastal management area. The determination of marine and coastal water quality objectives usually involves a two-step process:

- Establishment of a set of Beneficial Uses for the area.
- Establishment of scientifically well-founded water quality criteria (desirable range and maximum concentration levels) corresponding to each Beneficial Use.

The **Beneficial Uses** for an area require identification of the natural catchments, marine and coastal resources, social interests, actual and potential economic opportunities and administrative areas. Stakeholder discussions would be expected to assist planners and technical specialists in identifying resources issues and identifying present environmental and future values that may be needed and achievable. Initial nomination of Beneficial Uses from such a consultative process will need to be reviewed in the context of scientific and economic assessments for the management area, thus yielding a final list of Beneficial Uses.

The **water quality criteria** describe the water characteristics that need to be maintained to sustain specified uses or to protect specific Beneficial Uses. These are usually set conservatively (at low levels of pollution) to ensure long-term protection of the Beneficial Uses and are expressed as a desirable range and a maximum level of concentrations. In some cases, a value judgment is required as to the acceptable risk to human health or ecosystem impairment. Where several Beneficial Uses are set for a defined water body or ecosystem assemblage, the most limiting or stringent guidelines would need to be met in determining the local area water quality objectives. The AMWQC provide a benchmark for this action.

The resultant **water quality objectives** reflect specific water quality decisions and targets for a defined area. The outcome of this process contributes to both the broader management goals and specifically defines the water quality objectives that need be met by the management plans and actions for the area.

The process can be informed by the development of guidelines that relate to Class Designators for specific ecosystems and water-body types (see below). The water quality objectives form a foundation for the development of water quality standards, if required, to control effluent emissions from industry and public sector infrastructure, such as sewage treatment plants.

Water quality standards represent an enforceable target of pollutant concentration that an activity is permitted to discharge into receiving waters, from permitted discharges or accidental spills. Standards will provide metrics for regulated limits of pollutant discharges vital to ensuring enforcement, compliance and pollution control. Thus, water quality standards can provide a performance benchmark that is auditable. In turn water quality standards provide a target for measuring success of immediate management actions and by providing an assessment of compliance options put in place in the management plans for a defined water body or coastal area.

Water quality standards are usually required as a management action to ensure that the water quality management objectives will be met in local receiving waters in the presence of socio-economic developments that can contribute pollutants. Through such management actions, including setting of industry-specific and facility-specific emission standards, management objectives can be proceeded towards or achieved in order to maintain or remediate water and broader environmental quality of the local area. The determination of water quality standards for emission control or industry-specific discharges will be site- related, will require knowledge of the existing local water quality, and usually will acknowledge dilution potential and the assimilative capacity of the receiving waters (and allied ecosystems) in ascribing a mixing zone for discharged waters and for receiving waters. A key element in the setting of standards will be the application of risk assessment methods to gauging the probability of adverse effects on the Beneficial Use (e.g., environmental or human health) that may potentially or actually result from the convenience of discharging pollutants to the receiving waters. This type of risk assessment is generally referred to as ecological risk assessment (ERA), which has a systematic and increasingly quantitative methodological foundation, and is itself increasingly integrated into environmental impact assessment (EIA) protocols. Consideration of the pollutant loadings are generally considered as total mass loadings (concentration of pollutant x volume), although standards are then usually derived as concentrations (e.g., upper levels and concentration limits for compliance, or a weighted average over, say, 24h or 48h).

Knowledge of conceptual models of the pollutants and their relationship with the receiving waters environment and usually dynamic models appropriate to the local conditions will be involved in the determination of water quality standards by environmental managers in consultation with the relevant industrial sector stakeholders. Sector-specific effluent quality guidelines and standards (e.g., for municipal water treatment facilities, steel manufacturing, pulp and paper production, oil and gas

refining, abattoirs, livestock rearing, mariculture operations) may be developed to regulate waste and pollutant levels consistent with best available technology that is economically achievable (BATEA), as exemplified by ANZECC. Such an approach can offer useful planning tools for both industries and environmental managers and which can be fine-tuned locally to meet the site condition as a setting for economic development and the maintenance (or improvement) of water quality. In some cases, it could be expected that concentration metrics of a specific Class Designator (where applied) may be sufficient and thus obviate the need for additional determination of local, site-specific water standards.

Overall, the management goals for an area remain as the primary target for the integrative and adaptive management actions. In this context, ambient water quality values (e.g., the AMWQC) often represent the ultimate desirable endpoints for marine and coastal management actions. The management reality of the concentrations of pollutants and physical parameters in waters of interest requires application of an iterative process, such as the ICM cycle - management goals and action plans (including setting site specific water quality standards), monitoring the effectiveness of the management actions and adjustment of the management actions and, often, the metrics of the water quality standards being enforced. In this way, water quality and wider environmental characteristics can be managed for improvement and remediation directed to an ultimate goal expressed by the ambient water quality values. This type of management action requires time for achievement of goals and invariably incurs a cost. Hence, management actions should be allied with cost-benefit analyses and usually require medium to long-term commitments by governance institutions for achievement of the goals.

7.6 Class Designators

There is considerable variability in coastal and marine settings and marine water quality within AMS's and across the ASEAN region. Thus, the national use of a single-value marine water quality standard for all settings and water types is unlikely to meet the demands for judicious use and conservation of marine and coastal resources, and also to meet the needs for sustainable development.

The ASEAN Working Group on Coastal and Marine Environment, in association with the earlier ASEAN-Canadian project, developed the ASEAN Marine Water Quality Criteria for 17 water quality parameters (see Table 1, above), following a transparent procedure founded on scientific knowledge (McPherson et al. 1999). These Criteria provide a single maximum concentration value, based on the premise of long-term no-effect concentration, which if not exceeded should provide for protect to all forms of aquatic life and aspects of aquatic life cycles. The addition of the bacterial water quality parameter provides a metric consistent with no adverse human health outcomes in marine waters. In essence these Criteria represent the basis for development of two water quality suites that are generic Class Designators, and offer an idealised yardstick

by which to gauge broadly the variation in water quality across the ASEAN region – water quality variation both due to natural conditions and settings and resulting from human impacts.

As a water quality management tool the Criteria are of limited use, especially at the level of site or management unit. There is a need to consider the development of different water classes to address diverse settings if water quality management is to achieve both conservation and specific socio-economic development goals appropriate to coastal management actions. A single set of water quality metrics for management application does not allow for the different ecosystem water quality requirements nor does it offer an environmentally sound basis for sustainable economic development. The "aquatic life" parameters of the Criteria could be broadly viewed as an optimal set of water quality metrics that might characterise a relatively pristine coral reef environment. Clearly, seagrasses and mangrove systems that are relatively unperturbed could be expected to be sustained in conditions that have more relaxed values for nutrient and sediment loads in the water column; examples of such conditions and sustained systems are common across the ASEAN region. The adoption of the single metric suite of water quality values represented in the single "aquatic life" parameters could severely curtail socioeconomic development opportunity by not reflecting the environmental reality of sustainable and well-managed development within the majority of estuarine and coastal systems.

These issues have been appreciated by AMS's and effort continues to be directed to the evolution of marine water quality criteria and standards.

A system of Class Designators that specify limit metrics for pollutant levels and biophysical conditions for specific ecosystems and water-body types provides a useful basis for integrated water quality management. Within ASEAN, Malaysia has been using such a system for managing freshwaters for several decades, and beyond the ASEAN region there are examples of application of Class Designator systems to management of marine and coastal waters, e.g., in the European Union and in Australasia. These Class Designator systems can be transparently related to Beneficial Uses for estuarine and marine areas. The application of a graduated series of guidelines encompassing a series of Class Designators supports an adaptive management approach to water quality and coastal systems, as represented in the management model (Figure 3, above). The development of Class Designators for application at local and sub-national levels should be derived at national levels.

ASEAN member states are at different development stages in their capacities and application of coastal management approaches, including the derivation of marine water quality management regimes. Thailand and the Philippines have adopted a water quality designator approach and developed a set of Class Designators that provide for management of marine water quality across conservation and sectoral socioeconomic development activities (Table 3). To a lesser extent, Indonesia and Viet Nam have proceeded along similar lines. Other AMS's have water quality monitoring

programs, some of which are directed towards specific management targets, for example, Singapore harbour monitoring. The status of water quality monitoring regimes across ASEAN is summarised in the Phase I report to this project (AMSAT 2005); Table 3 briefly updates the earlier assessment.

Table 3. Marine water quality Class Designators for Beneficial Uses currently applied in some ASEAN Member States.

Country	Class Designator Categories
Brunei Darussalam	Applies a partial sector-based approach e.g., discharge standards associated with Pollution Control Guidelines for Industrial Development
Cambodia	Coastal waters
Indonesia	Marine tourism activities Harbour waters Marine biota goals for other waters
Malaysia	A Class Designator approach to marine waters is in progress and scheduled for introduction in 2008. An effective water classes approach to freshwater management has been in place for several decades.
Myanmar	Early stage of water quality development
Philippines	**SA** (commercial shellfish tourism and marine park zones, coral reef parks) **SB** (Recreational Water Class I e.g., swimming; Fisheries Water Class I e.g., spawning areas) **SC** (Recreational Water II e.g., boating; Fisheries Water Class II e.g., commercial and sustenance fishing; Mangrove & wetland sanctuaries) **SD** (Industrial Water Supply Class II e.g. cooling.
Singapore	Singapore's approach to water quality issues is sector-based rather than from a Class Designator perspective, e.g., the Maritime and Port Authority of Singapore is responsible for shipping-related water quality, the Agri-Veterinary Authority of Singapore for aquaculture-related water quality, the National Environment Agency for land-based discharge and recreation-related water quality, and the National Parks Board for marine biodiversity-related water quality
Thailand	**Class I** (Natural Resource Preservation) **Class II** (Coral Reef Conservation) **Class III** (Aquaculture) **Class IV** (Recreation) **Class V** (Industry or Ports) **Class VI** (Residential Districts)
Viet Nam	Bathing and Recreation Areas Aquatic Cultivation Areas Other areas

In the development of a Class Designator approach within AMS's, the suite of targeted physico-chemical parameters has usually encapsulated most, if not all, the parameters that are included in the set of AMWQC (Textboxes 1–3). In addition, some states have a more extended suite of targeted parameters underpinning their marine water management enterprise.

Box 1. Thailand: Marine Water Quality Class Designators and Beneficial Uses.

	AMWQC	I	II	III	IV	V	VI
Parameter	($\mu g\ L^{-1}$ unless otherwise stated)						
Temperature	≤2°C increase over maximum ambient	≤1°C increase	No change	≤1°C increase	≤2°C increase	≤2°C increase	≤2°C increase
Dissolved oxygen	4 mg L^{-1}	4 mg L^{-1}	6 mg L^{-1}	4 mg L^{-1}	4 mg L^{-1}	4 mg L^{-1}	4 mg L^{-1}
Total suspended solids	≤10% increase over seasonal average						
Oil & grease	0.14 mg L^{-1}	Not unpleasant					
Mercury	0.16	0.1	0.1	0.1	0.1	0.1	0.1
Cadmium	10.0	5.0	5.0	5.0	5.0	5.0	5.0
Chromium (VI)	50	50	50	50	50	50	50
Copper	8	8	8	8	8	8	8
Arsenic (III)**	120	-	-	-	-	-	-
Lead	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Zinc**	50	50	50	50	50	50	50
Cyanide	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Ammonia – N	70	70	70	100	70	70	70
Nitrite – N	55	-	-	-	-	-	-
Nitrate – N	60	20	20	60	60	60	60
Phosphate	15 (coastal) 45 (estuarine)	15	15	45	15	45	45
Phenols	120	30	30	30	30	30	30
Tributyltin (TBT)	10 ng L^{-1}	0.010 (TBT)	0.010 (TBT)	0.010 (TBT)	0.010 (TBT)	0.010 (TBT)	0.010 (TBT)
Bacteria	100 coliform 100 mL^{-1} 35 enterococci 100 mL^{-1}	1000 total coliform 100 mL^{-1}	1000 total coliform 100 mL^{-1}	1000 total coliform 100 mL^{-1}	1000 total coliform 100 mL^{-1}	1000 total coliform 100 mL^{-1}	1000 total coliform 100 mL^{-1}
Beneficial Uses (or Environmental Values)	**Protection of Aquatic Life**	**Natural Resources Preserva-tion**	**Coral Reef Conserva-tion**	**Aqua-culture**	**Recreation**	**Industry or Ports**	**Residential Districts**

** Not formally adopted as AMWQC

Box 2. Philippines: Marine water quality Class Designators and Beneficial Uses.

The Philippines classifies its water bodies according to their Beneficial Uses. Four different types of water usage with their corresponding numerical values have been identified to maintain minimum conditions necessary to assure the suitability of water for its designated use and classification.

Water classifications are arranged in the order of protection required with class **SA** having generally the most stringent water quality for marine/coastal waters and class **SD** waters have the least stringent water quality for marine/coastal waters.

The scheme has been successfully implemented and serves as the basis for enforcement of the Department of Environment Administrative Order no.35 series of 1990, otherwise known as the Revised Effluent Regulations of 1990.

	AMWQC	**SA**	**SB**	**SC**	**SD**
Parameter					
Temperature	≤2C° increase over maximum ambient	<3C° rise	<3C° rise	<3C° rise	<3C° rise
Dissolved oxygen	4 mg L^{-1}	70% saturation	70% saturation	70% saturation	50% saturation
Total suspended solids	≤10% increase over seasonal average	30% increase	30% increase 30 mg L^{-1}	30% increase 30 mg L^{-1}	60% increase 30 mg L^{-1}
Oil & grease	0.14 mg L^{-1}	1000 (pet.ether)	2000 (pet.ether)	3000 (pet.ether)	5000 (pet.ether)
Mercury	0.16	2.0	2.0	2.0	
Cadmium	10.0	10.0	10.0	10.0	
Chromium (VI)	50	50	100	100	
Copper	8.0		20.0	50	
Arsenic (III)**	120	50	50	50	
Lead	8.5	50	50	50	
Zinc**	50				
Cyanide	7.0	50	50	50	
Ammonia – N	70				
Nitrite – N	55				
Nitrate – N	60				
Phosphate	15 (coastal) 45 (estuarine)				
Phenols	120	ND	10		
Tributyltin (TBT)	10 ng L^{-1}				
Bacteria	100 Faecal coliform 100 mL^{-1} 35 enterococci 100 mL^{-1}	ND	200 faecal colliforms 100mL^{-1}		
Beneficial Uses (or Environmental Values)	**Protection of Aquatic Life (ambient water quality criteria)**	**Commercial Shellfish, Tourism, Marine Park Zones, Coral Reef Parks**	**Recreational – Class I (e.g., swimming) Fisheries – Class I (spawning areas)**	**Recreational II (e.g., boating) Fisheries II (commercial/sustenance fishing) Mangrove & wetland sanctuary**	**Industrial water supply – Class II (e.g., cooling)**

** Not formally adopted as AMWQC
ND= not detectable

Box 3. Indonesia: Marine water quality Class Designators and Beneficial Uses.

Indonesia marine water quality standards are promulgated in the Decree of the State Minister of the Environment Number 51 of 2004 on Marine Water Quality Standard, revising the previous standard on the Ministerial Decree Population and Environment No. 2, 1988: Guidelines for Environmental Quality Standards for Water, Wastewater, Air and Seawater.

Article 7 is articulated in this regulation to address the absence/lacking of beneficial uses designation of coastal areas in some areas in Indonesia. Those coastal waters falling outside of Harbour Waters and Marine Tourism shall refer to the Marine Biota Quality Standard.

	AMWQC	**I1**	**I2**	**I3**
Parameter	(ug L^{-1} unless otherwise stated)			
Temperature	≤2C° increase over maximum ambient	Natural condition of environment	≤2C° increase over maximum ambient	Natural Coral: 28-30 Mangrove: 28-32 Seagrass: 28-30
Dissolved oxygen	4 mg L^{-1}	-	> 5 mg L^{-1}	>5 mg L^{-1}
Total Suspended Solids	≤ 10% increase over seasonal average	80 mg L^{-1}	20 mg L^{-1}	Coral: 20 mg L^{-1} Mangrove: 80 mg L^{-1} Seagrass: 20 mg L^{-1}
Oil and Grease	0.14 mg L^{-1}	5 mg L^{-1}	1 mg L^{-1}	1 mg L^{-1}
Mercury	0.16	3.0	2.0	1.0
Cadmium	10.0	10.0	2.0	1.0
Chromium (VI)	50	-	2.0	5.0
Copper	8.0	50.0	50.0	8.0
Arsenic (III)**	120	-	25.0	12.0
Lead	8.5	50.0	5.0	8.0
Zinc**	50	100	95	50
Cyanide	7.0			5.0
Ammonia-N	70	300	Nil	300
Nitrite – N	55	-		
Nitrate – N	60	-	8	8
Phosphate	15 (coastal) 45 (estuarine)	-	15	15
Phenols	120	2.0	Nil	2.0
Tributyltin (TBT)	10 ng L^{-1}	0.010		0.010
Bacteria	100 faecal colliforms 100 mL^{-1} 35 enterococci 100 mL^{-1}	Coliform 1000 MPN 100ml^{-1}	*E.coli* 200 MPN 100ml^{-1} Coliform 1000 MPN 100ml^{-1}	Coliform 1000 MPN 100ml^{-1}
Beneficial Uses (or environmental values)	**Protection of Aquatic Life (ambient water quality criteria)**	**Harbour Waters**	**Marine Tourism Activities**	**Marine Biota**

** Not formally adopted as AMWQC

The development and use of Class Designators for management of marine water quality is clearly linked with the determination of Beneficial Uses for estuarine and coastal waters as part of the broader ICM process, as outlined above. Indeed, this relationship is apparent in the management practices for marine water quality by the Philippines, Thailand, Indonesia and Viet Nam; practices that have been in place and evolving for up to a decade or longer. There remains a diversity of terminology and water groupings associated with the Class Descriptors and associated Beneficial Uses across the ASEAN region.

The ASEAN Regional Expert Review Workshop, which was held 19-21 November 2007 in Bangkok, Thailand reviewed the current application of Class Designators by AMS's and discussed the allied Beneficial Uses imperatives for management presently being applied. The meeting agreed that Beneficial Uses could be readily described by nine marine water groups for each of which it would be possible to develop an appropriate suite of metrics that sets limits to pollutant levels and/or physical changes (Table 4).

Table 4. Suggested Beneficial Use Class Designators for use in ASEAN.

1.	Marine biota and habitats
	1.1 Marine parks/reserves
	1.2 Coral reefs – coastal/offshore
	1.3 Seagrasses
	1.4 Mangroves - sediment high/low habitats
2.	Tourism/recreation
3.	Sustainable fishing (spawning grounds, benthic)
4.	Coastal community resource-based livelihood
5.	Aquaculture/mariculture
6.	Residential districts (urban waters)
7.	Harbours/ports/shipping
8.	Industrial (waters)

Further development of these initiatives in the development of water quality Class Designators and a their clear linkage to Beneficial Uses would contribute strongly towards attainment of a harmonised yet flexible approach to both marine and coastal environmental management and reporting and sustainable development across the ASEAN region.

7.7 Monitoring and Assessment

Monitoring and assessment are key actions in the ICM cycle and are pivotal to effective implementation of adaptive environmental management approaches; it is the structured monitoring results that inform managers as to the effectiveness (or otherwise) of the management strategy and accompanying actions. Water quality monitoring is an important issue, and one that requires serious consideration, planning and design to be effective for rigorous data and information provision and to be cost-effective.

Monitoring is the systematic collection of physical, chemical and biological data; water quality monitoring measures physical and chemical parameters of interest, and often includes related biological parameters—for example, phytoplankton and chlorophyll *a* concentrations, bacteriological moieties (e.g., *E. coli* concentrations). Sediment sampling is becoming increasingly included in water quality monitoring protocols, especially where heavy metals and organic contaminants are the chemical species of interest. Our evolving scientific understanding of heavy metals and organic toxicants is confirming both that there is a strong affinity with marine sediments and that the sediment milieu is an important locus for chemical transformations and speciation. Indeed, there is increasing evidence suggesting that monitoring of toxicants (e.g., trace or heavy metals, organic toxicants) may be more effectively achieved from sampling the sediment rather than the water column.

A fundamental tenet for any monitoring program is that it must have a clear purpose and be addressing an issue that relates to management questions such that the outcomes from the monitoring measurements will assist with management decision-making and usually actions. Implementing a monitoring program without such purpose can be considered a waste of resources and time; there are multiple examples of such wasted effort in global literature and agency reports around the world.

Generally, monitoring programs will be designed to provide environmental data to meet one or more of the following purposes:

- provision of baseline physical, chemical and biological data that describe existing water quality conditions;
- measurement of selected physical and chemical water quality data (and sometimes biological data) that can be used to determine trends in environmental changes or environmental health of a system;
- measurement of physical and chemical water quality parameters to ensure compliance with licensed or permitted limits for water quality discharges from a facility to receiving waters of a system; and
- determination of physical, chemical and usually biological data required for impact assessment, usually in relation to validating water quality objectives that form part of an overall management strategy and plan.

Approaches and methodologies for the design of monitoring programs are considered in detail in the following part of this document (*ASEAN Marine Water Quality Monitoring Manual)*, along with commentary and examples of various models and assessment tools, including risk assessment.

8 Regulatory and Allied Management Frameworks

A key component of water quality management approaches is the identification of opportunities where economic instruments can be applied to ensure a realistic valuation of water resources as an asset – conservation, use or improvement has a cost. The pricing of water resources should reflect the full social cost (financial and environmental) of using the resource as well as capital, operations and maintenance costs. Effective management of water quality depends on an enforcement program that encompasses recognition of contributions by point source discharges, diffuse sources and "natural" background conditions (see ANZECC 2000). Prescriptive regulation is one option for management and achievement of water quality goals. Education and the application of economic instruments such as the adoption of market incentives and sanctions offer opportunities for win-win achievement of water quality goals. Hence, in many cases a successful enforcement strategy for water quality management could be expected to involve a package of regulatory and market-based measures to capture the advantages of each approach.

8.1 Regulatory Management Approaches

The regulation of pollution discharges through licensing and permitted limitation arrangements is effective for point sources, such as industrial discharges and municipal sewage and waste treatment facilities. The setting of effluent standards and licensing of public and private sector discharges to defined water bodies requires an understanding of the ambient levels in receiving waters and effluent levels, usually based on monitoring, modelling and risk assessments. Regulatory effluent standards can be designed to make sure that the water quality goals and objectives for a system are met, and monitoring programs can underpin the assessment of compliance or decisions for application of penalties.

The determination of licensed discharge limits needs to be related to technological performance capabilities of the industrial process or the operational capabilities of the industrial facilities. Pollutant levels should be set consistent with levels that can be achieved by modern technology and economic viability. This is sometime referred to as BAT or BATEA – Best Available Technology Economically Achievable – for industrial processes and public treatment facilities. It ensures that from the

commencement of new installations there is the adoption of industrial technology that has demonstrated achievement for the desired effluent pollutant levels within a viable economic framework. Such technologies could be progressively phased-in by existing discharge facilities as part of a program of on-going pollution discharge licences. This approach introduces an economic element to the prescriptive regulation.

Diffuse sources of pollution are not amenable to the application of standard pollution management techniques that involve regulation and market-based approaches. Diffuse sources are difficult to address, because specific "cause-effect" relationships are rarely obvious and the pollution sources usually extend over wide areas. Rural industries and urban conurbations typify diffuse source settings. Changing land-use practices can be approached through education; market-based solutions including economic incentives and regulations can yield positive management outcomes. For example, changes in tillage periods on agricultural lands to avoid major rainfall periods, encouraging judicious application of fertilizer and herbicides/insecticides, and better management of solid wastes can provide marked changes to diffuse-source inputs from rural industries to aquatic systems. The utility of water detention/infiltration basins, installation of artificial wetlands, solids interceptors in stormwater flow infrastructure are some effective control measures being applied to diffuse source management in urban areas. The cleanup of the nitrogen and phosphorus levels and overall water quality of the Rhine River offers a salutary example of an effective management approach towards control of diffuse sources (Behrendt et al. 2002; Salomons 2004).

Introducing mandatory waste minimisation policies on communities and industries can make significant contribution to preventative approaches that reduce the amount of pollution from both point and diffuse sources. Increasingly, waste minimisation practices along with industry-based codes of conduct or best management practices are being applied across the world to assist in limiting pollution from diffuse and point sources and to reducing environmental degradation.

8.2 Alternative Instruments and Measures for Management

Globally, there is an array of economic instruments in place or being introduced to supplement regulatory mechanisms for the management of water quality. In cases where conventional regulatory approaches are of limited effect, such as controlling diffuse pollution from agricultural industries, market-based instruments offer approaches that are innovative and novel. Examples of these economic market-based mechanisms for water quality management include:

- Discharge levies or pollution charges such that the pricing of the water resources reflects the environmental impact of use and degradation of the water asset value where water quality degradation results, and elevated license fees to recover the costs of regulation and monitoring;

- Effluent permits, which can be traded;
- Performance bonds, repayable when compliance with standards is achieved, and offering incentives such as subsidies, low-cost loans and tax allowances to encourage on-site BATEA technologies and waste-minimization actions that reduce the load of pollutants discharged;
- Non-compliance fees, in which the levies charged for exceeding the permitted discharge limits mirror the profits gained through non-compliance;
- Application of levies and higher rates of charge on users of water, sewage and waste treatment services, and imposing special levies on stakeholders for the remediation of impacted estuarine and river systems; and
- Measures to increase community awareness and acceptance of improved water use practices.

There is evidence that regional or large-scale, multi-catchment planning approaches for socio-economic development and improved catchment management (e.g., port planning and large-scale developments with strategic planning bases) is leading to improved land-use actions. These initiatives are reflected by diminished loads from land-based pollution sources compared with the conventional "tyranny of small decisions" that in the past has tended to characterise coastal development. Economies of scale and active planning are leading to improved infrastructure and modern technologies, enhanced and planned waste-minimisation facilities and improved outcomes for coastal and estuarine waters.

8.3 International Conventions and Accords

International conventions, accords and regional programmes offer a valuable management framework especially for addressing water quality of oceanic and outer continental shelf waters (Table 5). For example, the International Maritime Organisation (IMO) is an increasingly valuable forum and institution that is diminishing the potential for pollutants from ship-borne sources through the establishment of various international conventions and accords. This approach is particularly useful when addressing transboundary issues such as those associated with shipping and ocean transport. Regional and bilateral accords may also prove valuable in addressing transboundary airshed pollution issues where particulate and volatile materials enter coastal and marine waters. A similar context of international accords may prove useful in the development of standards for water quality and the enforcement of the standards in key socio-economic activities carried out in territorial and Exclusive Economic Zone waters, for example the oil and gas industry.

Table 5. Some international conventions, accords and regional programmes relevant to marine water quality management in ASEAN.

ASEAN Agreement on Transboundary Haze Pollution, 2002
ASEAN Agreement on the Conservation of Nature and Natural Resources, 1985
International Convention for the Prevention of Pollution from Ships, 1973, as modified by the Protocol of 1978 (MARPOL 73/78), and amendments
International Convention on the Control of Harmful Anti-fouling Systems on Ships, 2001
International Convention for the Control and Management of Ship's Ballast Water and Sediments, 2004
UN Convention on Biological Diversity, 1992
UN Convention on Climate Change
UN Convention on Wetlands of International Importance (RAMSAR), 1971
UN Law of the Sea Convention, 1982
UNEP Global Programme of Action for the Protection of the Marine Environment from Land-Based Activities, 1995
UNEP Regional Seas (East Asian Seas) Programme and Action Plan, 1981

9 Management Auditing and Reporting

9.1 Effectiveness of Management Plans and Management Settings

The outputs from water quality monitoring programs contribute important elements to the application of the ICM cycle and to the effectiveness of adaptive environmental management approaches. Management goals and the allied strategic plans for management of a system will specify desired outcomes in relation to implementation of strategic management actions. Water quality is usually a key element of this overall management process. The effectiveness of the strategies put in place for management of a system require evaluation; here, the metrics from the component water quality monitoring actions will provide one dataset for determining the efficacy of the

management action to control water quality of the system. These should indicate if management targets have been met by the management strategy and actions and will indicate the need for revised goals or modified actions that may be needed to meet the overall management objectives. The timeframes for the management cycle may be brief or extended.

The reporting of water quality monitoring outcomes will need to reflect the monitoring design program and purpose for the targeted system and to meet the needs for clear and explicit values including the description of uncertainties and variability in sampling and analysis. Detailed consideration of the form and content, and examples of reporting formats, are provided in the following part of this document (*ASEAN Marine Water Quality: Monitoring Manual*).

9.2 Aggregated Information

In addition to the direct measurement and evaluation of specific metrics resulting from water quality monitoring to support specific site-related management plans, agencies responsible for marine and estuarine system management are required to report on their management performance and usually the status of the systems. This requires the aggregation of information relating to the extent and degree of various pressures of change (e.g., pollutant loads from point and diffuse sources to a system), the status of the environmental and/or public health quality of the system (e.g., water quality, environmental health of intrinsic ecosystems, human health impacts), and time trends that will be reflective of the effectiveness of management measures. The derived information will be a key part of management reporting about performance to policy and other decision-makers and to the wider community of stakeholders with interest in the quality and status of the relevant systems.

In this context, there are two avenues of interest for receipt of information assessed and compiled from water quality and allied environmental/ public health monitoring programs in the context of the management issues. First are the requirements within each AMS and second are the requirements of ASEAN.

National reporting of marine and estuarine water quality and environmental status of marine and estuarine systems will need to meet national legislative requirements as well as policies of the environmental management institution. Such reporting requirements will reflect existing national practices and will include specific types of information, measurement metrics and compliance indices; a diversity of formats and content that meet the relevant legal and policy institutions and technical needs of individual ASEAN member states. However, there will undoubtedly remain a desire and opportunity for national reporting of marine and estuarine water quality status and trends that integrate specific technical information and that represents a digest of management achievements relating to water quality status. In a similar context, reporting marine and estuarine water quality status across the ASEAN region will

add to the existing initiatives for State of the Environment reporting for the region (e.g., Third ASEAN State of the Environment Report 2006, ASEAN 2006).

The implementation of a Class Designators framework offers a useful approach that can help integrate and summarise national data and be readily input into a regional reporting system about water pollutants in the form of compliance and status. The AMWQC already adopted by ASEAN – protection of aquatic life and human health – provide an initial structure against which individual ASEAN member states and ASEAN can aggregate information. The types of information could be aggregated through construction of indices for, say, percentage compliance or number of times values have been exceeded, percentage of marine and water systems under active management, identification of hotspots.

Further division of the "protection of marine life" category within the management and reporting structures of individual ASEAN member states (for example, as currently developed by the Philippines and Thailand, Table 3), could be expected to provide a more detailed but still aggregated picture of water quality and marine and estuarine environmental status across the region. This would require harmonisation across ASEAN member states of a sub-set of water quality Class Designators that addresses ecosystem conservation objectives and relevant economic sector activities. Such an initiative would confer advantage to ASEAN environmental considerations and provide a common framework for exchange of ideas and mutual support in water quality management between ASEAN member states.

Globally, a number of effective approaches have been taken to aggregate water quality and environmental data in order to construct and communicate meaningful and effective status and trend information that can assist decision-makers and the wider community of stakeholders. For example, the global score-card system promoted by the World Resources Institute (WRI) (Appendix 1) and applied within the Millennium Ecosystem Assessment (MEA) (Appendix 2), and the regional assessment as an annual report card used by the Healthy Waterways Partnership in South-eastern Queensland coastal and estuarine systems in Australia (Healthy Waterways) (Appendix 3.a). Healthy Waterways has issued annual reports on the status and trends in water quality and system health for Moreton Bay, adjacent to Brisbane city, and for estuarine systems extending about 100km north and south of Moreton Bay. The report cards are derived from a water quality and other environmental monitoring programs and represent an aggregation of data and information (Appendix 3.b.) that is used by local government and other stakeholders in the planning and implementation of remediation and other management actions. Members of local communities are major stakeholders in the process and it is an effective communication tool for getting status/change reporting to multiple stakeholders e.g., politicians, community, industry, science.

Acronyms

AMSs	ASEAN Member States
AMSAT	Australian Marine Science and Technology Ltd
AMWQC	ASEAN Marine Water Quality Criteria
ANZECC	Australian and New Zealand Environment and Conservation Council
ASEAN	Association of Southeast Asian Nations
AWGCME	ASEAN Working Group on Coastal and Marine Environment
BATEA	Best Available Technology that is Economically Achievable
EEZ	Exclusive Economic Zone
EIA	Environmental Impact Assessment
ERA	Ecological Risk Assessment
ESD	Ecologically Sustainable Development
GEF	Global Environment Facility
HELCOM	Helsinki Commission (for the Baltic Sea region)
ICM	Integrated Coastal Management
IMO	International Maritime Organisation
MEA	Millennium Ecosystem Assessment
PEMSEA	Partnerships in Environmental Management for the Seas of East Asia
UNDP	United Nations Development Programme
WRI	World Resources Institute

Glossary

Ambient Water Values. The concentrations of chemicals or physical changes in water properties that will not cause adverse impacts to the health of biological organisms including humans (see US EPA website, www.epa.gov)

Beneficial Uses/Environmental Values. The particular uses or values of the environment that are conducive to public benefit, welfare, safety or health and which require protection from the effects of pollution, waste discharges and deposits (see ANZECC 1994). Operationally, the terms *Beneficial Uses* and *Environmental Values* tend to be used interchangeably.

Class Designator. A set concentration and biophysical change limitation metrics for water quality parameters which define different water types relating to a management purpose for a defined area. Class Designators are usually allied with specific Beneficial Uses.

Coastal and estuarine waters. The domain encompassing estuarine and adjacent marine coastal waters. Used descriptively, rather than with finite geo-spatial determinants, to differentiate from oceanic, atmospheric or terrestrial domains. The coastal extent is usually to the limits of land influence on marine waters (see coastal zone definitions, Crossland et al. 2005).

Ecosystem Approach. Usually the integrated management of human activities ine Water Quality Guidelines. The numerical concentrations or narrative statements recommended to support and maintain designated uses of the marine environment. These will generally discuss socio-economic information in principle.

Marine Water Quality Objectives. Numerical concentrations or narrative statements that have been established to support and protect the designated uses of the marine environment *at a specific site.* These will take consideration of relevant socio-economic data for a specific geospatial area.

Marine Water Quality Standards. Marine environmental quality objectives that are recognised in enforceable environmental control laws of a level of government. These are auditable performance benchmarks.

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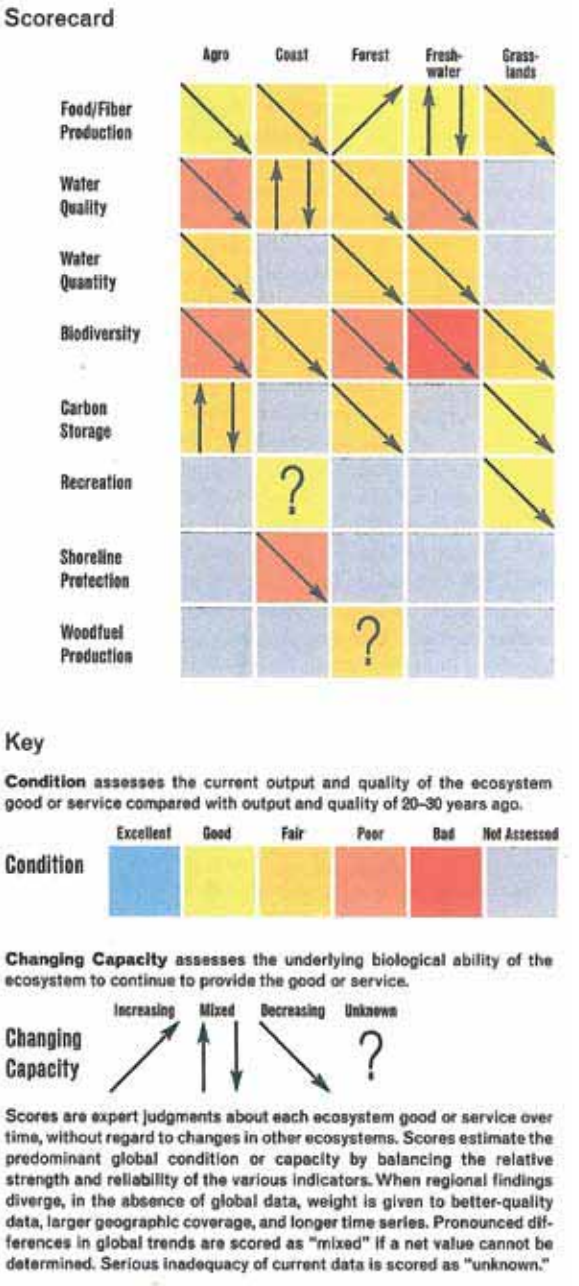
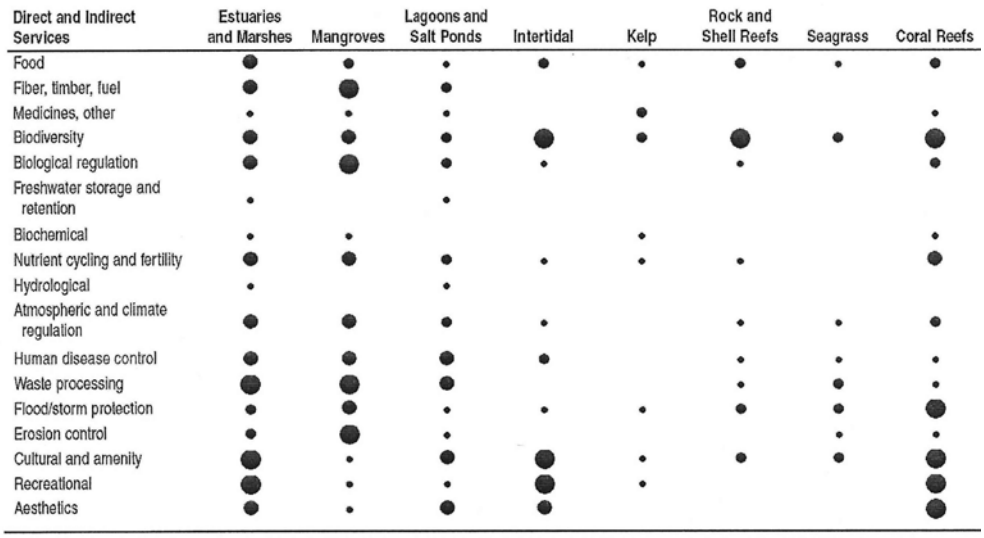
Appendix 1

Example of a scorecard system applied to global ecosystems status and trends (WRI 2000).



Appendix 2

Example of a scorecard system applied to the status and trends of environmental goods and services in coastal systems (MEA 2006).



Appendix 3a

Healthy Waterways Annual Report Card 2006 for northern estuaries and marine waters of Southeast Queensland, Australia. (from Healthy Waterways Partnership.)

October 2006

Northern Catchments

Sub-regional Summary

Printed on recycled paper.

The Northern sub-region comprises the catchments of the Noosa, Maroochy and Mooloolah Rivers – an area of 1,679km² (7% of the South East Queensland region). There are three local governments (Caloundra, Maroochy and Noosa Shires) within the sub-region with a significant portion of Caloundra City Council falling within the Moreton Bay sub-region. Several catchment, landcare and bushcare community-based groups are active within the region.
As the efforts of Caloundra City Council contribute to both Northern and Moreton Bay sub-regions, its management responses have been apportioned between the sub-regions for reporting purposes. This summary reports only on the management responses affecting the Northern catchments. Freshwater and estuarine Report Card results are included for the Pumicestone catchment for easy reference.

Freshwater Results

B Noosa Catchment — MAP REF 1

- Streams generally remain in good condition
- Results for both the physical-chemical and ecosystem process indicators remain strong
- Results for the aquatic macroinvertebrate indicator were very similar to those in the past, but scores for fish were substantially lower in both seasons

C- Maroochy Catchment — MAP REF 2

- Streams are generally in fair condition, with results this year very similar to those for 2003-04
- Available results for nutrient cycling were poor
- Physical-chemical, ecosystem processes and fish indicators have consistently been very good

B Mooloolah Catchment — MAP REF 3

- Streams are generally in good condition
- Results for all indicators, apart from nutrient cycling, continue to return consistently high scores
- In the long-term, results for autumn have tended to be better than those for spring

C- Pumicestone Catchment — MAP REF 4

- Streams are generally in fair condition
- Results for nutrient cycling in spring 2005 were well below those recorded in the past three years
- Ecosystem processes and physical-chemical indicators continue to score highly

Estuarine Results

B A Waterway name
2006 grade / 2005 grade

A- Noosa River — MAP REF 1

- Elevated inorganic nutrients in middle and upper reaches compared to 2005
- Intact channel and natural habitats throughout
- Some nutrient processing

D+ Maroochy River — MAP REF 2

- Increase in nutrient levels, especially total phosphorus in the middle and upper reaches
- Turbidity in middle to upper reaches remains low
- Modified river banks with urbanisation in the lower reaches and limited riparian habitat in the middle and upper reaches

B Mooloolah River — MAP REF 3

- Consistently good to excellent water quality
- Low nutrient and turbidity levels throughout
- Highly modified channel and river banks
- Some nutrient processing

B Pumicestone Passage — MAP REF 4

- Fair water quality throughout
- Increase in water clarity in southern reaches
- Decrease in phytoplankton abundance in southern reaches compared to 2005
- Intact natural habitats throughout

NATURAL RESOURCES AND WATER

QUEENSLAND MUSEUM

Report Card 2006 3A

Management **Responses**

Northern Catchments

Managing **Point Source Pollution**

There are seven council-owned wastewater treatment plants (WWTPs) operating within the Northern sub-region. During the 2001-06 South East Queensland Regional Water Quality Management Strategy (SEQRWQMS) reporting period, upgrades to four of the WWTPs were planned, designed or commenced, representing a significant financial commitment in wastewater infrastructure by councils and the State Government. For example between 2001 and 2006 the State Government has contributed over $17 million in subsidies to northern local governments for wastewater treatment plant upgrades, new plants, and reuse initiatives.

Maroochy Shire Council undertook significant upgrading to the largest of the northern subregion's WWTPs. The $34.3 million Maroochydore WWTP upgrade is due for completion in late 2006 and will cater for future growth in the area, while reducing the amount of nutrients released to the Maroochy River. Upgrading of the Coolum WWTP also commenced in 2005.

The Caloundra City Council upgrade to the Kawana WWTP (the second largest of the northern WWTPs) was completed during 2006. The $22.5 million upgrade boosted the capacity of the Kawana plant to treat all wastewater from Caloundra, allowing decommissioning of the Caloundra WWTP. Work on sewering the Glass House Mountains township was also completed in 2006. Once complete, all wastewater from the town will be treated at the Landsborough WWTP, which is in the process of being upgraded.

In Noosa Shire, a review of long term wastewater treatment options was commenced in 2004. The findings from the review have been guiding Noosa Shire Council in deciding the best approach to future wastewater management.

Northern sub-region councils (in collaboration with Caboolture, Kilcoy and Pine Rivers Shire Councils) also developed an information kit to assist residents in non-sewered areas manage their own on-site sewage facilities (e.g. septic tanks).

Planning and **Protection**

Noosa Council employed a Sediment and Erosion Control Officer and commenced the new Noosa Plan in 2006. This plan establishes provisions for protection of riparian areas and requires both the implementation of water sensitive urban design as well as improved sediment and erosion control on building sites. In 2005 Noosa Shire Council and the State Government developed The Noosa River Plan which facilitates a more coordinated approach to the planning, development and management of the Noosa River system.

Caloundra City Council, through its Waterways Operations Team, continued programs of water quality monitoring and litter removal as part of the on-going management of the City's waterways.

All local government planning schemes within the Northern sub-region implemented provisions for improving stormwater management, riparian areas, and protection of waterways.

Managing **Diffuse Source Pollution**

During 2001-06 councils and community-based catchment groups continued to reduce the impacts of diffuse sources of pollution from both urban and rural areas of the Northern sub-region. Expenditure on this work since 2001 exceeded $9 million. Maroochy Shire Council's Maroochy River Recovery Plan is a strong example of this work.

Riparian work in Northern sub-region 2001-06*	
Number of trees and shrubs	280,224
Length of streambank improved (km)	273
Number of volunteers actively involved	5,345
Funding for riparian work	$3,055,000

Funding for stormwater management in Northern sub-region 2001-06*	
Structural stormwater management	$3,451,000
Non-structural stormwater management	$3,540,000
Stormwater monitoring	$279,000

* Based on incomplete information and is therefore likely to underestimate the full extent of work completed. Riparian work includes a range of activities such as revegetation, weed and erosion control, fencing and on-going maintenance. Stormwater management includes installation and maintenance of stormwater treatment infrastructure (structural) and non-structural stormwater management initiatives including planning and regulatory controls, education and awareness raising, street sweeping, etc.

Maroochy River Recovery Project

The Maroochy River Recovery initiative commenced in July 2004 as a package of 15 individual but complimentary pilot and demonstration projects with an indicative funding level of $3.6 million over three years.

In the nature of a 'pilot', the purpose was to begin the process to reverse an apparent decline in the health of Maroochy waterways by targeting waterways contamination from point source sewage effluent, rural runoff and urban runoff. The 15 projects mirror the following actions endorsed by Council :

- appropriately vegetated riparian corridors;
- sediment and erosion control;
- an appropriate water sensitive urban design program;
- full program of domestic wastewater treatment monitoring and rectification by landowners;
- litter management;
- increased focus on sewer infiltration and inflow; and
- education.

In 2005-06 Council allocated the following funding to riparian rehabilitation alone to address diffuse catchment loads:

- $230 000 via the Waterways Community Grants;
- $400 000 for Large Scale Rehabilitation involving landholders within the Upper Paynter Creek subcatchment; and
- $400 000 for Large Scale Rehabilitation involving landholders within the North Maroochy subcatchment.

To date, these projects have involved

- 62 properties
- 100,000 native trees planted
- 20 kilometres of protective fencing installed
- 15 specially engineered low level crossings constructed; and
- 600 Camphor Laurels treated.

HEALTHY WATERWAYS
Because we're all in the same boat

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Appendix 3b

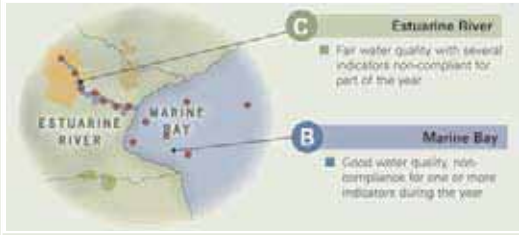
Healthy Waterways: calculation of report card. (from Healthy Waterways Partnership).

From a total of 248 estuarine and marine sites, maps are produced for each indicator which show the median values for each site from the reporting year.

Compliance scores are then calculated for each indicator as the proportion of the reporting zone that complies with the Water Quality Objectives, 0 representing non-compliance and 1 representing total compliance.

An **Ecosystem Health Index (EHI)** for the reporting zone is calculated by averaging the compliance scores for each indicator.

The Biological Health Rating (BHR) assesses those indicators measured by the EHMP without established objectives. The BHR ranges between 0 and 1 for each zone, with 1 representing an unmodified and healthy ecosystem and 0 representing a highly modified and unhealthy ecosystem.



A single EHI value and a single BHR value are calculated for each waterway by averaging the indicator ratings. These two values are combined together with expert opinion to provide a single value used to assign a Report Card Grade.

SEQRWQMS (2001) water quality objectives for key indicators monitored by the Estuarine/Marine EHMP

Performance Indicator	Performance Measure	Bay Objective	Estuary Objective
Chl *a*	Annual median	<1µg/L (<2µg/L for Bramble, Waterloo, Deception and Southern Moreton Bay)	<10µg/L
TN	Annual median	<0.22mg/L	<0.45mg/L
TP	Annual median	NA	<0.06mg/L
DO	Annual median	NA	80-100%
Secchi disc depth	Annual median	>1.7m	NA
Turbidity	Annual median	NA	<20NTU
Lyngbya	Annual maximum	<0% cover	NA
$\delta^{15}N$ (macroalgae)	Reporting zone max.	<4‰	NA

Part 2

Monitoring Manual

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Part 2

Monitoring Manual

1 Background

ASEAN environment ministers adopted the ASEAN Marine Water Quality Criteria (AMWQC) for 17 parameters in 2002. Part One of this Handbook outlines the history behind the Criteria and steps towards their implementation under the ASEAN Marine water Quality Project.

Part Three of the Handbook (which follows) consists of a Monitoring Manual wherein recommended methods for the implementation of monitoring programs suitable for the AMWQC are documented.

Methods for program design, sampling, analysis, data analysis and interpretation and reporting and information dissemination have been selected on the basis of their suitability to be used in the ASEAN region. The methods focus on the 19 parameters included in the initial AMWQC report (Anderson et al 1999) (18 where nitrate and nitrite are counted as one) even though only 17 of these were accepted by the ASEAN ministers in 2002 (Zn and As were exclude as needing further studies). One other parameter – chlorophyll – has also been included in the methods as it is now realised that this is a critical indicator of nutrient enrichment.

Where possible, alternate but acceptable, methods are given covering different levels of sophistication of instrumentation. This is in recognition that all states in ASEAN do not have access to the same capacity of analytical instrumentation. This Manual does not list all methods suitable for the AMWQC parameter list but attempts to include those we believe are most likely to be of use in the region.

Equal emphasis in this manual is given to project design and data analysis as are given to sampling and laboratory methods as it is commonly noted that many monitoring programs throughout the world have inadequate design and are not set up to answer a clear question (objective) or are designed such that the data collected is not adequate to answer the required question.

The structure of this manual is based on the document 'Australian Guidelines for Water Quality Monitoring and Reporting' and section headings loosely follow those laid out in the process flow charts of that document:

- Setting monitoring program objectives;
- Study design;
- Field sampling program;
- Laboratory analysis;
- Data analysis and interpretation; and
- Reporting and information dissemination.

This manual is designed to be used with a number of other publications from which many of the techniques, methods and information were drawn in preparing the manual. Some of those publications form an essential companion set for this manual. They are:

1. ANZECC 2000b. Australian Water Quality Guidelines for Fresh and Marine Waters, Australian and New Zealand Environment and Conservation Council, Canberra, ACT, Australia.
2. Grasshoff K, Kremling K and Ehrhardt M (eds) 1999. Methods of Seawater Analysis. Wiley-VCH.
3. APHA 2006 Standard Methods for the Examination of Water and Wastewater. American Public Health Association, 21st Edition, and online methods at www.**apha**.org/publications/
4. Johnstone R and Preston M 1993. Nutrient analysis in tropical marine waters. Practical guidance and safety notes for the performance of dissolved micronutrient analysis in seawater with particular reference to tropical waters. IOC UNESCO Manuals and Guides No. 28, UNESCO, Paris.
5. UNEP 1983. Determination of faecal coliforms in sea-water by the membrane filtration culture method. UNEP/WHO Reference Methods for Marine Pollution Studies No. 3 Rev. 1. 1983 Revised 1995.
6. Queensland EPA 1994. Water quality sampling manual. http://www.epa.qld.gov.au/publications/p00330aa.pdf/Water_quality_sampling_manual_for_use_in_testing_for_compliance_with_the_Environmental_Protection_Act_1994.pdf

1.1.1 Emerging issues

The original 17 parameters identified by ASEAN (McPherson et al. 1999) encompass a suite of parameters targeted primarily on trace metals, nutrients and suspended

sediment. Discussion at the ASEAN Regional Expert workshops (Viet Nam and Bangkok) identified some potential parameters which may be beneficial to add to future monitoring programs, e.g., chlorophyll *a*, polyaromatic hydrocarbons (PAHs) and some parameters that should be measured for background data, e.g., salinity, turbidity. Techniques for monitoring these parameters have been included in this manual.

New substances which may become pollutants are being introduced into human use in specific locations all the time. These include a whole range of new synthetic chemicals as well as changes in usage of older chemicals in new locations. In recent years, a number of studies have demonstrated the presence of contaminants that had not previously been measured in the environment (EPA USA). These include many commonly-used compounds such as insecticides, pharmaceuticals, antibiotics, hormones, fire retardants, detergents and other industrial chemicals that are produced in high volumes and can be introduced to the environment during their production, use or disposal. They have probably been present in the environment since they entered commercial use, but the technologies for their detection have only recently become widely available.

Types of Monitoring Programs

1.2.1 Introduction

Long-term environmental monitoring is essential to determine baselines, measure change and assess overall ecosystem health. Enhanced monitoring can improve the management and protection of marine resources and can also protect human health. The creation of an integrated monitoring network that encompasses estuarine, coastal and offshore waters allows documentation of status and change.

There are various reasons for monitoring water quality, some of which are listed below. A monitoring program may have objectives which encompass several of these objectives. The type of monitoring program that a country will eventually decide to use will depend on the type of information required by scientists and management within that country.

Consideration of the parameters needed to answer the monitoring question and linked to the conceptual model is essential. For example, to detect whether fertiliser residues of nitrogen are being discharged into a particular marine environment, it may be important to measure nitrate and not just total nitrogen, as fertiliser is lost as nitrate while total nitrogen contains nitrogen from many sources.

A properly designed sampling program will require an understanding of the environment as well as the wide variety of physical, chemical and biological processes influencing the distribution of the analyte(s) being monitored.

1.2.2 Background/ baseline monitoring

Background or baseline monitoring is carried out to understand the spatial and temporal range of water quality parameters important to aquatic ecosystem health in water bodies, or to assess natural variability of water quality parameters in time and space. This information can then be used in designing a monitoring program in which the signal (the result being sought) can be separated from the noise (the natural variability). Typically, long-term monitoring data is required to confidently identify the spatial and temporal patterns in marine waters (see Text Box 1).

It is important to distinguish between detection and assessment. There is a major difference in how two such monitoring studies would be planned. To *detect* an impact (see Section 1.2.4) it is only necessary to demonstrate an unambiguous effect on one component of the ecosystem. To *assess* the impact, or to detect long-term change, it is important to monitor all components that are indicative of that change. The spatial and temporal constraints on the monitoring will be defined by the intensity of the influence and extent of the ecosystem being monitored. In baseline monitoring, it is useful to monitor two systems, one in which there is evidence that change may have occurred and one in which the extent of change is minimised (i.e., a reference site). Monitoring information can then be analysed over time and over spatial scales. For example, the Long term Chlorophyll Monitoring Program in the Great Barrier Reef (Brodie et al. 2007) documents the regional cross-shelf patterns of phytoplankton biomass, recorded as chlorophyll *a* concentration, in Great Barrier Reef (GBR) waters. Decadal monitoring of monthly samples across and along GBR waters have demonstrated significant differences in chlorophyll *a* across the shelf, with mean inshore concentrations exceeding offshore concentrations and persistently higher inshore concentrations adjacent to catchments with higher occurrences of agriculture (De'ath et al. 2006). Further details on the design of the monitoring program can be found in Text Box 1.

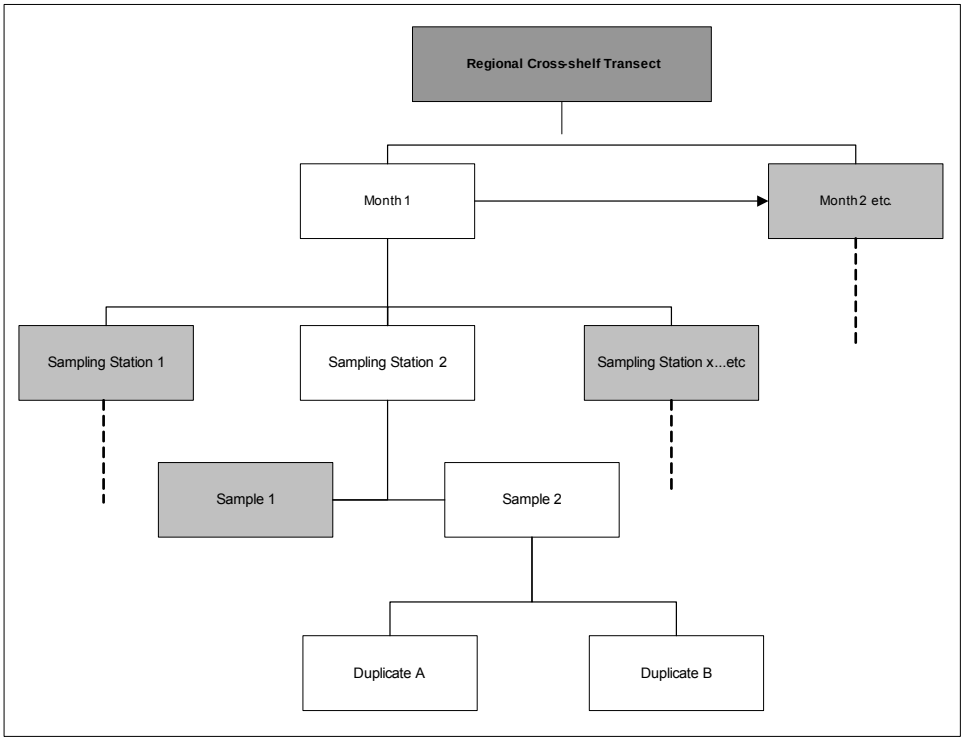
Box 1. Long-term monitoring of chlorophyll in the Great Barrier Reef

Surface chlorophyll a concentrations in the Great Barrier Reef (GBR) lagoon have been monitored monthly since 1992 at 86 stations in the GBR lagoon within five broad regional zones. The stations are located on eight sites across the shelf from 13°S to 23°S. The monitoring program was established to detect changes in nearshore water quality as a result of increasing loads of nutrients being exported from the catchments adjoining the GBR. In most regions, sampling is conducted monthly including both nearshore (<25 km from the coast) and offshore (>25 km from coast) conditions. Sampling was conducted at approximately monthly intervals in order to quantify seasonal changes in phytoplankton biomass. More frequent sampling was constrained by operational costs.

The actual date for sampling within a calendar month was determined by the individual collecting institution and depended on logistics such as prevailing weather conditions.

The duration and time-span of individual data sets vary between regions. At each sampling station, weather and physico-chemical measurements (salinity, temperature, secchi depth, depth, presence of *Trichodesmium* spp. and weather conditions) are also collected to aid interpretation of the chlorophyll *a* results.

A schematic of the chlorophyll *a* sampling strategy for each cross-shelf region is presented below. For more information, see www.gbrmpa.gov.au



1.2.3 Issue-based monitoring

Issue-based monitoring is carried out to identify particular water quality issues, such as:

(a) Is a water body contaminated – that is, are there detectable contaminants in the water body at concentrations above those that occur naturally?

(b) Is a water body polluted – that is, are there detectable contaminants at concentrations known to cause adverse ecosystem effects or human health effects?

(c) Does the water body meet water quality guidelines/criteria?

(D) Are there detectable adverse biological effects or human health effects?

Text Box 2 illustrates a compliance monitoring program set up to identify non-compliances in bathing waters in European waters. The quality of bathing waters relates directly to human health, so it is important to have a compliance-based monitoring program which can identify problem areas quickly and instigate management actions to avoid impacts on human health.

1.2.4 Identifying contaminant sources

Monitoring may be carried out to identify (and quantify) the sources of contaminants from the land to waterways, in terms of land uses and activities. In these programs the land area will be divided into different land uses, potential pollutant sources (e.g., oil palm cultivation, metallurgical industry, urban area, sewage treatment plant) determined and the water quality characteristics of each source identified through monitoring sites downstream of each land use/source.

An example of a monitoring program set up to identify contaminant sources in land use is the Healthy Waterways program running in the Mackay-Whitsunday region adjacent to Southern Great Barrier Reef waters (Rohde et al. 2006). The project was designed to quantify pollutants generated by the major land uses in rainfall runoff events in the Mackay-Whitsunday region and to quantify pollutants discharging to the inshore areas of the GBR lagoon. Further details of the design of the program are given in Text Box 3.

Box 2. The European Bathing Waters Directive

The aim of this monitoring program is to inform the public of the quality of bathing areas in each Member State of the European Community over the previous year and the frequency of non-compliances of bacteriological thresholds. It also covers trends in bathing water quality in relation to previous bathing seasons (from 1990 to 2006).

Sampling of bathing waters during the bathing season.
Samples are taken at the place where most bathers can be found and with a specific frequency. The basic rule is fortnightly sampling, visual and olfactory inspection of the water during the bathing season, plus one additional sample 14 days before the start of the bathing season.

Analysis of the water samples by a qualified laboratory.
The samples are analysed as quickly as possible. The samples should be analysed for all parameters listed in Annex 1 to the Directive. Priority is given to two microbiological parameters:- total and faecal coliforms—and three physico-chemical parameters:-mineral oils, surface-active substances (lasting foam as a result of the presence of detergents) and phenols.

Compliance reporting.
European states shall set for all bathing areas or for each individual bathing area, the values applicable to bathing water for the parameters given in the table below. The values measured in the bathing water programs may not be less stringent than those given in the mandatory column.

Reporting to the European Commission. Member States report the results of the sampling to the Commission. On the basis of these results, the Commission then publishes this report with the quality assessments and updates the website before the start of the new bathing season.

Information to the public.
Most Member States distribute information on the quality of their bathing areas through the press, television, Internet, etc. during the bathing season and present a summary report shortly after the end of the bathing season.

For further information, please refer to
www.europa.com or www.environment-agency.gov.uk

	Micriobiological parameters	***Guide***	***Mandatory***	***Minimum sampling frequency***	***Method of analysis and inspection***
1	*Total coliforms/100ml*	*500*	*10 000*	*Fortnightly (1)*	*Fermentation in multiple tubes. Subculturing of the positive tubes on a confirmation medium. Count according to MPN (most probable number) or membrane filtration and culture on an appropriate medium such as Tergitol lactose agar, endo-agar, 0.4% Teepol broth, subculturing and identification of the suspect colonies. In case of 1 and 2, the incubation temperature is variable according to whether total or faecal coliforms are being investigated.*
2	*Faecal coliforms/100ml*	*100*	*2 000*	*Fortnightly (1)*	
3	*Faecal streptococci/100ml*	*100*	*-*	*(2)*	*Litsky method. Count according to MPN (most probable number) or filtration on membrane. Culture on appropriae medium.*
4	*Salmonella/litre*	*-*	*0*	*(2)*	*Concentration by membrane filtration. Inoculation on a standard medium. Enrichment - subculturing on isolating agar - identification*
5	*Enteroviruses PFU/10 litres*	*-*	*0*	*(2)*	*Concentrating by filtration flocculation or centrifuging and confirmation*

1.2.5 Monitoring transport and processing of contaminants in catchments

This type of monitoring is designed to interpret and quantify the transport of contaminants (concentrations and loads) from the generation point (land use, specific activities) to and within the water body of concern. During transport, the determination of rates of trapping and removal of contaminants and storage times is necessary. Important trapping/removal mechanisms include sedimentation, burial, chemical detoxification, denitrification, biological uptake of nutrients and storage in vegetation and animals, evaporation, dilution and complete removal from the system. In this type of monitoring program, sampling sites spaced out longitudinally from the source to the ecosystem of concern may be used to measure the changes occurring to key pollutants. Programs such as the Healthy Waterways program described in

Box 3. Mackay Whitsunday Healthy Waterways Program

This program was set up to:

(i) Quantify pollutants generated by the major land uses in rainfall runoff events in the Mackay Whitsunday region.
(ii) Quantify pollutants discharging to the inshore areas of the Great Barrier Reef (GBR) lagoon.
(iii) Obtain baseline data to support regional and local target-setting and water quality improvement plans.

The Mackay Whitsunday region covers an area of approximately 9000 km^2 along the central Queensland coast with 4 major rivers and a number of smaller streams discharging directly to the GBR lagoon and the western Coral Sea.

Major land uses in the region include sugarcane growing, beef grazing and urban/industrial, with considerable areas of national park/state forest. Significant water quality issues have been identified in the region in previous studies, and include fish kills associated with low dissolved oxygen, mangrove dieback and high concentrations of nutrients and herbicide residues in major stream-flow events.

A total of 21 sites was selected to represent runoff from single land use sub-catchments (forest, sugarcane, grazing and urban) and mixed land-use catchments. Sampling was targeted at flood events where sites along the rivers and smaller streams were sampled in combination with marine sampling. Total suspended solids, nutrients, pesticide residues and organic compounds from event flows were measured from different land uses and catchments. The extent and nature of plumes in the marine environment were quantified using aerial mapping and satellite imagery. Nutrients were analysed to give full speciation and the pesticides targeted were those known to be used in the region, particularly a number of herbicides.

Results from the monitoring program have led to some preliminary observations about the source of contaminants and their impact. In comparison to forest sub-catchments, it was found that:

- Sugarcane-dominated sub-catchments exported moderate to low concentrations of total suspended solids (TSS), high concentrations of dissolved nutrients (particularly nitrate and phosphate) and herbicides (particularly diuron).
- Grazing-dominated sub-catchments exported moderate concentrations of TSS and nutrients and low concentrations of some herbicides.
- Urban sub-catchments exported high concentrations of total phosphorus (TP) and filterable reactive phosphorus (FRP) and moderate concentrations of trace organics (herbicides and hydrocarbons).

For more information on the design of the monitoring program, please refer to Rohde et al. 2006 and www.actfr.jcu.edu.au

Text Box 3 are also useful in quantifying contaminants from specific land uses. Many monitoring programs use *in situ* measurements from rivers to calculate daily loads of nutrients into adjacent coastal waters. Typically these monitoring programs have *in situ* high frequency measurements of flow rates coupled with less frequent nutrient measurements (Mitchell and Furnas 1997).

Box 4. Long-term monitoring of chlorophyll in Chesapeake Bay, USA

Background

Over a period of decades, significant changes are believed to have occurred in the phytoplankton dynamics of Chesapeake Bay, including elevated nutrients inputs, particularly nitrogen and phosphorus, which has promoted a significant increase in phytoplankton biomass since the early 1950s. Other changes include a shift in the size structure of the phytoplankton (Malone et al. 1991) and a change in the phytoplankton species composition (Marshal and Lacouture 1986)

Understanding trends in phytoplankton dynamics is complicated by large seasonal and interannual variations in the distribution and abundance of phytoplankton, superimposed on changes that are thought to have occurred over a period of several decades.

However, trend analysis shows a quantitative evidence of change in Chesapeake Bay by reporting a long term increase of phytoplankton biomass, measured as surface chlorophyll a concentrations (Harding Jr and Perry, 1994). Chlorophyll is useful expression of phytoplankton biomass and is arguably the single most responsive indicator of N and P enrichment in this system.

Model

The analysis allowed for the removal of freshwater flow and attendant properties by developing regional models to predict chlorophyll *a* from physical variables and use of the models to resolve an historical increase of chlorophyll *a* from variability. The models developed quantified a long term chlorophyll *a* trend by simplifying the processes controlling the variables to be predicted.

Trend analysis

Analysis of chlorophyll *a* is from data collected between 1950 and 1994 and relies on models of mean monthly chlorophyll for 6 separate regions.

Data from each annual set of chlorophyll *a*, salinity (S) and temperature (T) were grouped by month and region and mean monthly (arithmetic mean) $\log_{10}$ chlorophyll *a* was computed for each of the 6 regions selected to represent the 6 salinity types of the Bay. A set of autoregressive moving average models with structural components reflecting the contributions of freshwater flow, S and T was developed to predict the mean monthly chlorophyll *a* concentration by region.

1.2.6 Trend monitoring

This type of monitoring is carried out to identify trends (commonly temporal trends) in contaminant generation and water-body concentration, typically looking for conclusive evidence of change, e.g., increases/decreases in contaminant loadings or contaminant concentrations through time, improving/deteriorating biological indications of ecosystem health. Trends may occur at a range of temporal scales, such as daily (day and night), monthly (tidal/lunar), seasonally (summer/winter or wet season/dry season) or multi-year (El Niño, sunspots cycle).

The problem of detecting a trend is usually compounded by limited spatial and temporal coverage for indicators of water quality. Sparse data can make it difficult to identify seasonal and interannual variability and to correct for any external influences on the variability such as prevailing weather conditions. In most estuarine and coastal systems, detecting changes in water quality over periods of years is complicated by high variability. Few data sets are available with the time-span of coverage or consistency of methodology to identify the signals of change, particularly for water quality indicators. However, with the right planning and the right questions, long-term monitoring can be analysed for trends that identify anthropogenic influences. Confidence in the detection of trends is a powerful tool for both management and public awareness. In Chesapeake Bay, data for chlorophyll *a* has been collected for five decades (Harding Jr 1994; Harding Jr and Perry 1997). This long-term data set, though not optimally distributed in time and space, represents a potentially informative indicator of ecosystem change. Text Box 4 describes the long-term monitoring program and the statistical techniques used in the trend analysis.

1.2.7 Management effectiveness monitoring

Such monitoring is carried out to determine whether management intervention to reduce contaminant sources is changing contaminant concentration/loadings. This will normally incorporate a trend or comparison element, such as comparison data from before and after the intervention was done. In this type of monitoring, issues such as those associated with comparisons between adjacent sites, 'reference' sites, before and after comparisons incorporated into the multiple before-after control-impact (MBACI) system have to be resolved.

An example of this type of monitoring is sampling in different sub-catchments and in adjacent coastal waters. This ties specific river sources to outputs (Rohde et al. 2006). Information can directly inform management of problem sources and how best to reduce deleterious anthropogenic inputs.

It is important to manage management expectations. Any information depends on the parameter being measured, and direct relationships can not always be inferred. For example, interannual variability in sediment loads can be much larger than any reductions in sediment loss from catchment areas. It may take years to see an impact from a management decision. By identifying where the management action is most likely to be affected, e.g., at a sub-catchment scale, it may be possible to measure change. At a larger ecosystem scale (coastal/offshore) such identification is much more difficult.

Different sources of parameters react differently. For example, coastal erosion can be reduced by planting trees, but there may be a decadal response before sediment reduction in rivers and the sea. Nutrient reduction strategies may be measurable in rivers and sub-catchments but it may take many years before resultant biological

change in marine waters is measurable. However, if there is a reduction in pesticide input, the effects can be seen within a year or two, as pesticides are not found naturally and have a short half-life. Management strategies need to understand the nature of the parameter and its occurrence in marine systems (natural or non-natural). There is a need to be aware when identifying a trend analysis and the knock-on effect to biological communities.

Box 5. Ecosystem Health Monitoring Program (South East Queensland)

The Ecosystem Health Monitoring Program (EHMP) is one of the most comprehensive marine, estuarine and freshwater monitoring programs in Australia. It delivers a regional assessment of the ambient ecosystem health (or 'pulse') for each of South East Queensland's (SEQ) 18 major catchments, 18 river estuaries and Moreton Bay, highlighting where the health of our waterways is getting better or worse.

Ecosystem health is assessed using traditional water quality parameters, complemented by a limited range of biological indicators. Water quality parameters are measured every month at 254 sites, whilst sampling of biological indicators varies according to the characteristics of these particular indicators.

Estuarine/Marine Report Card
From the 254 sites, **maps** are produced for each indicator which show the median values for each sites for the reporting year.

Compliance scores are then calculated for each indicator as the proportion of the 'reporting zone' that complies with Queensland Water Quality Guidelines. (0=non-compliance, 1= compliance).

An E**cosystem Health Index (EHI)** for the reporting zone is calculated by averaging the compliance score for each indicator.

Biological Health Rating (BHR) assesses the biological indicators for estuaries and the bay. BHR ranges from 0 to 1 for each zone, with 1 representing an unmodified and healthy ecosystem. For more information, please refer to www.ehmp.org/index.html

Parameters
Water quality is comprised of physico-chemical parameters, dissolved and total nutrients, water clarity and phytoplankton abundance. Water quality information is collected to assess baseline ecosystem processes and to track changes in the zones of human impact.

Sewage nitrogen mapping assesses the extent of sewage treatment plant discharges into SEQ waterways through measurements of the uptake of the stable nitrogen isotope ^{15}N by the macroalga *Catenella nipae*.

Lyngbya majuscula is a toxic filamentous cyanobacterium found in tropical and sub-tropical marine and estuarine environments worldwide. EHMP compiles data from a variety of sources to create an annual distribution map of *Lyngbya* in Moreton Bay.

Coral community structure of hard substrates is monitored at five sites throughout Moreton Bay. Data are collected from 5 sites on three 20 m long transects at each site. Each transect runs parallel to the shoreline to ensure that data are recorded from similar depths across the site. Queensland Parks and Wildlife Service marine park rangers capture video footage along the transect lines using an underwater digital video camera. The film is analysed for total percent cover. The substrate is categorised into 5 groups; hard coral, soft coral, sponge, macroalgae and bare substrate. Incidents of coral bleaching are also recorded.

The **seagrass depth range** (SDR) is the difference in elevation (m) between the upper and lower depth record of the seagrass *Zostera capricorni* at a site. The distribution of seagrass in Moreton Bay is mapped every three years using a combination of remotely sensed images, underwater camera observation and visual estimation of seagrass cover.

1.2.8 Status report monitoring

One common use of monitoring programs is to collect data which can be used in an assessment/ranking scheme for the environment, such as a water quality report card or a 'State of the Environment' report. The Healthy Waterways Program is a well-developed setup in south-eastern Queensland in which a score-card system is associated with a comprehensive monitoring program (Text Box 5). See The ASEAN Marine Water Quality: Management Guidelines for further information.

Monitoring outputs via a report card or state of environment reporting process can manage expectations far better than reporting data (especially with lack of long-term data). They can identify where the problems are occurring, and are easily understood by non-scientists.

1.2.9 Research monitoring

Some water quality/ecosystem health questions only require a short study at limited locations to reach a conclusive answer and in these cases the boundary between monitoring and research becomes blurred.

1.2.10 Reactive water quality monitoring

Programs set up to link to a pollution event are known as reactive water quality monitoring events. They are typically triggered by a pollution event such as an oil spill and usually have contingency plans to be implemented very quickly after the pollution incident. These types of programs are usually associated with high public awareness of the pollution event and should be managed transparently with a well-developed public information and communication system in place. Information relating to the pollution event is not always able to be collected at the time of the sampling and links to long-term monitoring programs (where existing) and other sources of information are essential for the long-term management of the impacts of the pollution event. Text Box 6 illustrates a oil spill contingency program set up by the Australian National Maritime Safety Authority.

Box 6. National Maritime Safety Authority: National Marine Oil Spill Contingency Plan

The National Plan has been in operation since 1973 and brings together the combined resources of the Commonwealth, State and Northern Territory (State/NT) Governments, and the oil, shipping and exploration industries, to provide a level of preparedness to the threat posed to the marine environment by oil and chemical spills. For full response scenario, refer to www.amsa.gov.au/publications/OILSPILL

RESPONSE

Measures to be employed

In the event of an oil spill in the marine environment the following measures should be employed according to the circumstances of the spill and conditions prevailing:

• if possible prevent, control or stop the outflow of oil from the source;
• if coastal or marine resources are not threatened or likely to be threatened, monitor the movement and behaviour of the oil spill;
• if coastal and marine resources are threatened, activate response operations to protect sensitive resources;
• if possible, contain the spread of oil; and • if, due to weather and sea conditions, a response at sea is not feasible, or the protection of sensitive areas is not feasible, or these have already been affected, determine appropriate cleanup priorities and other response measures. The importance of human health and safety in any response operation cannot be overstressed.

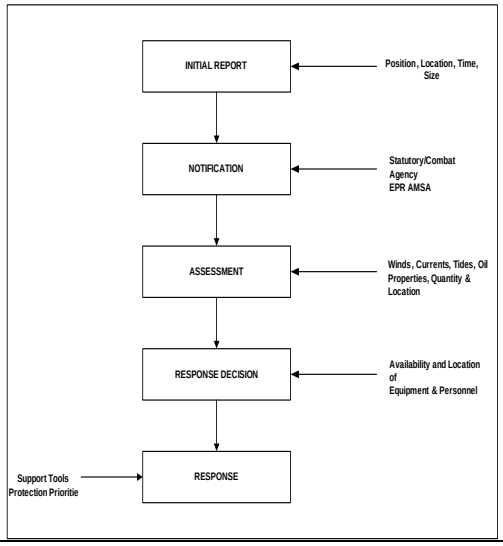
Overall Protection Priorities

Protection priorities to be employed during a response to an oil spill are, in order of descending priority:

• human health and safety; / • habitat and cultural resources; /• rare and/or endangered flora and fauna;
• commercial resources; and • amenities.

Incident Reporting and Response Activation

Notification of a pollution incident will normally be made from observations by Government agencies, shipping or aircraft, by the public, or by those responsible for the incident. The response procedures that shall be followed are summarised below:



1.3 The Steps Required to Design and Implement a Monitoring Program

Figure 1.1 outlines the six step process involved in the design and implementation of a marine monitoring program. Each step will be discussed in more detail in subsequent sections. Monitoring programs require identification of a set of objectives, or what is required from the monitoring program. The objectives of the program will then decide the structure of the study design, the field sampling program, laboratory analysis, data analysis and the final reporting and information outputs. Each section is covered within separate chapters.

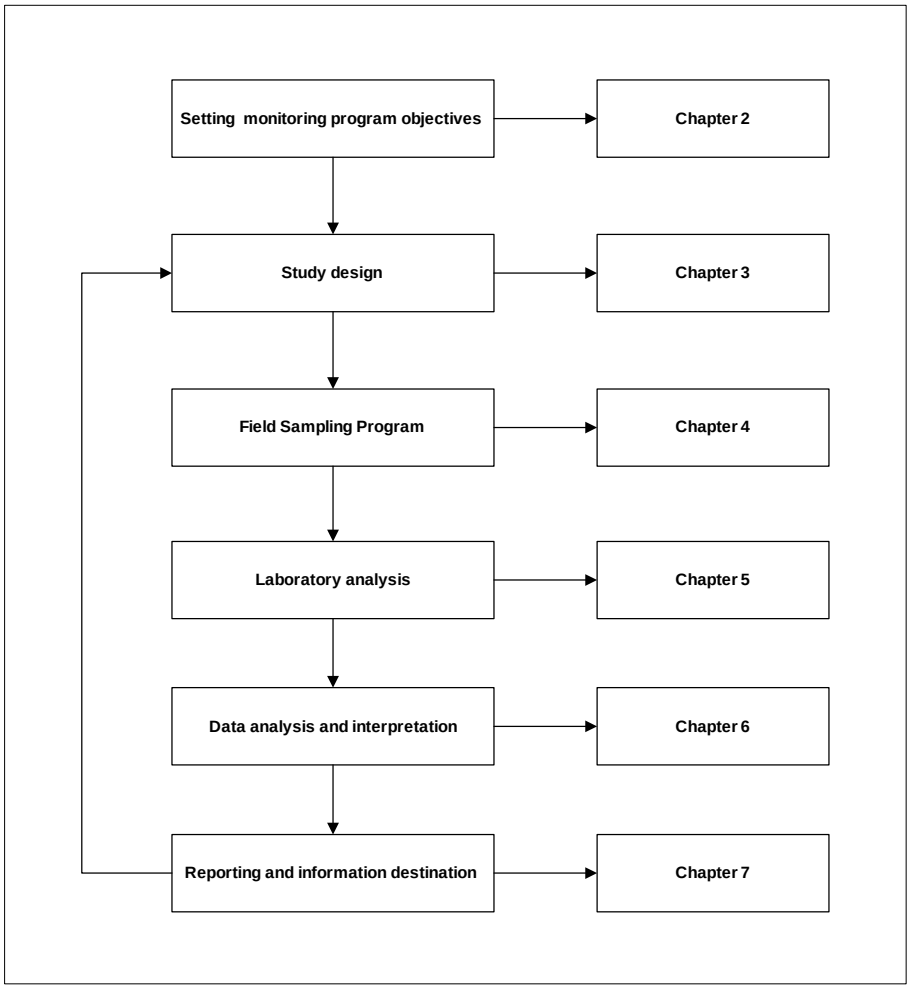


Figure 1-1. Framework for a water quality monitoring program. Each box is dealt with in individual chapters in this manual.

2 Setting Monitoring Program Objectives

The establishment of monitoring program objectives is facilitated by a series of steps, including definition of the issue, definition of the information requirements, compilation of all available information and development of a conceptual model. The recommended steps in the development of the monitoring program objectives are shown in Figure 2-1.

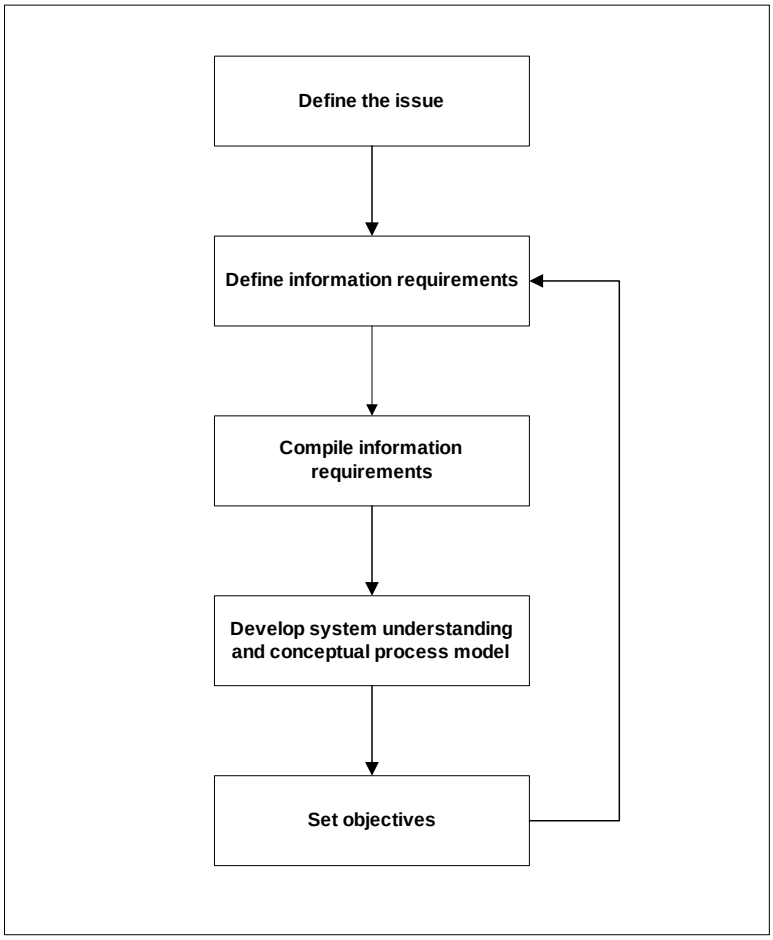


Figure 2-1. Framework for setting monitoring program objectives.

2.1 Define the Issue

When designing a monitoring program it is essential to define the issue, or the reason why it is important to monitor information on the parameters. One way to define the

issue is to identify the risks to the environment from anthropogenic influences. For example, the program 'Reefs at Risk' shows a risk-based analysis of the cumulative threats of over-exploitative land-use, pollution and coastal development, coupled with the effects of global climate change (www.wri.org/**reefs**at**risk)**. Despite widespread recognition that coral reefs are severely threatened, information regarding particular threats to specific reef areas is limited. Only a small percentage of reefs has ever been studied, and even fewer have been monitored over time using consistent methods. In addition, these data are rarely consolidated in a central repository where copies would be widely accessible.

This lack of information inhibits effective decision-making about coastal resources. The Reefs at Risk in Southeast Asia (RRSEA) project was designed to address this information deficiency through an extensive data compilation and improvement effort. Understanding which human activities negatively impact which reefs is a key to future conservation and planning efforts. The goal of the RRSEA project is to raise awareness about threats to coral reefs and provide resource managers with specific information and tools to manage coastal habitats in Southeast Asia more effectively.

2.2 Define Information Requirements

It is important to consider over what area in the marine environment the key pollutants are likely to be dispersed and whether the monitoring program will cover this whole area or concentrate on some smaller part, perhaps considered to be representative of the whole.

An idea of the likely duration of the study is also important. This may depend on natural variability of the system (long enough to distinguish the signal being detected from the 'noise'), seasonal factors, logistics constraints, budgets and other factors.

2.3 Compile Available Information

Before starting any monitoring program, it is important to review all existing data, in both scientific and grey literature. A review of existing water quality information and ecosystem health information in the study area provides background information for the new monitoring program. Historical data can be invaluable in investigating trends in the water quality parameters, identifying the approximate ranges of concentrations that exist in the monitoring areas, and can increase confidence in potential outcomes. Spatial analysis of the existing data is useful in identifying areas of interest and areas of concern.

2.4 Develop System Understanding and Build a Conceptual Model

This is a critical initial step in monitoring design. A clear understanding/model of how the identified key pollutants cause loss of ecosystem health or values in the region of concern is necessary to interpret the meaning of the data collected.

Most monitoring programs use a qualitative monitoring model to deal with the monitoring results because it is not initially clear what a change in a key pollutant concentration means for the ecosystem/value at risk. Models can be simple or complex and can be pictorial or 'arrows and connections' (Figure 2.2).

A conceptual model of the system with a simple diagram showing the boundaries of the system of concern and showing the important inputs and outcomes of interest is all that is needed (Maher et al. 1994). Its production may help ensure inclusion of all the important processes and critical measurements. Conceptual models are important in defining the 'why' question. These diagrams enable explicit knowledge about an aquatic system, especially assumptions of how a system functions and the important (or dominant) processes. Models can help define:

- What to measure and with what precision;
- Site selection;
- Spatial boundaries; and
- Time and seasonal considerations.

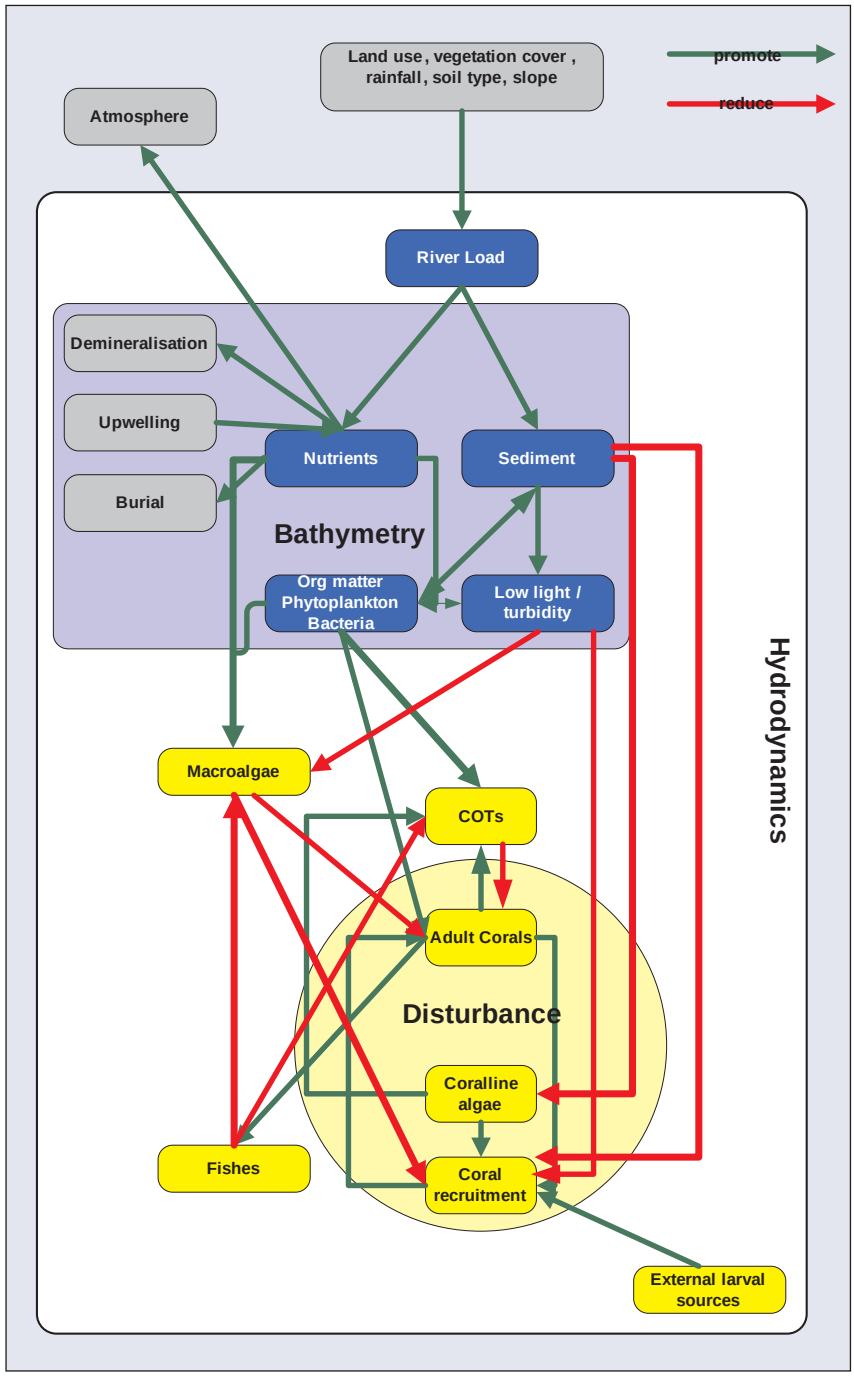


Figure 2-2. Conceptual model of the effects of sediment and nutrient loads on coral reefs (from Fabricius 2007). (COTs = Crown of Thorns Starfish)

2.5 Set Objectives

It is critical that the monitoring program has a question or an objective which is believed to be able to be answered using the data to be collected. Many water quality monitoring programs globally to date did not have a clear question, so that years of data collection did not answer the question that needed an answer. By setting the questions(s) early, the monitoring program can be designed with the correct sampling locations, frequencies, parameters etc to specifically answer the actual question. Examples of hypothetical questions would be:

- Is a marine system improving due to management actions?
- If so, how can you tell which way it is heading?
- Which area is most at risk?
- What is the source of risk?

2.6 Summary

Specific monitoring objectives should be considered for each ecosystem or Beneficial Use identified in the monitoring program.

A comprehensive water quality monitoring program in a tropical environment should include biological monitoring in parallel with water column monitoring (these are closely inter-linked and related). Biological monitoring manuals can be found readily in literature (e.g., www.aims.gov.au; Christie et al. 1995). This present monitoring manual focuses on the water column. Practical cost-effective approaches are included with a range of methodologies referred to but not always described in detail. It is recommended to use this manual with the supporting appendices and referenced literature.

3 Study Design Issues

3.1 Introduction

The ultimate goal of any sampling strategy is to guarantee the acquisition of valid data. The sampling strategy is governed by the *objectives* of the monitoring program and by the expected or known *spatial and temporal variability* of the analyte concentrations.

For a monitoring program to produce the maximum amount of useful data with the minimum of effort, information is needed regarding:

- Nature of the area;
- Location and nature of pollution sources;

- Areas of immediate concern;
- Sampling stations;
- Number of samples and frequency of sampling; and
- Hydrological and climatic information.

A well-planned monitoring study needs to take into account all potential sources of variability. When a clear understanding of the natural variability is understood, anthropogenic influences can be identified.

Monitoring should involve a multi-scaled sampling approach with a focus on key parameters that are expected to respond to management actions. In addition to the techniques presented in this manual, it is important to identify relevant but simple measurements to encourage community participation and strategic fixed stations to improve our understanding of temporal variability in key parameters (Falkowski and Kolber 1995).

3.2 Location

Before commencing a sampling program it is important to understand the area to be sampled. The first requirement is a large-scale map which illustrates the topography and catchment. The location of inflowing streams and rivers and urban centres are particularly relevant and should be noted for future analysis, together with any special features which can be used as reference points for sampling stations. Aerial surveillance can give a valuable and unique perspective of a catchment, estuary or coastal environment which is difficult to achieve by other means. During a physical inspection of the area, the aerial map should be annotated to show any useful shore-based reference points. Every estuary and coastal area has particular tidal patterns, sediment loads, salinities and temperatures and when the basic characteristics of an area are understood, many predictions about these and other parameters can be made.

Information gleaned from local maps and visual inspection should assist in identifying the major potential sources of pollution to be monitored. While sampling is invariably used as a regulatory or control mechanism to manage existing problems, it is equally important and more cost effective to use it to identify unaffected areas. The identification of reference sites can be invaluable in assessment of anthropogenic change.

3.2.1 Definition of sampling area

For the purposes of this manual and interpretation of the ASEAN water quality criteria, definitions of estuarine and coastal areas need to be clarified. The definitions of estuarine and coastal waters are based on the traditional definition of an estuary

where the estuarine areas are contained between river banks and up to the tidal limit as shown in Figure 3.1. In some interpretations the coastal area outside the estuary is known as a river plume influenced estuarine area but in this manual we will call this the coastal and/or marine area. The estuarine area is typically a heterogeneous system with highly variable salinity regimes, influenced by both freshwater intrusion and tidal currents. It is a dynamic system where salinity may vary between 0 and 35 psu depending on the season and freshwater inflow. This variable environment influences all biological and chemical processes and should be accounted for in any data analysis. In contrast, the coastal area is characterised by consistent salinities between 30 to 35 psu for most of the year (excluding periods of high riverine discharge).

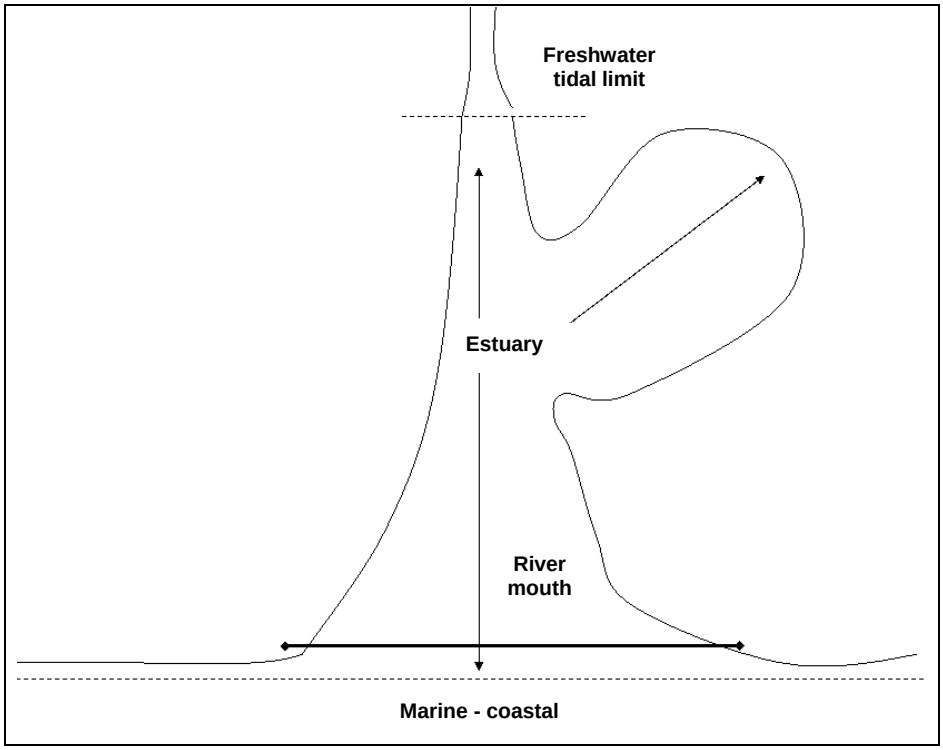


Figure 3-1. Freshwater, estuarine and coastal areas.

3.3 Selection of Field Sampling Sites

Sampling sites should form a gradient away from the source (e.g., river mouth, sewage treatment plant (STP) outfall) towards the ecosystem of concern (coral reef, fishery area, aquaculture intake, recreational area, tourist area). Logistics of access to sites—vessel access, weather constraints, water depth, safety issues and vessel traffic—also need to be considered.

The distribution of analytes in seawater varies according to locality, depth and time (season) due to changes in physical and biogeochemical processes. Thus, the water sampled should be representative of the conditions of the water body being studied or at a given geographical location. The selection of sampling sites and depth is dependent on the objectives of the monitoring program. Due consideration should be given to variability of the analyte concentrations among sampling locations e.g., coastal urban and industrial areas, and to prevent memory effects from samplers used when the concentrations of the target analytes vary greatly at different depths from the same station or at a certain area.

However, for monitoring programs focusing on the baseline for a well-mixed seawater body, such variations are expected to be minimal. Sampling at uniform depth, e.g. 30 cm below surface, and a fixed distance from the coast, may be selected for a sampling program.

Sampling sites should be located in order to determine not only the impact of direct sources but also the extent of impact on the whole estuary or coastal water body. For sampling programs to be effective it is necessary to take samples at extremes of tide and season, often over considerable periods of time (months to years) and/or at long time intervals. Fixed sampling sites enable repetition and comparison over time. This is where good planning of the program in advance is particularly important.

Figure 3-2 illustrates some of the major types of hydrographic situations which may be encountered and some types of sampling programs. In areas where large intertidal flats are exposed, sampling carried out at or near low water is necessarily greatly restricted by the limited volume of water available. While the examples given are illustrative, it should be noted that when the main waterway is joined by tributaries or creeks as in B, samples should be taken in the side channels and just below their confluence with the main channel. This will be useful in isolating local sources and determining their contribution to pollution in the main water body.

The design of a sampling plan should ensure that samples are collected at sites and times that provide a representative sample, thus providing an accurate description of the water body. Sampling sites should be located in areas that are safe to access, accessible under all weather conditions, be well-mixed to ensure homogeneous samples and be easily locatable for routine sampling. Permanent sampling locations should be established to ensure that representative samples can be compared over time.

To examine the effect of a point-source discharge, sites should be arranged in such a way that the end-of-pipe, upstream and downstream zones of the discharge water are sampled. The degree of mixing within the water body will determine the proximity of sites to each other—where mixing is strong (the water is homogeneous) sites may be spread further apart.

It is often sufficient to take discrete samples just below the surface at a given site, particularly in shallow and well-mixed environments. In deeper or poorly-mixed waters, a 'surface sample' may not accurately describe the characteristics of the entire water column and other sampling techniques should be employed. These can include integrated vertical column samples or discrete samples at given depths.

There are no strict rules regarding sampling frequency, but this will be dictated by the known variability of the parameters of interest and the objectives of the plan. Careful planning should determine a sampling frequency that has the best chance of providing the information required to meet the objectives of the plan. It is important to consider the frequency carefully. If samples are not taken frequently enough, the characteristics of the water body might not be adequately described, resulting in a poor understanding of the system and potentially inaccurate reporting of compliance or non-compliance. On the other hand, overly frequent sampling may be a waste of time and resources.

In general, if a measurement parameter has a predictable pattern which has been shown statistically or through a pilot study (e.g., discharge at a certain time of day) the sampling plan can be tailored to sample at regular intervals. Alternatively, if the system or processes are highly variable or unpredictable, the sampling should be undertaken more regularly over several time-scales.

3.4 Data Variability

A major consideration in the design of any program is the natural variability of the response variable. The spatial scale of variation of the biological variable involved will determine the size and number of sampling units and the number of levels of sampling in a hierarchical design. Integrating these considerations with the requirements imposed on the sampling by the spatial scale of the impact can be tricky. Similarly the temporal variability (speed and magnitude of change in seasonal patterns) will influence the duration, frequency and pattern of sampling.

The most appropriate sampling technique will be the one that provides the necessary data at the required time and space intervals in the most cost-effective manner. Some examples of different sampling frequency are

- Continuous measurement by a probe *in situ;*
- Intermittent measurement by a probe *in situ;*
- Automatic sampling based on flow, time or other basis;
- Manual sampling based on flow, time or other basis;
- Processing sample on site or returning to laboratory; and
- Remote sensing via satellite or aircraft.

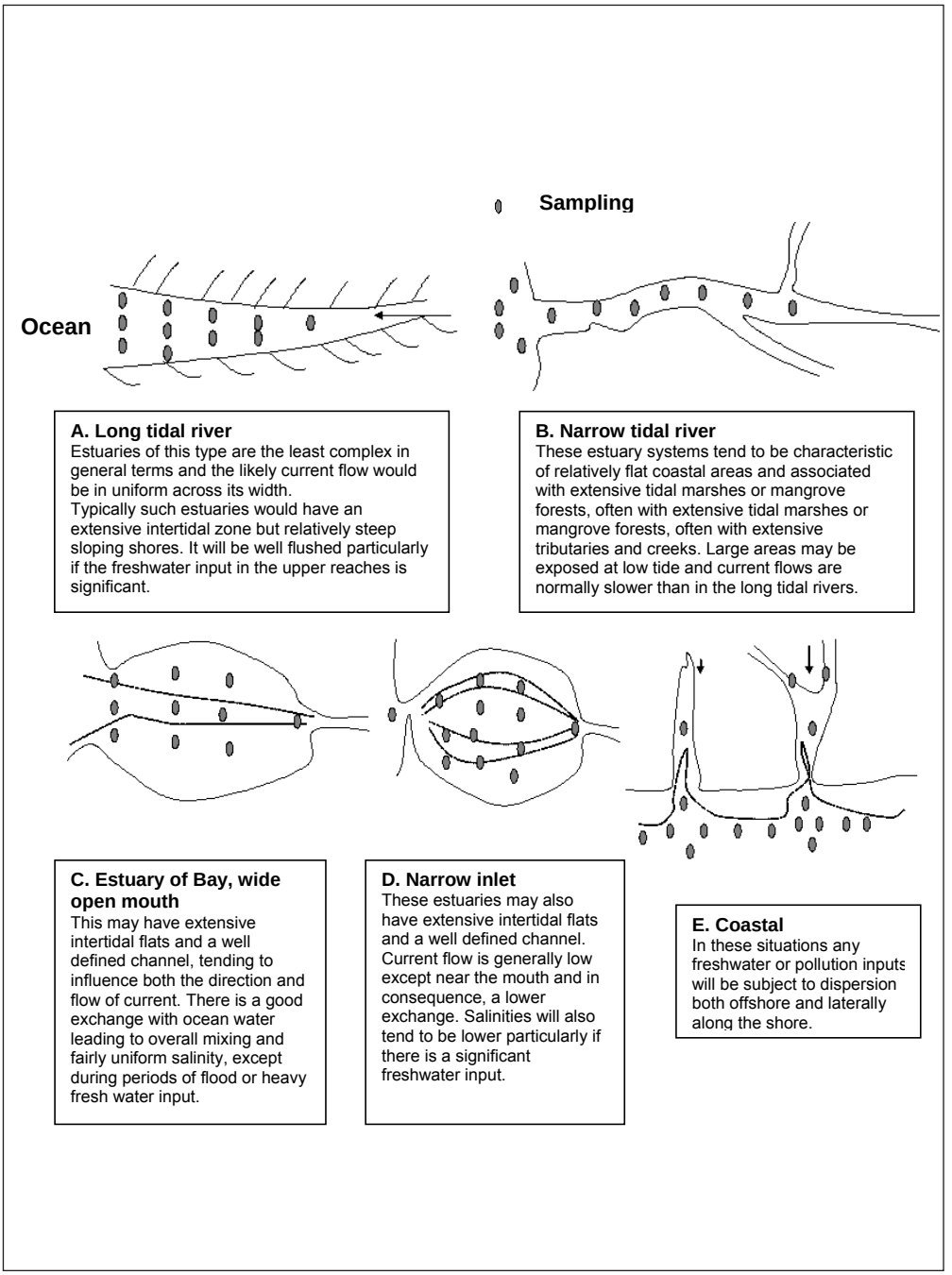


Figure 3-2. Suggested sampling sites for different types of estuaries and coastal waters.

3.5 Spatial and Temporal Variability

It is important to determine the number and arrangement of sites to capture the spatial variability of the water body and pollutant transport. Currents, prevailing winds, size of discharge, dilution factors and processes happening to the pollutants, e.g., sedimentation, biological uptake, evaporation, photodegradation may all affect the placement of sampling sites.

Sampling needs to be carried out at frequencies which capture the natural variability and/or event-based nature of the system. Thus consideration is necessary of seasonality, e.g., wet season/dry season, monsoon pattern; discharge events, e.g., outfall dynamics, river flow patterns; pollutant process dynamics, e.g., periods of sedimentation, biological uptake, benthic sediment resuspension. Logistics may also be a constraint on design, e.g., sampling from a tourist vessel constrained by its commercial timetable.

The timing of sampling may range from continuous to intermittent, even during an annual visit. Sampling frequency should not be driven by cost or convenience, but by the hypothesis being tested. One consideration in timing is the process under investigation. In an algal bloom development, the numbers of algal cells may double every two to three days. If the question relates to nutrient fluxes, then sampling needs to reflect flow events that transport materials into and through the aquatic system.

Variability in time and space of the parameters of interest is a significant aspect to be considered in the sampling design. This variability will determine the number of sites, number of replicates and the frequency of sample collection.

Examples of variation include:

- Short term variation – particular events such as scheduled discharges of wastewater might always occur on the same day of the week leading to a consistent pattern of variation in the quality of the discharge;
- seasonal variation, such as that experienced in specific industries with a seasonal activity pattern;
- event variation – the influent (and effluent) from sewage treatment plants varies after a rainfall event due to the infiltration and inflow into the sewage system diluting the concentration but increasing the volume of wastewater;
- diurnal variation – changes in dissolved oxygen and pH throughout the day due to respiration and photosynthesis, changes in water temperature;
- depth variation – stratification effects can occur in lagoons and lakes as well as in rivers, creeks, marine water and estuaries;
- seasonal and event variation – variations in flow and salinity due to rainfall and temperature;

- tidal variations – variation in flow direction and volume due to tide (in rivers and estuaries as well as the marine environment); and
- spatial variation – occurs in the receiving environment due to a range of factors from natural biological variability, wave action and turbulence through to flow and concentration modifications around structures such as jetties and weirs.

Knowledge of the variations likely to affect monitoring results is important in selecting the frequency and pattern of sampling as well as sampling points.

A major consideration in the design of any program is the natural variability of the response variable or parameter of interest. The spatial scale of variation of the biological variable involved will determine the size and number of sampling units and the number of levels of sampling in a hierarchical design. Integrating these considerations with the requirements imposed on the sampling by the spatial scale of the impact can be tricky. Similarly, the temporal variability (speed and magnitude of seasonal changes) will influence the duration, frequency and pattern of sampling. The following list of considerations will be important at the design stage:

- Location – accessibility, proximity to pollutant sources;
- Depth – homogeneity of water body, stratification;
- Current – source of sampled water;
- Tidal conditions – source of sampled water, mixing regime;
- Timing – natural variability – tides, river discharge, rain, algal blooms;
- Salinity – river discharge gradients;
- Frequency;
- Spatial and temporal variability issues; and
- Cost versus confidence – sampling frequency.

3.6 Precision and Accuracy Required

In developing a monitoring program, the number and frequency of samples needs to be sufficient to provide certain levels of confidence in the interpretation of results. Most of the sampling undertaken for regulatory purposes is a form of hypothesis testing—i.e. testing the hypothesis that environmental harm has not occurred. In hypothesis testing there are two types of errors to aim at avoiding: falsely detecting environmental harm when it has not occurred (false positive, Type I error) and not detecting environmental harm when it has occurred (false negative, Type II error). The probability of a false positive is called the level of significance. The probability of a false negative (Type II error) is related to the power of the test. Further information on determining the probabilities is provided in texts such as ANZECC (2000). Depending

on the circumstances it may be preferable to minimise each type of error (or both) for particular regulatory requirements.

The method detection limit (MDL) is defined as the 'minimum concentration of an analyte that can be measured and reported with a 99% confidence that the analyte concentration is greater than zero'. The practical quantification limit (PQL) is the minimum concentration of an analyte that can be accurately and precisely quantified. In general, the PQL is 5 to 10 times the MDL, depending on the analyte. Statistical tests are more accurate when most data values are above the PQL.

4 Field Sampling Program

4.1 Sampling Methods and Equipment

4.1.1 Introduction

This chapter outlines the various sampling methods that could be used in the sampling of marine waters. Some sampling protocols are common to many of the parameters.

The methodology associated with field sampling is outlined, including the requirements of sample collection, identification of field measurements, sample container requirements sample preservation, quality assurance and control.

4.1.2 Common sampling protocols

The sample collection process should be co-ordinated with the laboratory so that analysts know how many samples will be arriving, the approximate time of their arrival and the analyses that are to be carried out, and can thus have appropriate quantities of reagent chemicals prepared.

It is good practice to prepare a checklist such as the one on the following page, so that nothing is missing or forgotten before a sampling expedition is undertaken. Many of the items in the list are self-explanatory; others are described more fully in later sections of this chapter.

Personnel trained in both sampling techniques and field test procedures should also be aware of the objectives of the monitoring program. The choice of a representative sampling point and the use of appropriate sampling techniques are of fundamental importance

A checklist of equipment is vital for a successful field trip. On-site testing is common for certain variables, especially those that may change during transport. Dissolved oxygen, turbidity, transparency, conductivity, pH and temperature are most often measured on site. Procedures for carrying out analyses in the field are covered in Section 4.9.

4.1.3 Sample container requirements

Selection of sample containers is dependent on the analyte being considered. Containers for the transportation of samples should be provided by the laboratory. This ensures that large enough samples are obtained for the planned analyses and that sample bottles have been properly prepared, including the addition of stabilizing preservatives when necessary. It is essential to have enough containers to hold the samples collected during a sampling expedition. Sample containers should be used only for water samples and never for the storage of chemicals or other liquids. Glass containers are commonly used and are appropriate for samples for many analyses, but plastic containers are preferred for samples intended for certain chemical analyses. Plastic has the obvious advantage that it is less likely to break than glass.

Sample containers must be scrupulously clean so that they do not contaminate the samples placed in them. Table 4-1 provides general information on appropriate types of sample container and the recommended procedures for cleaning them when water samples are to be used for chemical analysis. Some water quality variables are unstable and, unless an analysis can be carried out immediately after the sample is obtained, it is necessary to stabilise the sample by adding a chemical preservative. It is often convenient to add chemical preservatives to containers in the laboratory. It is essential that the containers be clearly labelled with the name, concentration and quantity of the preservative chemical, the volume of the sample to be collected and the variables for which the sample is to be analysed. If preservatives are not added to containers in the laboratory, the chemicals, pipettes and directions for adding preservatives must be included in the kit of supplies and equipment taken on the sampling expedition.

Table 4-1. Sample containers and requirements for field sample collection.

Variables to be analysed	Recommended container	Washing procedure
Aluminium, Barium, Beryllium, Cadmium, Chromium, Cobalt, Copper, Iron, Lead, Lithium, Manganese, Molybdenum, Nickel, Selenium, Strontium, Vanadium, Zinc	500-1,000 ml polyethylene (depending upon number of metals to be determined)	Rinse 3 times with tap water, once with chromic acid, three times with tap water, once with 1:1 nitric acid and then 3 times with ultrapure distilled water.
Acidity, Alkalinity, Arsenic, Calcium, Chloride, Colour, Fluoride, Hardness, Magnesium, pH, Potassium, Sodium, Sulphate, Turbidity	1,000 ml polyethylene	Rinse 3 times with tap water, once with chromic acid, three times with tap water, once with 1:1 nitric acid and then 3 times with ultrapure distilled water.
Carbon, total organic Nitrogen as ammonia Nitrogen as nitrate, nitrite Nitrogen, total	250 ml polyethylene	Rinse 3 times with tap water, once with chromic acid, three times with tap water, and then 3 times with ultrapure distilled water.

Phosphorus, total	50 ml glass	Rinse 3 times with tap water, once with chromic acid, three times with tap water, and then 3 times with ultrapure distilled water.
Cyanide Chlorophyll (spectrophotometric methods), Suspended Solids	1000 ml plastic	Wash 3 times with water and detergent.

4.1.4 Sample types

Two different types of sample can be collected. The simplest, a discrete sample, is taken at a selected location, depth and time. Normally, the quantity of water collected is sufficient for all the physical and chemical analyses that will be done on the sample. Sometimes, if the sampler is small and many analyses are to be done, two samples will need to be taken at the station and will be mixed in the same transport container. Discrete samples are also known as 'spot' or 'snap' samples. Composite or integrated samples, i.e., combined samples, may be needed to fulfil some specific monitoring objectives. There are several types of integrated samples:

- *Depth-integrated:* most commonly made up of two or more equal sub-samples collected at predetermined depth intervals between the surface and the bottom. A piece of flexible plastic piping several metres in length and weighted at the bottom provides a simple mechanism for collecting and integrating a water sample from the surface to the required depth. The upper end is closed before hauling up the lower (open) end by means of an attached rope. Integrated samples can also be obtained using a water pump (submersible pumps are available which allow sampling at depth) which is operated at a steady pumping rate while the water inlet is drawn upwards between the desired depths at a uniform speed.
- *Area-integrated:* made by combining a series of samples taken at various sampling points spatially distributed in the water body (but usually all at one depth or at predetermined depth intervals).
- *Time-integrated:* made by mixing equal volumes of water collected at a sampling station at regular time intervals.
- *Discharge-integrated:* for such samples it is first necessary to collect samples and to measure the rate of discharge at regular intervals over the period of interest. A common arrangement is to sample every 2 hours over a 24-hour period. The composite sample is then made by mixing portions of the individual sample that are proportional to the rate of discharge at the time each sample was taken

4.1.5 Sampling at depth

The depth water sampler is designed in such a way that it can retrieve a sample from any predetermined depth. There are several types of water-sampling containers, named after their designers: Nansen, Niskin and Van Dorn. Plastic buckets may be used in an emergency but they do not sample a known depth of water.

4.1.5.1Equipment for sampling at depth

a. The Nansen bottle

The Nansen bottle, a metal or plastic cylinder, is lowered on a cable into the water. When it has reached the required depth, a brass weight called a 'messenger' is dropped down the cable. When the weight reaches the bottle, the impact tips the bottle upside down and trips a spring-loaded valve at the end, trapping the water sample inside. The bottle and sample are then retrieved by hauling in the cable.

A second messenger can be arranged to be released by the inverting mechanism, and slide down the cable until it reaches another Nansen bottle. By fixing a sequence of bottles and messengers at intervals along the cable, a series of samples at increasing depth can be taken.

The sea temperature at each water sampling depth is recorded by means of a reversing thermometer fixed to each Nansen bottle. This is a mercury thermometer with a constriction in its capillary tube which, when the thermometer is inverted, causes the thread to break and trap the mercury, fixing the temperature reading. Since water pressure at depth will compress the thermometer walls and affect the indicated temperature, the thermometer is protected by a rigid enclosure. A non-protected thermometer is paired with the protected one, and comparison of the two temperature readings allows both temperature and pressure at the sampling point to be determined.

The Nansen bottle has largely been superseded by the Niskin bottle and now is no longer under manufacture, though is still in use.

b. The Niskin bottle

The Niskin bottle is a development of the Nansen bottle. The 'bottle' is a tube, usually plastic to minimise contamination of the sample, and open to the water at both ends. Each end is equipped with a cap which is either spring-loaded or tensioned by an elastic rope. The action of the messenger weight is to trip both caps shut and seal the tube.

A reversing thermometer may also be carried on a frame fixed to the Niskin bottle. Since there is no rotation of the bottle to fix the temperature measurement, the thermometer has a separate spring-loaded rotating mechanism of its own tripped by the messenger weight.

A modern variation of the Niskin bottle uses actuated valves that are either preset to trip at a specific depth detected by a pressure switch, or remotely controlled to do so via an electrical signal sent from the surface. As many as 36 Niskin bottles can be mounted together in a circular frame termed a *rosette*.

Thermistor temperature sensors are more commonly employed on Niskin bottle rosettes due to their higher accuracy compared to mercury thermometers.

c. The Van Dorn bottle

This is another open sampler with spring-loaded closures but because it can be used in a horizontal fashion it can sample a thinner layer of the water body and may be useful in very shallow waters. It is normally constructed of plastic but also available with Teflon coated surfaces which are useful for sampling for metals.

Table 4-2. Description of sampling containers and suggested parameters to be collected in each type of container.

Sampling container	Composition of sampler	Suggested parameters	Use
Niskin	Plastic	Nutrients, metals	
Nansen	Metal	Nutrients, pesticides	Not used for metals
Van Dorn	Plastic	Nutrients, metals	Shallow water sampling
Bacteriological	Sterilised glass	Bacteria, pesticides	
Bucket	Plastic, open	Nutrients	

The use of Niskin bottles (or other sampling bottles) will depend on the depth of the water and the required number of samples. The methods described below refer to Niskin bottles.

If surface and bottom samples are required, the following applies:

For deeper waters (>10 metres), it is suggested that samples be taken 1 m below the surface and 3 m above the bottom. For shallower waters (5-10 metres), it may be more appropriate to take samples 1 m below the surface and 1 m above the bottom. For well-mixed waters less than 5 m, it is appropriate to take a surface sample. For stratified waters of any depth, it will be more appropriate to sample above and below the stratified layer. Thus samples should be taken in the top layer, through the stratified layer and below the stratified layer. Table 4.4 outlines the equipment required for water sampling and their uses.

If waters are to be collected through the water column, then Niskin bottles can be set at equal distances along the sampling wire. For example, if the water at the sampling site is 20 m depth, Niskin bottles could be set out at 1, 6, 11 and 17 m.

Table 4-3. Equipment required for the sampling of water in a marine environment (assumes sampling from a boat).

Equipment	Use
Niskin bottles	Sampling of surface and sub-surface waters
Holding Rack	Holding the Niskin bottles in upright position
Thermometers	For measurement of water sampled. Niskin bottles can use reversing thermometers
Section pulley	To carry weight of Niskin bottles
Messengers	To relay down wire to close Niskin bottles at required depths
Secchi disc	Divided into black and white quadrants and used for estimation of turbidity (reference)
Field data sheets	All sampling and field details should be included on the field data sheets
In situ logging	Refer to Section 4.9

4.1.5.2 Typical procedure for depth sampling

1. At a sampling site, record the acoustic depth as measured by the depth sounder.
2. Record weather details and geographical position on the field data sheet. Note station name, date and time. Observe water surface for presence of *Trichodesmium* and other algal blooms and record observations on data sheet.
3. Take a Secchi disc reading from the sampling side of the vessel and record value on data sheet.
4. Set the Niskin bottles for sampling by locking the top and bottom lids into the open position and connecting the thermometers to the front of the bottle.
5. Secure the reversing thermometers within a holder which is screwed to the bottle. Care should be taken when attaching the screws to the Niskin bottle. Instructions on temperature readings will be specific to the manufacturer's guidelines and should be applied.
6. Attach the dump weight to the end of the sampling wire. The cable should be kept clean at all times to minimise contamination from oil or rust and should be replaced if they are rusted.
7. Take the first Niskin bottle from the rack, set and attach it to the wire, approximately 0.5 m above the lead weight. Lower the wire until the Niskin

bottle is 5 m above the acoustic depth. Attach the second cocked Niskin bottle to the wire. Lock the messenger onto the quick release pin located just below the top Niskin bottle. Allowing 1 m for the freeboard of the boat, lower the second Niskin bottle 1 m from the side of the boat.

8. Wait approximately two minutes for the reversing thermometers to equilibrate with the surrounding water, then attach and release a second messenger down the wire. Place a hand on the wire to feel the vibrations as the Niskin bottle fires. The messenger triggers the closing mechanism on the Niskin bottle, reverses the thermometers to take the *in situ* temperature reading and releases the attached messenger to trigger the lower Niskin.
9. Wait 10 seconds and winch the bottles up. Release each Niskin bottle from the wire and replace in the bottle rack. Secure the bottles in the rack with elastic cord.
10. Read the *in situ* water temperature by swiping a magnet over each reversing thermometer. Immediately record the temperature and time on the field data sheet.
11. Commence laboratory water sampling procedures once the Niskin bottles have been secured in the rack.

4.1.6 Samples for physical and chemical analyses

The minimum sample size varies widely depending on the range of variables to be considered and the analytical methods to be employed, but it is commonly between 1 and 5 litres. The volumes required for individual analyses are summarised in Table 4-3, which also describes the holding time, or the maximum time that the stabilised sample can be stored. Further storage after the maximum holding time would result in degradation of the sample.

Table 4-4. Water quality parameters, recommended sampling volume, preservative required for storage, and maximum holding time in storage.

Parameter	Sample Volume (mL)	Container type	Preservative/ Storage	Holding time
Alkalinity	250	Plastic	Refrigerate	24 hrs
Aluminium	250	Plastic	Nitric acid (1%m/V)	1 month
BOD	1,000	Glass or plastic	Refrigerate in dark	24hours
Calcium	250	Plastic	Nitric acid (70%m/V)	1 month

Chlorophyll	100 /1000	Plastic	Freeze filter medium in dark	1 month
Chloride	250	Plastic	-	6 months
Cyanide	1000	Plastic	NaOH, refrigerate in dark	24 hours
Fluoride	250	Plastic	-	1 month
Iron	250	Plastic	Nitric acid (70%m/V)	1 month
Magnesium	250	Plastic	Nitric acid (70%m/V)	1 month
Manganese	250	Plastic	Nitric acid (70%m/V)	1 month
Ammonia nitrogen	100	Plastic	Refrigerate	6 hours
Kjeldahl nitrogen	250	Plastic	Refrigerate or Freeze	24 hours 1 month
Nitrate nitrogen	100	Plastic	Refrigerate or Freeze	24 hours / 1 month
Nitrite nitrogen	100	Plastic	Freeze	48 hours
Phosphorus	100	Plastic	Freeze	48 hours
Phaeophytin	100 /250	Plastic	Freeze filter medium in dark	1 month
Potassium	250	Plastic	-	1 month
Selenium	250	Plastic	Nitric acid (70%m/V)	1 month

- The following general guidelines can be applied to the collection of water samples to be analysed for physical or chemical variables from freshwater and marine areas:
- Before collecting any sample, record the location and position of the sampling site.
- Avoid touching and disturbing the bottom of a water body when taking a depth sample, because this will cause particles to become suspended.
- Sampling depth is measured from the water surface to the middle of the sampler. Samples taken to describe the vertical profile should be taken in a sequence that starts at the surface and finishes at the bottom. When taking the sample at the maximum depth it is important to ensure that the bottom of the sampler is at least 1 m above the bottom.
- Do not lower a depth sampler too rapidly. Let it remain at the required depth for about 15 seconds before releasing the messenger (or whatever device closes

the sampler). The lowering rope should be vertical at the time of sampling. In flowing water, this will not be possible and the additional lowering necessary to reach the required depth should be calculated.

- The container to be used for transport or storage of the sample should be rinsed three times with portions of the sample before being filled. This does not apply, however, if the storage/transport bottle already contains a preservative chemical.
- The temperature of the sample should be measured and recorded immediately after the sample is taken.
- Separate portions of the sample should be set aside for pH and conductivity. The same portion must not be used for both determinations because of the possibility of potassium chloride diffusing from the pH probe.
- Whenever the sample bottles are open, their tops must be kept in a clean place.
- A small air space should be left in the sample bottle to allow the sample to be mixed before analysis.
- All measurements taken in the field must be recorded in the field notebook before leaving each sampling station.
- All supporting information should be recorded in the field notebook before leaving each sampling station. Conditions such as ambient air temperature, weather, presence of dead fish floating in the water or of oil slicks, algal growth or any unusual sights or smells should be noted, no matter how trivial they may seem at the time. These notes and observations will be of great help when interpreting analytical results.
- Samples should be transferred to sample bottles immediately after collection if they are to be transported. If analysis is to be carried out in the field, it should be started as soon as possible.

4.2 Nutrients

4.2.1 Introduction

Nutrient determinations in estuarine and coastal water matrices present unique problems due to the highly variable salinities of the samples and the wide and dynamic range of nutrient concentrations encountered. This is particularly pertinent in tropical waters where oceanic surface waters have exceedingly low nutrient concentrations but inshore anthropogenically-affected waters may have high concentrations and the deeper ocean waters are also high in nutrients. Internationally, a great deal of

work has already gone into documenting the methods needed to monitor nutrients in tropical marine waters: see for example Johnstone and Preston (1993) and Devlin and Lourey (2000).

Tropical marine ecosystems generally exhibit high levels of production (in the same range as some of the most productive terrestrial ecosystems in the world) in waters with nutrient concentrations which are typically very low or undetectable. Oceanic coral reefs characteristically exhibit high levels of productivity and species diversity (parallel to rainforests) even though they exist in nutrient-poor oceanic waters. As a result, nutrient studies in tropical marine ecosystems often require the detection of compounds at extremely low levels, often at the lower limits of the methods presently available (Johnstone and Preston 1993). Consequently, nutrient analysis in tropical waters requires considerably greater vigilance than might be needed when working with, for example, sewage waters or samples from 'richer' temperate coastal marine ecosystems where the majority of nutrient investigations have historically been carried out. In addition to the lower levels to be detected, tropical environments often also present a more demanding environment with regard to the storage of samples and the analysis procedures. High temperatures and humidity can present considerable problems for both the chemist and chemistry, significantly increasing the difficulty in achieving accurate results. All analyses should be conducted in an environmentally controlled laboratory, but this is not always achievable and many researchers have to work under difficult and less than ideal circumstances.

4.2.2 Sampling strategy

There are considerable problems involved with the treatment and storage of samples for nutrient analysis, many of which arise from the very rapid rate at which nutrients are assimilated by both phytoplankton and bacteria. In addition there are difficulties with possible changes occurring because of sample manipulation. In general it is recommended that samples be filtered if there is visible turbidity but not if the water is clear. It is more likely to be necessary to filter waters from close to the land than those from samples obtained from 'blue' waters off the continental shelf. If filtration is necessary, it should be performed as soon as possible after collection and the exposure of the sample to the air must be minimised. Dispensing of samples in a way that generates bubbles, for example, should be actively avoided. Suitable containers are made by a number of manufacturers but the product made by Millepore may be taken as a reference point. Filter units should be properly cleaned before use and filters, once installed, should be pre-cleaned by passing distilled de-ionised water through them. At least two sample volumes of distilled water should be used for cleaning purposes. Also, it is important to check that the filter is not ruptured during the installation process. This may easily be done with syringe/filter combinations by gently trying to push air through the wetted filter. If when the plunger is released it springs back, then the filter is in good order; if the plunger does not spring back, it is

likely that the filter is either not correctly sealed or it is ruptured. A glass-fibre filter such as a Whatman GF/F is an appropriate choice (except if silicate analysis is to be conducted on the sample material). Pore size of the filter must be kept constant for comparability of results. GF/F filters have a nominal pore size of 0.7 µm whereas Millepore makes a cellulose acetate of pore size 0.45 µm. If the sample contains very fine particulate material containing reactable phosphorus, for example, this may pass through the 0.7 µm GF/F filter and be included in the 'phosphate' result but might not pass the 0.45 µm Millepore filter resulting in different results for the two products.

Special attention must be paid to possible nutrient sample contamination generated by the boat. Wastewater discharged from washbasins, showers and toilets contains significant amounts of phosphorus and nitrogen compounds which can contaminate surface waters to be sampled. For this reason, the water sampler must be deployed far from wastewater outlets, even if no waste is being discharged at the time of sampling. There are also potential problems with kitchen waste.

Mixing by the ship's propeller can disturb the natural distribution of the determinands in the surface layer, particularly 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 a ship. In view of the potential for contamination, the analyst should 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.

4.2.3 Collection, treatment and storage

The general rule for nutrient analysis is that sample storage should be avoided and that analysis should take place immediately after collection. 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 immediate analysis is not practicable, it is essential to adopt appropriate storage measures. Samples should be stored protected from light and refrigerated. The best option, especially in warmer tropical environments, is for the samples to be rapidly cooled as soon as possible. In the field, options may include packing the samples in ice in an insulated container (an insulated picnic/food storage box is very convenient for this purpose). Freezing samples in the field may be difficult unless

there is access to an electricity supply, but it is possible to generate solid carbon dioxide (dry ice) from a compressed gas cylinder using a simple attachment. This process is not very efficient, however, and involves access to gas cylinders, but might be worth considering; especially where access to electricity or a field freezer is limited. If methanol or ethanol are available, these can be mixed with dry ice to produce a freezing bath which can be used to snap freeze your samples in be field. The frozen samples can then be held on dry ice or in an insulated container until they can be analysed or transferred to a freezer.

All samples must be frozen in an upright position. Differential freezing can occur, leading to a spatial concentration of some compounds within the sample container and, if this happens to be on the lid, the material can be easily lost and/or contaminated. The effect of this is negated if, as standard practice, all samples are treated the same way and frozen in an upright position.

All storage of samples for nutrient analysis will involve some alteration in the amount of nutrient eventually measured. This is particularly true for ammonia for which any kind of storage should be avoided if at all possible. Whilst some workers have attempted to calibrate for this by storing a sample with a known concentration of the nutrient involved, there can still be many problems and should not necessarily be relied on. It is always best to conduct the analyses as quickly as possible.

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

If for practical reasons samples cannot be analysed within these time limits, the corresponding data should be identified with the duration of time from sampling to analysis unless the storage method has been validated. The following methods of storage can be recommended (Table 4-5).

Table 4-5. Methods of storage for nutrients analysis.

Silicate	0-4°C protected from light. Do not freeze (polymerisation 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 minimised and optimum disposal procedures should be followed. Since no preservation method for nutrients can, at present, be recommended for general use, each laboratory must validate its storage methods for each nutrient.

In most tropical waters nutrient concentrations are extremely low and frequently close to the detection limits of the standard methods. It is therefore extremely important that the greatest care is taken to minimise contamination of either the samples or the reagents required. This can be improved by the use of spectrophotometer cells of the greatest practicable length. A 10 cm cell will provide a tenfold increase in the measured value of sample absorbance relative to a l cm cell for a constant value of cell-to-cell blank and instrumental noise. The same result cannot be obtained by range or scale expansion facilities which amplify signal and noise equally. For tropical waters, cell lengths smaller than 4 cm should not be used and 10 cm cells are probably best if this can be accommodated within the cell compartments.

The warm temperatures and generally high humidity in the tropics often present problems for analysts. Many laboratories in the tropics still lack adequate air conditioning and the laboratories are kept cooler by either their location in a shaded section of the building or by the use of ceiling fans. Provided the temperature obtained is within the limits for the chemistry, these methods are adequate. However, the movement of warmer humid air through the laboratory by ceiling fans is a vector for airborne contamination. Consequently, it is advisable to work within a small cabinet or under some type of transparent cover so that sample contact with this turbulent air is minimised. Further, given that samples and reagents are undoubtedly less stable in warm climates, every precaution should be undertaken to preserve their integrity. Reagents should be freshly prepared and kept in well-stoppered or firmly-closed containers. They should be kept cool and away from light; a laboratory refrigerator is an obvious choice for storage purposes. Some specific precautions are given in the individual method sections.

The laboratory must be kept free of potential contaminants. These may arise from obvious sources such as ammonia solutions or nitric acid being used by other workers but may also come from less obvious sources. For example, many domestic cleaning

products contain ammonia and, if used by laboratory cleaning staff, these can lead to contamination problems. Similarly, certain marine animals (e.g., prawns) are a rich source of ammonia and should be kept away from the analytical facilities. This includes the refrigerator or freezer where reagents or samples are stored. Cigarette smoke and the fingers of cigarette smokers are rich in nitrogen oxides and any smoker who is taking samples for nitrate or nitrite analysis must wear gloves and must not smoke during sample collection or around sampling equipment.

4.2.3.1 Quality control procedures during sampling

1. Clean all field equipment with deionised freshwater before and after each field trip.
2. Keep the vessel and sampling equipment clean throughout the trip. Take on board a freezer dedicated to sample storage. *Do not allow the freezer to be used for any other purposes.*
3. Smoking is not allowed in the vessel whilst sampling is in process.
4. Use common sense during the sampling process and be aware of potential sources of contamination. These include sweat, sunscreen lotions, washing detergent, clothes, food and fishing equipment.
5. Do not handle the inside of Niskin bottles, nutrient tubes and caps during the sampling and filtering process.

4.2.4 Blanks and standards for nutrient samples

Seawater blanks can be made up from reagent grade (AR) NaCl and Super QTM water (36 g L^{-1}). Before each field trip, fill six 10 mL polypropylene tubes with seawater blanks and a further six with seawater standards. These will be stored for the duration of the voyage either on the vessel or at the laboratory.

One litre of the seawater blank is stored on the boat at room temperature for the duration of the sampling trip. Dispense seawater blanks into appropriately labelled polypropylene tubes after every fourth sample taken throughout the trip. *This will account for any contamination occurring during the sampling and filtering process.*

1. Pre-label the acid-washed 10 mL tubes (Tables 4-1 and 4-2) and place in a clean rack (numbers depend on sampling regime). Water for nutrient samples should be taken first to minimise any risk of contamination. Remove the lids of the nutrient tubes and lay them upright to avoid contamination from the bench. Rinse the tubes twice with the water from the Niskin bottle. Flick the sample tubes dry and place back in the rack.
2. Rinse a 50 mL syringe, (connected to a 0.45 µm filter device), with seawater from the first Niskin bottle. Fill the syringe completely and insert the plunger.

Gently push the plunger down until a steady flow occurs, allowing at least 10 mL of the sample to flow through the filter. Without stopping, place the syringe over the top of the first rinsed nutrient tube and gently push the plunger down until the tube is filled to 80% capacity. Do not draw back on the syringe while the filter is in place as this will displace the filter paper.

3. Repeat until the labelled nutrient tubes have all been filled to 80% capacity. Close the tubes and place them in the rack until all sampling has been completed. Do not overfill the tubes as they may burst when frozen. Replace the filter device after every 8 samples.
4. If duplicate subsamples are being collected, repeat steps 1 and 2 with the same Niskin bottle. If only one subsample is required, carry out steps 1 and 2 with the next Niskin bottle.
5. For each set of nutrient samples per site, dispense three seawater blanks in the labelled containers (Tables 4-1 and 4-2). One blank will be used for total dissolved nutrients, one for dissolved inorganic nutrients and one for silica analyses.
6. Once nutrient subsampling has been completed, place the racks of tubes in their designated storage areas. Total and Dissolved subsamples and corresponding blanks are stored frozen. Silica subsamples and corresponding blanks should be stored at room temperature.

4.3 Trace Metals

4.3.1 Introduction

This section focuses on guidelines on aspects for field sampling program specifically for trace metals in marine waters. For more detailed discussions, refer to Grasshoff, Kremling and Ehrhardt (1999), PSEP (1997) and other documents quoted in this section.

The monitoring of trace metals concentrates on the dissolved form of the metals in the water column, in line with the objectives of the AMWQC. The field sampling program developed here is limited to surface water sampling, mainly in estuarine and coastal waters.

Field sampling represents not only the first but often the most critical step of trace metals in seawater monitoring programs owing to the potential of contamination. The quality of data obtained from seawater studies is related to three principal factors:

1. design and performance of a representative sampling program;

2. adoption of analytical protocols which enable measurements of appropriate accuracy and precision; and
3. selection and use of suitable storage procedures for samples to minimise changes in analyte concentrations and speciation prior to further treatment in the laboratory.

Dissolved trace metals are affected by both the physical processes described previously and by the biological processes of uptake, excretion and biodegradation. Physico-chemical exchange reactions taking place at surfaces of lithogenic and organic particles and at boundaries (air-sea or water-sediment interfaces) will further affect the distribution of dissolved trace metals in seawater. Pressure- and temperature-dependent equilibria readjustments are expected to influence the distribution of dissolved trace metals in seawater.

4.3.2 Sampling strategy

The ultimate goal of the sampling strategy is to guarantee the acquisition of valid data. The sampling strategy is governed by the *objectives* of the monitoring program and by the expected or known *spatial and temporal variability* of the analyte concentrations.

The distribution of trace metals in seawater varies according to locality, depth and time (season) due to changes in physical and biogeochemical processes. Selection of sampling sites and depth is dependent on the objectives of the monitoring program. Consideration should be given to variability of analyte concentrations with sampling locations, e.g., coastal urban and industrial areas, and to prevent memory effects from samplers used when the concentrations of the target analytes vary greatly at different depths from the same station or in a particular area.

For monitoring programs focusing on the baseline for a well-mixed seawater body, such variations should be minimal. Sampling at uniform depth, e.g., 30 cm from surface, and a fixed distance from the coast, may be selected for well-mixed areas.

4.3.3 Sampling techniques

To avoid contamination of seawater with trace metals during sampling, the parts of the sampler in contact with seawater should be made of materials which should not absorb or adsorb trace metals on its surface, e.g., Teflon, PTFE, polypropylene or polyethylene. A common choice would be a Van Dorn sampler (such as a Model Horizontal Beta Plus™) which can be obtained with internal Teflon coating, making it suitable for trace metals and organic constituents.

For estuarine and coastal water sampling, small boats may be moored at fixed positions located with the use of GPS equipment. After stopping the engine, sampling of seawater with the Van Dorn sampler may be taken at the side of the vessel in the upwind direction. If a bigger boat is used, a moored buoy may be necessary. Water is

drawn from the buoy position via polytetrafluoroethylene (PTFE) tubing to a pump on the sampling vessel. This mode of seawater sampling may be combined with in-line filtration.

Standard physico-chemical parameters, such as conductivity (salinity), temperature and dissolved oxygen may be measured *in situ* while taking samples using appropriate field measurement instruments, such as a multiparameter sonde, equipped with the necessary sensors.

4.3.4 Sampling errors

There are two types of sampling errors: *systematic (bias)* errors and *random* errors.

Systematic errors tend to influence the measurement, producing results that are either biased low or biased high. Systematic errors may occur in the sampling procedure, contamination from the ship and sampling equipment used. For example, the use of metallic messengers may be responsible for severe contamination of seawater meant for trace metals analysis. Effective control and quantification of systematic error is difficult and remains a challenging problem. Often, systematic error in sampling may be detected by means of an obvious departure from known historical or concentration trends of the target metals.

Random errors are variation in the results of repeated analyses under identical conditions. These errors are caused by the type of equipment used, personnel errors and inherent errors in the method. Random errors may be estimated through repeated independent analyses performed by different analysts. Random errors are always present and exist independently from systematic errors.

4.3.5 Quality control

For the purpose of this manual, the quality control measures include *precision*, *accuracy* and *limit of detection*.

Precision or reproducibility of a method involves all steps from sampling of the water to the final data. Precision is often expressed as relative standard deviation (RSD) or coefficient of variation (CV). Repeated analyses of the same sample establishes the 'within laboratory precision'. Systematic errors due to sampling and subsampling may distort the normal (Gaussian) error distribution. Generally RSDs tend to increase with decreasing concentration levels. *Intra*-laboratory reproducibility refers to RSD measurements within a measuring laboratory. *Inter*-laboratory reproducibility refers to RSD measurements from different measuring laboratories obtained through inter-comparison exercises.

Accuracy is a measurement of the correctness or exactness of data (determined value) as compared to a known (true) value. Deviation from accuracy (inaccuracy) may arise due to random and systematic errors in the measurement process. Evaluation

of the accuracy of the measuring laboratory may be achieved through the analysis of certified reference materials or the recovery of samples spiked with target analytes.

The *Limit of Detection* (LOD) for a given analyte is defined as the lowest concentration level that is statistically different from a blank at a specified level of confidence. In practice, the LOD for a given analyte is often limited by the level and variability in the blank value rather than by the sensitivity of the analytical technique. The analytical result (X_A), is considered as 'real' and different from the blank if it is at least as great as the mean blank value (X_{bl}) plus 3 standard deviations of the blank value (s_{bl}):

$$X_A \geq (X_{bl} + 3s_{bl}) \text{ or}$$

$$X_A - X_{bl} \geq 3s_{bl}$$

Measurements below $3s_{bl}$ (=LOD) should be reported as 'not detected' (ND) with the LOD given in parentheses. As a rule of thumb, the blank level should always be kept at approximately ≤10% of the measured quantity.

In trace metal analysis, the LOD can be estimated from the standard deviations (s) of several measurements of a low concentration sample. This may be performed during a field trip by repeatedly taking duplicate samples of the same type at different stations. The standard deviation (s) can then be estimated by:

$$\sqrt{\frac{\Sigma d^2}{2n}}$$

where d is the difference between pairs of results, n is the number of pairs. A reasonable estimate of the LOD is given by a $3s$ value.

4.3.6 Filtration techniques

Water samples for dissolved metals are filtered though 0.4 to 0.45 µm membrane filters prior to preservation. Filtering must occur as soon as possible after sampling and always within 24 hours. For this reason, field filtering is preferred but may not always be practical. When it is not feasible to filter samples for dissolved metals within 24 hours of collection, sample results may be qualified to reflect this. The filtrate, which contains the dissolved fraction, is preserved to pH <2 with ultrapure nitric acid. Samples collected for trace metals are particularly prone to contamination during filtering and great care must be taken to minimise it.

An alternative 'storage' procedure involves the on-board separation of trace metals from seawater by ion exchange resins (Chelex 100) with the subsequent elution and analysis in onshore laboratories. This procedure offers the advantage of reduced sample storage but requires the provision of a clean bench to minimise contamination risks.

It is usually necessary to filter marine water samples prior to addition of any preservation agent (e.g., HNO_3 to adjust pH to 2.0) to the samples. The main purpose of filtration is to separate the suspended particulate material (SPM) prior to analysis. For open seawater, including coastal waters under calm conditions, filtration may not be needed since if the SPM level is relatively low, unless iron (Fe) is to be determined.

4.3.6.1 Without on-site filtration:

Filtration on-site may be difficult, and the risk of contamination high, especially if the target trace metals are present at ultratrace levels (<1 µg/L). The concentrations of trace metals measured in marine water samples preserved with acid (for adjustment to pH 2.0) may be deemed to be equivalent to the dissolved trace metal concentrations.

Filtration is necessary for estuarine water due to the elevated levels of SPM in these waters. If it is not possible to carry out on-site filtration, this should be done in the nearest laboratory on land, under clean environmental conditions, to avoid any potential contaminations.

4.3.6.2 With on-site filtration:

The generally accepted operational definition for 'dissolved' refers to the fraction of seawater and its trace constituents that has passed through a 0.45 µm (or 0.4 µm) filter.

Filtration offers the advantage that the labile fraction of particulates does not release trace metals into the dissolved phase and also avoids interference from particles during spectrophotometric analysis, e.g., for Cr(VI) determination. The only drawback is possible adsorption losses of trace metals onto reactive particles such as bacterial walls during filtration.

The dissolved concentration of trace metals can be influenced by the filter type, filter diameter, method of filtration, concentration of SPM, size of particles and the volume of sample processed. The final selection of filter is often a compromise between the numerous requirements of the analysis. For most trace metal analyses (except for Hg), polycarbonate filters (e.g., Nucleopore type) are commonly used. For Hg, glass-fibre filters may be used instead.

The filters must be cleaned thoroughly prior to use. This is often done by leaching the filters with a mild acid solution and due care must be made to pre-qualify the filters to ensure the target analytes are absent. This may be performed by passing ultrapure water (resistivity of 18 $M\Omega cm^{-1}$ or equivalent) and analyzing the filtrate for the target analyte. Commercial acid-leached filters are available from Environmental Express, SKC Inc. and other scientific equipment suppliers.

A range of filtration techniques is available, including vacuum, pressure, or *in situ* filtration.

Vacuum filtration is performed by suction under aspirator vacuum and the filter support is constructed of either sintered glass or ceramic material. The drawbacks of vacuum filtration include potential contamination risks from the multitude of transfer steps involved whereby the water samples may be in contact with different materials and the laboratory air. Commercial all-plastic filtration apparatus are available from suppliers such as Sartorius and are recommended for trace metal analyses.

Pressure filtration involves the pressurisation of the water sample to force it through an in-line filter into the receiving bottle. This technique reduces the number of transfer steps, thus minimizing the risk of contamination. Purified compressed air is generally used but ultrapure nitrogen is required if the redox state of the water sample needs to be maintained. Various filter holders are commercially available e.g., Sartorius, Millepore.

In situ filtration avoids the settling bias that can alter the partitioning between dissolved and particulate size fractions in seawater. *In situ* filtration uses pump systems that may be deployed from moorings and offers the additional advantage of *time-integrated* water samples (see Section 4.1.4). Pumping rates of between 1 and 200 L h^{-1} can be selected.

4.3.7 Storage, preservation and treatment

Two important considerations in storage of seawater samples are biological activity in seawater and interaction of trace metals with the material of the storage vessel. Biological activity in seawater continues after sampling due to physiological activities (digestion and excretion) of micro- and nano-plankton. The walls of the storage vessels may often enhance bacterial growth by several orders of magnitude. In addition, the material of the storage vessels may adsorb dissolved trace metals.

For trace metals in seawater, the widely accepted procedure is to acidify the seawater samples to pH 1.5 to 2.0 with ultrapure acid (approximately 1 mL L^{-1} of water sample) and to store the acidified water samples in acid-leached containers constructed of either Teflon, high-density polyethylene or quartz. The pH of the samples should be confirmed to be <2 at time of preservation by pouring off a small amount of sample and checking it with short-range pH paper. The pH should be checked again at the time an aliquot is removed for analysis. Excess acid should be avoided, however, as preconcentration techniques are strongly dependent upon pH. This pre-treatment allows the water samples to be kept for at least 6 months and up to 2 years. Seawater samples meant for Hg determination should be stored in Pyrex or quartz bottles, acidified to 2% HNO_3 and kept under cool (4°C) and dark conditions.

If speciation of trace metals is required, measurements should be made as soon as possible after sampling. If immediate analysis is not possible, the samples should be stored unacidified but frozen to preserve as much as possible of the original distribution of species. For Cr(VI) samples, the water samples should be analysed as soon as

possible after sampling. coastal seawater for Cr(VI) determination is stable for up to one month if stored at room temperature under natural pH and for up to 8 months if stored frozen (Sirinawin and Westerlund 1997). For tributyltin (TBT), filtration (0.45 µm), acidification to pH <2 followed by storage at 4°C in amber borosilicate glass bottles is acceptable (Tong et al. 1996). Analysis for TBT (as Sn) is performed within 30 days of sampling.

Samples are brought to the laboratory for preservation should be kept cool (4°C) during transportation and be preserved within 24 hours of sampling. When this is not practical, samples should be preserved as soon as possible and preserved samples must sit at least 16 hours prior to analysis to allow metals that may have plated onto the walls of the sample container to resolubilise.

4.3.8 Typical sampling procedures

1. Pre-sampling preparation:

1.1 Follow planning of sampling program.

1.2 Prepare sampling log sheets and chain-of-custody forms.

1.3 Prepare sampling equipment, containers, storage box.

2. Identification of sampling location:

2.1 Identify specific sampling location by descriptions of site and GPS positioning.

2.2 Ensure that Sampling locations are at least 100 m from the low tide line of beach.

3. *In situ* Measurements:

3.1 Parameters: temperature, pH, turbidity, conductivity, salinity and dissolved oxygen.

3.2 Equipment: YSI 6000 water quality logger or equivalent; equipment should be pre-calibrated in laboratory prior to the field sampling trip. Standard calibration solutions should be available for checking of equipment performance at regular intervals.

3.3 Measurement: taken at bow of sampling vessel anchored with bow facing into the current.

3.4 Depth of water quality sonde logger: 30 cm.

3.5 Frequency of sampling: 1 minute intervals for 10 minutes

4. Water Sampling:

4.1 Sampler: van Dorn with internal Teflon coating and no metal contact parts

4.2 Model: Horizontal Beta Plus™ Bottles (Wildlife Supply Company, USA) or equivalent

4.3 Quality control: field blanks and field duplicates

4.4 Sampling container and preservation (trace metals): see Table 4.5.

4.5 On-site and transportation sample storage container: all samples to be kept in cold storage container box maintained at 4°C and sent to testing laboratory within 24 hours.

5. Sample transportation and chain-of-custody documents:

5.1 Prior arrangement should be made to ensure the storage box for the sample collected can be transported to the testing laboratory on time. Transportation by public transport, such as bus, train and commercial flights may be feasible.

5.2 A copy of the chain-of-custody documents should accompany each shipment of the sample storage containers. The monitoring program manager should keep records of the chain-of-custody documents and the information should be included in the reports of the monitoring results.

Table 4-6. Preservation methods for trace metals collected in the field.

Parameter	Bottle Type	Bottle Size	Preservation
As, Cd, Cu, Pb, TBT, Zn	High density polyethylene	1 L	HNO_3 to pH 2, 4°C
Hg (total)	Glass, amber	250 mL	HNO_3 to pH 2, 4°C
Cr(VI)	High density polyethylene	250 mL	4°C

4.4 Chlorophyll a and Phaeophytin

4.4.1 Introduction

Plant pigment concentrations in natural waters provide a semi-quantitative index of phytoplankton biomass. From a practical perspective, the pigment most useful for estimating total phytoplankton biomass is chlorophyll *a*. Concurrent concentrations of chlorophyll *b* and *c* are usually much smaller and vary in response to community floristic composition.

All materials chlorophyll containing are fluorescent. When the organisms are microscopic, such as phytoplankton, this fluorescence may be measured directly in bulk water solutions or extracts of filtered materials.

The concentration of photosynthetic pigments is used extensively to estimate phytoplankton biomass. All green plants contain chlorophyll *a,* which constitutes approximately 1 to 2 % of the dry weight of planktonic algae. Other pigments that occur in phytoplankton include chlorophylls *b* and *c*, xanthophylls, phycobilins and carotenes. The important chlorophyll degradation products found in the aquatic environment are the chlorophyllides, pheophorbides and pheophytins. The presence or absence of the various photosynthetic pigments is used, among other features, to separate the major algal groups.

Direct estimations of chlorophyll *a* concentration from fluorescence can be misleading due to interferences caused by the fluorescence of chlorophyll decomposition products (e.g., phaeophytin). In some circumstances, chlorophyll degradation products can form a significant fraction of the total plant pigment in a seawater sample (Parsons et al. 1984). The concentration of chlorophyll degradation products can be determined by acidification of the original sample.

4.4.2 Sampling

Water column samples can be collected using opaque containers and should be protected from excess heat and light. Filtration should be performed as soon as possible after sampling and preferably within one hour of sampling. Zooplankton, where present, may continue to consume chlorophyll.

Tests should be performed on new containers to verify that the material and construction of the container does not give rise to unacceptable changes in the sample over the stated storage time and under actual storage conditions.

Where pumped systems are employed, cell rupture and subsequent loss of pigment should be a consideration. Where there may be doubt as to the suitability of a pumped supply, comparative studies should be carried out to verify that sample integrity is not being compromised.

The method of sample collection must be recorded.

In estuaries, where plankton populations can vary with depth, collect the samples from all major depth zones or water masses. The sampling depth will be determined by the water depth at the station, the depth of the thermocline or isohaline, or other factors. In shallow areas of 2-3 m depth, subsurface samples collected at 0.5 to 1 m may be adequate. In deeper areas, samples should be collected at regular depth intervals. In estuaries, samples should be taken above and below the pycnocline. Depth intervals for sampling vary for estuaries of different sizes and depths, but depths representative of the vertical range should be sampled. Composite sampling above and below the pycnocline is often used. In marine sampling, the intent and scope of study will determine the type and number of samples collected

Over the continental shelf, samples should be taken at stations approximately equal distance from the shore seaward. Take a vertical series from surface to near bottom at each station, gradually adding more station across the shelf. It is important to sample the entire vertical range over a continental shelf. Sampling depths vary, but often are at 10 to 25 m intervals above the thermocline, then at 100- to 200 m intervals below the thermocline to 1000 m, and thereafter at 500- to 1000-m intervals.

Sampling frequency depends on the objective of the study as well as the range of seasonal fluctuations, the immediate meteorological condition, adequacy of equipment and availability of personnel.

The Van Dorn sampler is usually the preferred sampler for standing crop, primary productivity and other quantitative determinations because its design offers no inhibition to free flow of water through the cylinder. In deep-water situations the Niskin bottle is preferred. It has the same design as the Van Dorn sampler except that the Niskin sampler can be cast with a series on single auxiliary messengers.

4.4.3 Sample pre-treatment

Glass-fibre or membrane filter papers are mostly used and the final choice of filter media will depend on the subsequent analytical methods to be used. The use of pressure or vacuum should not be excessive as to avoid cell rupture.

The pore size of the filter media should be small enough to capture picoplankton and GF/F (0.7 µm pore size) is recommended.

The actual size and type of the filter media should be chosen prior to testing of analytical methods since the amount of water retention can significantly reduce the attack strength of the solvent used during the analytical stage. Once the amount of water retained is known for the media and filtration method used, the concentration of solvent added can be adjusted to compensate and to ensure that the attack strength of the solvent is optimal.

Removal of zooplankton is desirable because they can contain chlorophyll. They can be removed by pre filtering through a 100-150 µm mesh or can be picked off filters using tweezers.

Care should be exercised where large colonial phytoplankton are present as these may also fall victim to pre-filtering.

4.4.4 Filtering

1. Rinse the 100 mL measuring cylinders twice with water from the appropriate Niskin or other sampling bottle. Fill each cylinder from the sampling bottle and expel the excess water using a Teflon displacing cap.
2. If required, add 0.1–0.2 mL $MgCO_3$ (10 g L^{-1}) to the sample prior to filtration.

This buffers the sample against low pH, which can cause degradation of chlorophyll into phaeophytin during storage.

3. Pour the collected sample into a filter funnel and filter through the selected filter paper. Filter the sample under low vacuum pressure (<1/3 atm). Fold the filter paper in half using forceps to avoid loss of sample.
4. Place the folded filter paper carefully onto a piece of aluminium foil. Wrap the foil around the filter paper, avoiding touching the filter paper. Label and freeze the wrapped filter papers (Aminot and Rey 2000; Jeffrey et al. 1997).

4.4.5 Sample storage

Concentrate the sample by centrifuging or filtering as soon as possible after collection. If processing must be delayed, hold samples on ice or at 4°C and protect from exposure to light. Use an opaque bottle because even brief exposure to light during storage will alter chlorophyll values.

Storage conditions will have the greatest impact on the end result. Storage up to one month is possible when considering only chlorophyll *a* since early degradation products have spectral properties close to those of chlorophyll *a*. For prolonged storage, deep freezing to –18°C or below is both practical and the favoured option where the objective is to estimate total biomass. Where pigment information is required, more rigorous storage conditions may be needed.

Record the method of sample storage.

4.4.6 Quality control

To quantify uncertainty resulting from the sampling and sample handling procedures, duplicate samples and field blanks should be incorporated into sampling procedures and then submit this as supporting QA Data (http://www.quasimeme.org/index.htm).

4.5 Suspended Solids

4.5.1 Introduction

Suspended solids are a measure of the total amount of particulate matter in a water sample. An increase in the amount of suspended sediment, phytoplankton cells or other solids within the water column can lead to a reduction of light penetration into ocean waters. Such a reduction in ambient light can be detrimental to biota whose survival is dependent on sunlight. Sediment loading can be increased as a result of natural and human disturbances, including river input, storms, strong winds, trawling and dredging (Hatcher 1989). Extraction of the suspended material from a water sample is a necessary step in this procedure to permit easy calculation of total

suspended solids. One of the most widely used and popular concentration methods is filtering of the sample onto a pre-weighed filter paper (Gibbs 1974).

4.5.2 Sample collection

1. Label the 1 L plastic bottles, rinse twice with water from the appropriate Niskin bottle, then fill to the 1 L mark.
2. Place the pre-weighed polycarbonate membrane filters on the filtering apparatus. This consists of a 47 mm diameter filter funnel and base connected by a clamp.
3. Filter the sample under low vacuum pressure (<1/3 atm) until dry.
4. Rinse thoroughly with approximately 10 mL of deionised water to remove particulate matter adhering to the funnel and to wash salts out of the filter paper.
5. Once dry, turn the vacuum off, remove the funnel and fold each filter paper in half using fine forceps. Place each filter paper back into its labelled scintillation vial. Store the vials in a box at room temperature.

4.6 Bacteria

4.6.1 Introduction

Faecal coliforms should be used as the indicator organism for evaluating the microbiological suitability of recreation waters. A recommended technique is the multiple-tube fermentation or membrane filter procedures, a minimum of five samples taken over not more than a 30-day period. the faecal coliform content of primary contact recreation waters must not exceed a log mean of 100 mL, with not more than 10 percent of total samples during any 30-day period exceeding 400:100 mL.

4.6.2 Method

4.6.2.1 Sample collection – planning and preparation

- Determine the extent of the sampling effort and the equipment and supplies needed.
- Obtain necessary sampling equipment and supplies, including pre-sterilized sample containers.
- Prepare scheduling, and coordinate with microbiology laboratory to assure someone will be available to process microbiological samples within the 6-hour sample holding time.

- Verify that sampling will occur at a time that will allow the sample to arrive at the microbiology laboratory within the sample holding time.
- Using local tide tables, estimate the approximate time and heights for high and low tides spanning planned sample time if a beach is to be sampled. If possible collect sample at high tide, but only if this does not risk exceeding the sample hold time.
- Note the location of the sampling stations to provide this information on the Beach Sampling Data Sheets. The sampling stations for a given beach monitoring event will generally be located in the middle of the stretch of beach most commonly used for water contact recreational activities.

4.6.2.2 Sampling surface waters

Attach a clean sterilised sample bottle (Figure 4.1) to the clean sampling rod. Immediately before submerging the sample bottle, remove the ground-glass stopper from the bottle without touching the stopper cone. Immerse the bottle from the bow of the boat or from the windward side while the boat is moving forward slowly. Push the bottle with the sampling rod 25 cm under the water surface with the mouth of the bottle downwards, in order to avoid contamination by surface film, then turn the sample bottle upwards and take the sample (Figure 4-1). The sterilized sample bottle may also be filled directly by hand.

Retrieve the bottle and discard some water, if necessary, so that some air space remains in the closed bottle. This space is need for homogenizing the water sample at the receiving laboratory. Replace the glass stopper and store the samples in the clean thermoisolated box with cooling pads at about 4°C. Keep samples in the dark avoiding exposure to more than +10°C. Separate bottles from each other with wrapping paper to avoid breakage. Check the temperature with a thermometer every three hours. Report irregularities in the test report. Label sample bottles indicating sampling time, sampling station and other factors relevant to the interpretation of the results.

Note: It is known that the die-away rate of coliforms at ambient temperature in the presence of light is very high. Therefore, all efforts should be made to not collect more samples than can be filtered and incubated the same day. If this is not possible the samples should be stored at 4°C and analysed not later than 24 hours after sampling.

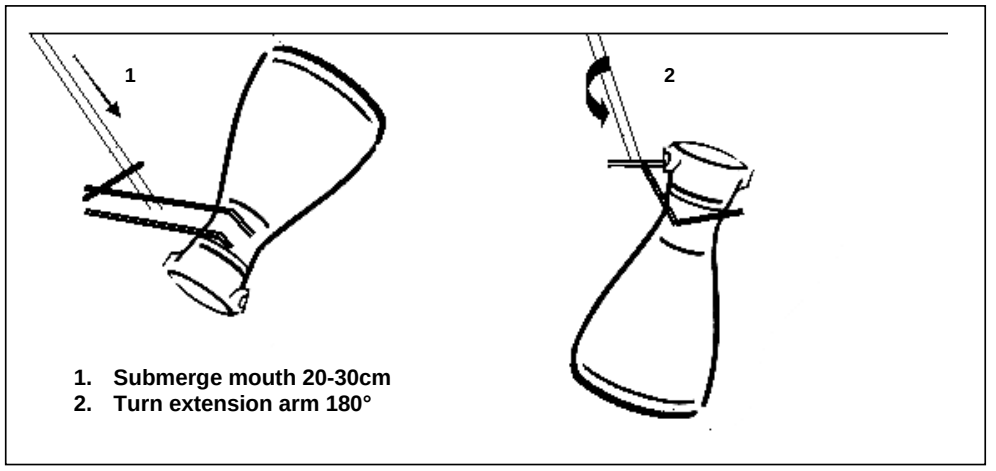


Figure 4-1. Sub-surface sampling with extension arm.

4.6.2.3 Sub-surface sampling

Lower the sterilised subsurface sampler (Figure 4-2) after attaching it to a clean plastic rope, without letting the weight disturb the bottom sediments. Release the messenger and after one minute retrieve the sampler and store it in a thermoisolated box. Proceed as for sampling of surface water.

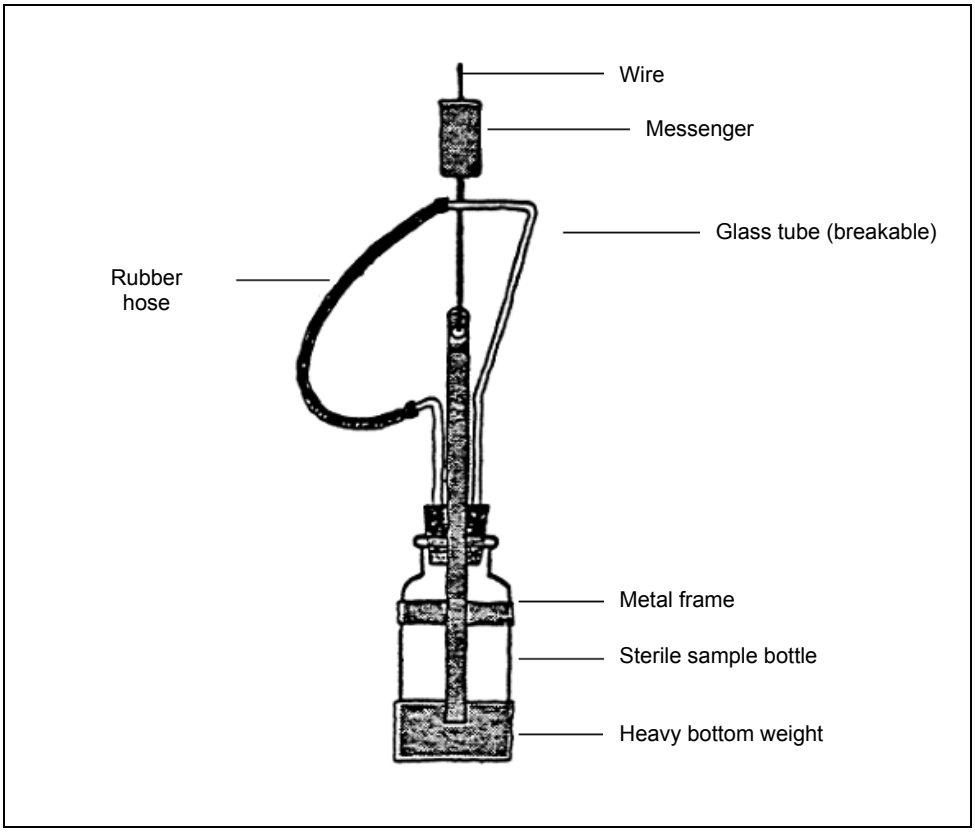


Figure 4-2. Sampler for sterile sub-surface sampling (from UNEP 1983).

4.7 Physical and chemical parameters

4.7.1 Salinity

4.7.1.1 Introduction

Salinity is formally defined as the total amount of dissolved inorganic salts in seawater. The technical term for saltiness in the ocean is *salinity*, from the fact that halides-chloride specifically, are the most abundant anions in the mix of dissolved elements. This was traditionally expressed as parts per thousand (‰) by weight, when all the carbonate has been converted to oxide, the bromide and iodide to chloride, and all organic matter is completely oxidised. In oceanography it has been traditional to express salinity not as percent, but as parts per thousand (ppt or ‰), which is approximately grams of salt per liter of solution. Other disciplines use chemical analyses of solutions, and

thus salinity is frequently reported in mg L^{-1} or ppm (parts per million). Prior to 1978, salinity or halinity was expressed as ‰ usually based on the electrical conductivity ratio of the sample to 'Copenhagen water', an artificial seawater manufactured to serve as a world standard. In 1978, oceanographers redefined salinity in the Practical Salinity Scale (PSS) as the conductivity ratio of a seawater sample to a standard KCl solution. Ratios have no units, so it is not the case that a salinity of 35 exactly equals 35 grams of salt per litre of solution. Salinity is now expressed in practical salinity units (psu).

Marine waters are those of the ocean, another term for which is euhaline seas. The salinity of euhaline seas is 30 to 35. Brackish seas or waters have salinity in the range of 0.5 to 29 and metahaline seas from 36 to 40. These waters are all regarded as thalassic because their salinity is derived from the ocean and defined as homoiohaline if salinity does not vary much over time (essentially invariant). Salinity is an ecological factor of considerable importance, influencing the types of organisms that live in a body of water.

Salinity, in conjunction with temperature, largely determines the density of seawater and as a conservative property can be used to identify specific water masses.

The salinity in a marine ecosystem may be affected by a number of factors. An increase in freshwater runoff due to high rainfall, coastal land clearing and urban development may cause a reduction in salinity, whereas evaporative concentration near shallow reefs may lead to an increase in salinity levels (Hatcher 1989). One obvious way of measuring salinity is to take a known mass of seawater, evaporate it to dryness and then weigh the remaining salt. In practice, this method tends to be highly variable and unpredictable. As a result, salinity is rarely determined directly but is routinely computed from chlorinity, electrical conductivity, refractive index or some other property where a functional relationship to salinity is well established. The conductivity of seawater is proportional to the salinity. With the appropriate corrections for temperature and pressure, the measurement of conductivity has become the most generally used method of determining salinity. Electrical conductivity is a measure of total electrolyte concentration in seawater and it is a technique which can be performed rapidly and with great accuracy, both in laboratories and *in situ.*

4.7.1.2 Sample collection

1. Label the 700 mL plastic bottles and rinse the bottle and lid twice with water from the appropriate Niskin bottle.
2. Fill the bottle to the top to avoid air bubbles from forming.
3. Place Parafilm over the opening of the bottle and screw lid on tightly.
4. Store the bottles in a dry environment at room temperature until returned to the Laboratory.

4.7.2 Clarity, turbidity and light in marine waters

Attenuation of light in the sea in nonalgal bloom areas is determined principally by the amount of suspended matter present, but in estuaries and nearshore coastal waters, colour from humic-like materials may significantly compete with particulate material in light attenuation. In moderately turbid coastal waters, 1% of the surface visible light energy may penetrate to a depth of 10 to 20 m, but in shallow estuaries penetration is often as little as 10 cm. In temperate estuaries there is typically a strong seasonal variability in water clarity between the active growing season and the winter, and in subtemperate to tropical estuaries water clarity is usually a function of the wet season.

4.7.2.1 Turbidity

Human-induced turbidity can result from a range of land management practices that increase sediment loads within streams and hence to the sea. These include clearing of vegetation (particularly of riparian zones), excessive irrigation and drainage which can lead to riverbed and bank erosion, and increased soil erosion associated with rainfall run-off.

Turbidity is the measure of the light-scattering properties of water and depends on the amount, size and composition of the suspended matter such as clay, silt, colloidal particles, plankton and other microscopic organisms. It is often measured in nephelometric turbidity units (NTU).

Turbidity is a primary water quality indicator. High turbidity and suspended solids together with corresponding low transparency are important limiting characteristics when assessing water quality. High turbidity resulting in low levels of transmitted light limits aquatic primary production. Turbidity is easy to measure and is sometimes used as a surrogate for suspended solids, but this is not straightforward. The same instrument must be used for all measurements (not just the same technique—nephelometry or transmissometry). The turbidimeter must be calibrated with a turbidity standard and suspended matter from the waters to be monitored and the particle size and composition should not change over the monitoring period. Turbidity is likely to be measured in many marine waters but decisions would be needed on where to measure suspended solids.

The most appropriate measure to use will depend upon the aquatic environment is being studied. For wetland, estuarine and marine systems transparency, measured by the Secchi disc method, may be the most appropriate measure, although this method has obvious limitations in shallow waters.

For methods of determining turbidity and suspended solids refer to APHA (1998).

4.7.2.2 Secchi depth

The Secchi disc, a simple and inexpensive tool, has been a mainstay in estimating water clarity (Holmes 1970). Secchi depth measurements are obtained with a 40 cm plastic or metal Secchi disc that is either white or is divided into black and white quadrants on a nonstretchable line that is calibrated in decimeters. The disc should be weighted to maintain a level position, especially under strong current conditions. The disc is lowered into the water until it disappears from view and the depth is recorded. The disc is then slowly raised to the point where it reappears and the depth is recorded again. The mean of these two measurements is the Secchi depth. Observations are made from the shady side of the vessel to reduce problems of glare; however, when a small boat is used for field work a "viewing tube" allows readings under full sunlight conditions. Measurement should be made without sunglasses.

While Secchi depth measurements often provide a longer historical record than electronic measurements, the Secchi disc does not provide all of the information required to distinguish the light attenuation effects of living phytoplankton pigments (i.e., traditionally estimated by chlorophyll *a*) from other factors (e.g., inorganic suspended sediments, organic non-chlorophyll-based detritus, and humic-like materials) that reduce water clarity. In turbid coastal waters, the analyst should be aware of lower values for the constant 1.7 to estimate the light attenuation coefficient (Giesen et al. 1990). More precise estimates of the light attenuation coefficient can be made with electronic submersible light meters including PAR meters (photosynthetic active radiation) and submersible spectral radiometers. These meters are now in widespread use, and their use should be encouraged because they give a direct measure of light attenuation, especially in shallow water where depth may limit use of the Secchi disc.

4.7.2.3 Light Attenuation

For aquatic plants, the sub-surface light climate has a major influence on growth (Boynton et al. 1982; Bricker et al. 1999; Gallegos 2001) particularly in inshore and near-shore environments where high levels of suspended particulate material may severely restrict the availability of light (Painting et al. 2007).

To calculate light attenuation, measurements of down-welling PAR are recorded using a suitable instrument, typically LI-COR (LI-192) underwater quantum sensors. The instrument should be protected in a stainless steel protective frame and interfaced with a solid state logger sampling at preset intervals. Care should be taken to minimise the influence of shading on measurements with the irradiance sensors by profiling on the illuminated rather than the shaded side of the sampling platform.

The attenuation coefficient, K (m^{-1}) of photosynthetically available radiation (PAR) is estimated using the Lambert-Beer Equation (Dennison et al. 1993) from vertical

profiles of downwelling irradiance. K_d is calculated from the slope of irradiance and depth:

$$K = \ln (L_z/L_o) - z$$

where K represents the light attenuation coefficient, L_z = light at depth, L_o = light at surface and z = depth.

4.7.3 Temperature

Temperature is measured with standard multiprobes (normally measuring conductivity, salinity, temperature, dissolved oxygen, pH and often depth and turbidity) which may be able to collect 'continuous' data and record through a logger. Individual temperature loggers for autonomous deployment are also useful for recording long-term temperature at fixed sites.

4.7.4 Dissolved Oxygen

Dissolved Oxygen (DO) is traditionally been determined by titration utilising the oxidative power of DO (Winkler method). However DO is now determined in the field using a DO electrode. The electrode will frequently be incorporated into a multiprobe unit. The electrodes are relatively robust but do need frequent checking for physical damage. Regular calibration checks are also recommended.

4.7.5 pH

pH is measured using a dedicated electrode also often incorporated into a multiprobe unit. pH electrodes are the least robust of the electrodes/sensors in the multiprobe set and need to be checked for physical damage and also calibrated for the expected/desired range frequently.

5 Laboratory Analysis Methodologies

5.1 Introduction

5.1.1 Methods strategy

The recommended steps in the development of the laboratory analysis methodologies are shown in Figure 5-1.

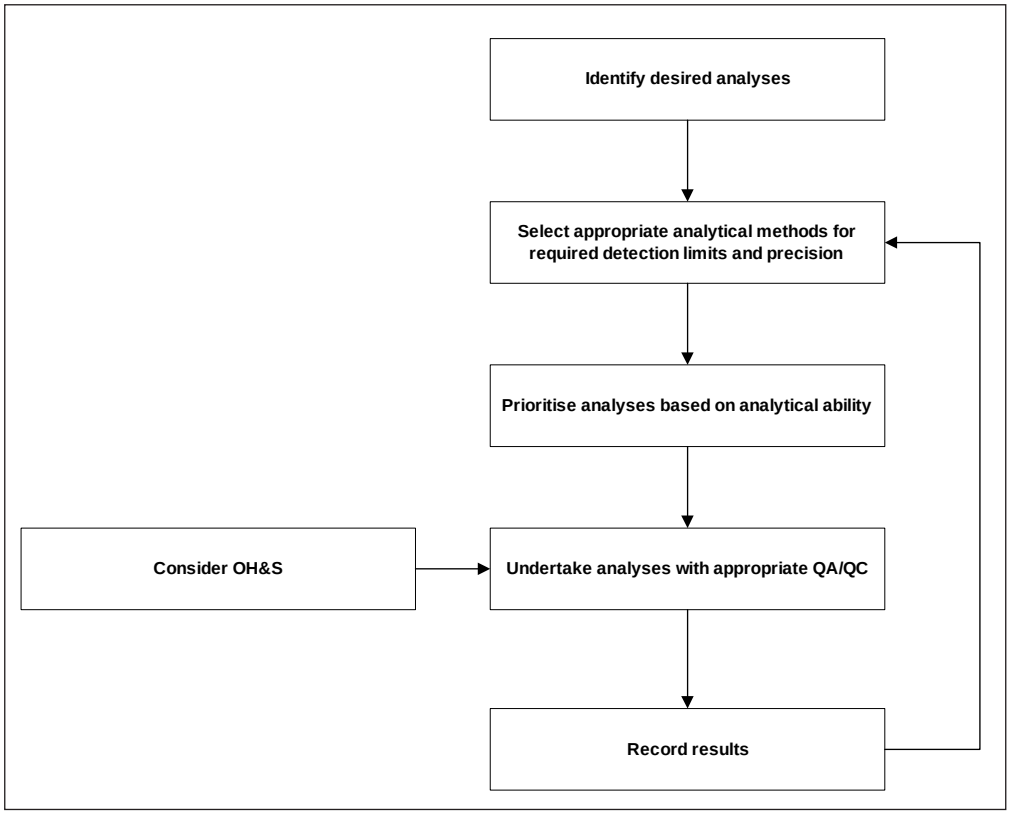


Figure 5-1. A framework for designing an analysis program.

The following sections describe the analytical methods involved with the detection of nutrients, chlorophyll, suspended solids, heavy metals, trace metals, phytoplankton, oil and grease, phenols and cyanide.

5.1.2 Method-defined parameters

A distinction needs to be made within the AMWQC, between those parameters in which the analyte is a well-defined chemical substance and those parameters where the analytical method defines the parameter. An example of the first type is hexavalent chromium (CrVI), where to a large extent any method which measures specifically this parameter should give the same result as any other i.e., the real value is largely method-independent. On the other hand, a parameter such as Oil and Grease (OG) is completely defined by the method used to measure it. OG represents a basically unknown mixture of different substances in any one sample which are extractable into the solvent used for the solvent extraction and then measurable by the detection technique used, e.g., gravimetry or infrared (IR) absorption. For these method-defined parameters (as also known as operationally defined parameters) it is

critical that only a single standard method is used. Changes to the method will mean that a different parameter is being measured and data cannot be compared to results from the original method unless extensive comparisons have been made and it can be shown conclusively that the methods give equivalent results. A good discussion of the issues associated with method-defined parameters can be found in Simonet et al. (2006). An example of how modification to an existing method-defined parameter, in this case OG, can be made is given in Farmaki et al. (2007) where a change in the solvent used for extraction for infra-red detection is discussed.

- Parameters in the AMWQC where a methodological step is important include:
- Total suspended solids (TSS) – the nominal pore size of the filter paper is the method-defined component in this case. Papers of pore size 0.4 µm are recommended. If papers of a different pore size e.g., 0.2 µm are used a different result, not comparable to results from the original method, will be obtained.
- Bacteria – results are completely dependent on the exact method used.
- Oil and Grease – the method is dependent on the extraction technique and solvent used and on the detection method. Different solvents, e.g., hexane *versus* Freon 113 *versus* tetrachloroethylene, will extract different amounts of the components of OG and the detection method i.e., gravimetry or IR absorption will measure different amounts of OG. Sometimes this method is further method-defined and may be called 'n-hexane extractable material' but this is now a different parameter than 'Oil and Grease'.
- Phenol – 'Phenol' can refer to either the chemical phenol by itself or a 'total phenols' in which other phenolic-based substances are included. The AMWQC refer to 'phenol' as the chemical phenol alone as so this becomes a well-defined substance and not method-defined. However if 'total phenols' or 'phenol compounds' are referred to this means the sum of all phenolic-based chemicals and the method for this will be method-defined, e.g., the 4-aminoantipyrine colorimetric method discussed in McPherson et al. 1999

Analyses for trace metals may also have method-defined parameters, because samples can be analysed for either 'total metals' which includes both particle-bound and dissolved metals or 'dissolved-form metals' which excludes particle-bound metals, usually by a filtration step. As the AMWQC refer to trace metals in the dissolved form this parameter needs to be measured. This is discussed further in Sections 5.3.2.2 and 5.3.2.3. It is important to note that the AMWQC specifies particular valence states for some metals e.g. hexavalent chromium (CrVI). Chromium is likely to exist in marine waters in a number of valence states including particularly trivalent and hexavalent so that a measure of 'total chromium' will not satisfy the need to measure CrVI (see sections 5.3.4.1, 5.3.4.2, 5.3.4.5 for methods to measure trivalent and hexavalent chromium separately).

Analyses for nutrients are often also method-defined to some extent. This is an important consideration for the parameter 'phosphate'. This parameter, because it has a number of chemical and physical forms included, is known by a large number of method-defined terms including 'dissolved reactive phosphorus', 'dissolved inorganic phosphorus', filterable reactive phosphorus' as well as 'orthophosphate'. As the intent is to separately measure a 'dissolved' fraction by separating out particulate forms by filtration (Section 4.3.2) the pore size of the filter used becomes a critical method-defined step. Thus, measurements which have used a Millepore 0.45 µm filter (cellulose acetate) may give different results from those obtained when using a GF/F (glass-fibre) filter of nominal pore size 0.7 µm.

Nutrient methods for 'dissolved or filterable organic nutrients' and particulate nutrients are also partially method-defined as method elements such as filtration pore size (see above), digestion technique and calculation by difference versus direct analysis all play a part in the final result.

5.2 Nutrients

5.2.1 Introduction

One scheme used for nutrient analysis is outlined in this section. Other schemes and a comparison of their capabilities can be found in:

- http://www.epa.qld.gov.au/publications/p00330aa.pdf/Water_quality_sampling_manual_for_use_in_testing_for_compliance_with_the_Environmental_Protection_Act_1994.pdf
- http://www.sepa.org.uk/pdf/marine/green_book/appendices.pdf

Phosphorus occurs in water samples as free or esterified phosphates-orthophosphates, polyphosphates and organically bound phosphates. The major forms of nitrogen present in seawater are nitrate, nitrite, ammonia, organic nitrogen and particulate nitrogen (Franson et al. 1980). The inorganic forms of phosphorus and nitrogen can be readily analysed using a variety of methods, but organically bound forms must also be measured to determine the total amount of dissolved phosphorus or nitrogen in a sample. This is done by converting organically bound forms to a more readily analysed form.

5.2.1.1 Nitrogen

Several methods have been used to determine the concentration of nitrogen (N) species in the marine environment. For eutrophication evaluation, the most common forms of N in order of decreasing oxidation state are nitrate, nitrite, ammonia and organic N. The sum of these is expressed as TN (total nitrogen) and is not to be confused

with total Kjeldahl N (TKN), which is the sum of organic N and ammonia. TN can be determined through oxidative digestion of all digestible N forms to nitrate, followed by quantitation of the nitrate. Nitrite is an intermediate oxidation state of N, both in the oxidation of ammonia to nitrate and in the reduction of nitrate. Such oxidation and reduction may occur in wastewater treatment plants, water distribution systems and natural waters. Ammonia is produced largely by deamination of organic N-containing compounds and by hydrolysis of urea. The two major factors that influence selection of the method to determine ammonia are concentration and presence/absence of interferences (e.g., high concentrations of coloured organic substances such as humic-like materials or paper-mill effluents).

Total N is measured by the persulfate method, which digests all compounds containing both organic and inorganic N. All N-containing materials (except N gas) are measured after sample digestion has occurred. Kjeldahl N minus the ammonia concentration is the surrogate measurement for all organic N-containing compounds.

Ammonia/ammonium

Ammonia is measured by the indophenol blue (= phenate method) after conversion of ammonia and ammonium to ammonia. This is done by raising the pH of the sample above 11. This method has some good features (e.g., minimal interference from waters highly stained with humic materials and paper mill effluents); however, the detection limit is relatively high, e.g., <0.04–10 µM NH_3-N (Parsons et al. 1984). In spectrophotometric methods, the ammonia is reduced to monochloramine and then reacted with phenol to form a blue colour. Ammonia can also be measured using selective ion electrodes but as detection limit is quite high (>2 µM) it is not recommended for most tropical marine waters. This method may be suitable in polluted estuarine waters.

Nitrates and nitrites

Nitrates and nitrites are measured in combination using the cadmium (Cd) reduction procedure of Wood et al. (1967). This colorimetric method determines the concentration of these two materials after reaction of nitrites to produce an azo dye, the colour of which is proportional to the concentration of the combined nitrates and nitrites. Total nitrate is determined by subtracting the concentration of nitrite from the combination of the two. The process for measurement of nitrite produces the same azo dye as the combined measure, but without the Cd reduction. The difference in these two measures is the nitrate concentration.

5.2.1.2 Phosphorus

The target detection limit for measurement of phosphorus (P) in seawater is ~ 0.3 µM. The procedures for the measurement of total particulate and dissolved P as well as orthophosphate in seawater provide detection limits that are less than this value

(US EPA 1996). These procedures convert the phosphorus-containing compounds to orthophosphate through the digestion of the sample with alkaline persulfate. This treatment is then reacted with ammonium molybdate and antimony potassium tartrate in acidic solution to produce an intense blue complex with ascorbic acid. Interferences with elevated concentrations of silicon can be avoided by maintaining an acid concentration in the reagents and analysing the material at elevated temperatures of ~37°C. The resulting phosphomolybdic acid reduction produces a purple-blue complex that is measured at 885 nm on a spectrophotometer. This method of measuring reactive phosphorus is recommended in Millero (1996).

5.2.1.3 Silica

The target detection limit for measurement of silicon (Si) in seawater is ~0.7 µM. Pigmented silicomolybdate complex produced by procedures contained in US EPA (1996) provides adequate sensitivity after the samples are filtered (0.45 µm GF/F filter) to remove interfering particles and turbidity, and after phosphates and arsenates are removed with oxalic acid. The resultant filtrate is treated with a solution containing metol-sulfate (p-methyl-amino-phenol sulfate) to produce a blue colour that is evaluated more efficiently than the yellow colour recommended for evaluation in US EPA (1996), with a spectrophotometer at 812 nm (Strickland and Parsons 1968). This method of measuring reactive silicate is also recommended in Millero (1996).

5.2.2 Synopsis of the technique

The major forms of nitrogen present in seawater are nitrate, nitrite, ammonia, organic nitrogen and particulate nitrogen (Franson et al. 1980). Phosphorus occurs in water samples as free or esterified phosphates-orthophosphates, polyphosphates and organically bound phosphates. The inorganic species of phosphorus and nitrogen can be readily analysed using a variety of methods, but organically bound forms must also be measured to determine the total amount of dissolved phosphorus or nitrogen in a sample. This is done by converting organically bound forms to a more readily analysed form. There are a number of methods in use to oxidise dissolved organics in water samples. These include acid digestion methods to hydrolyse esterified phosphorus in samples, and Kjeldahl digestion for organic nitrogen (Wangersky and Zika 1978). The method described here utilises strong ultra-violet light to simultaneously photo-oxidise organic nitrogen and phosphorus fractions. This is a technique that is commonly used to oxidise organics in seawater samples (Manny et al. 1971). The technique uses a high intensity ultraviolet light source to irradiate samples so the organic nitrogen is oxidised to nitrate and nitrite, while organic phosphorus is converted to orthophosphates. This method gives an accurate and precise indication of organically bound nutrient fractions (Strickland and Parsons 1972).

Filtered water samples are stored frozen in 10 mL, acid-washed nutrient tubes. Before analysis, water samples are thawed and placed under ultra-violet photo-oxidation

to convert organic nutrient components to inorganic forms. Samples then undergo colorimetric analysis using a flow-through auto-analysis system. As the samples are initially filtered to remove particulates, the final result gives total dissolved (or filterable) phosphorus and nitrogen. The dissolved inorganic value is subtracted from the total to give the organic value.

5.2.3 Equipment

- Ultra-violet photo-oxidation unit
- Silica sample vials (24)
- Silica stoppers with Teflon sleeves (24)

5.2.4 Method

General

1. Place frozen nutrient tubes into microwave oven for two minutes on 'high' setting to thaw samples.
2. Remove the cap from each nutrient tube and pour the entire contents into a clean, dry quartz sample vial. *Ensure no hand contact is made with the top of the nutrient tube or the inside of the cap.*
3. Select a silica stopper fitted with a Teflon sleeve. *Care should be taken to avoid touching the stopper surface*. Place the stopper tightly into the quartz sample vial and recap the plastic nutrient tube.
4. Place the quartz sample vials in holders.
5. Set the power and lamp switches to the 'on' positions. Place the timer switch on automatic and set the timer to 7 hours.
6. Upon completion of the oxidation period, remove the holders from the photooxidation unit and remove the individual quartz sample vials. Transfer the contents of the vials back into the original nutrient tubes.
7. Refreeze the samples to await inorganic nitrogen and phosphorus analyses.
8. Wash the quartz vials and stoppers thoroughly using distilled pure water and place them into a 60°C oven to dry.

Ammonia

Ammonium in the sample reacts with phenol and alkaline hypochlorite to form indophenol blue. The blue colour is intensified with sodium nitroferricyanide. The absorbance of measured at 640 nm is linearly proportional to the concentration of ammonia in the sample.

Nitrate and Nitrite

Nitrite is diazotised with sulfanilamide and coupled with N-(1 naphthyl) ethylenediamine dihydrochloride to form an azo dye. The absorbance of the azo dye, measured at 540 nm is linearly proportional to the concentration of nitrite in the sample. In a second analysis, nitrate is quantitatively reduced to nitrite by passing the sample through a copperised cadmium coil. Then nitrate+nitrite, (that is reduced nitrate plus nitrite already in the sample) is measured as noted above. Nitrate concentrations are obtained by subtracting nitrite from nitrate+nitrite.

Phosphate

Orthophosphate reacts with molybdenum (VI) and antimony (III) in an acidic medium to form an antimonyphosphomolybdate complex. This complex is subsequently reduced with ascorbic acid to form a blue complex and the absorbance is measured at 660 nm.

Silicate

Silicate reacts with molybdate in an acidic solution to form silicomolybdate. Silicomolybdate is then reduced with ascorbic acid to molybdenum blue. The absorbance of the molybdenum blue measured at 660 nm is linearly proportional to the concentration of silicate in the sample.

5.2.5 Quality assurance/quality control for nutrients

All laboratories involved in measurement and analysis of nutrients in marine waters should participate in international intercalibration exercises. The Australian Low Level Nutrient Intercalibration exercise is run annually by Queensland Health Scientific Services for laboratories in Australia and the Asia-Pacific region. ASEAN laboratories participated in this exercise in 2005.

It is highly recommended to take field blanks through the field sampling process and to incorporate laboratory blanks within the laboratory procedures.

Using segmented flow analysis, the limits of detection for nitrate/nitrite, ammonia, ortho-phosphate and silica are 0.007 µmoles L^{-1}, 0.007 µmoles L^{-1}, 0.009 µmoles L^{-1} and 0.071 µmoles L^{-1} respectively.

5.3 Trace Metals

The methods chosen for trace metals analysis should be able to meet the very low levels set by the AMWQC. It is almost impossible to measure trace metals at such low levels by direct instrumental measurement methods, because of the salt matrix i.e., the very high NaCl content of seawater. Common methods of trace metal analysis in seawater often involve a sample pretreatment/ preconcentration step

followed by measurements using highly sensitive instruments. During pretreatment and preconcentration, 'clean techniques' which minimise all possible sources of contamination have to be employed.

The guidelines for ASEAN applications for the monitoring of trace metals in marine water are based on or extracted from the well-tested methodologies reported in the publications by PSEP (1997) and Grasshoff et al. (1999). After the pretreatment and preconcentration steps, trace metals may be measured by the APHA (2006) and US EPA (1996) standard instrumental methods.

5.3.1 Introduction

The background or baseline trace metal concentrations in estuarine, coastal and offshore marine waters should be reviewed before selecting any analytical methods. In Table 5-1, where the data were obtained using 'clean' techniques, most trace metals reported were in the sub-ppb range, i.e. <1 µg L^{-1}–0.001 µg L^{-1} in marine water and often also in estuarine water. For baseline trace metals monitoring, the methods selected must be sensitive enough to detect at the required concentration range.

The concentration of trace metals in estuarine waters and sometimes in coastal waters is subject to variation due to non-equilibrium processes in these regions. The non-equilibrium processes are a direct result of more intense inputs from rivers, the atmosphere or sediment and removal of elements by biological uptake or sorption onto sedimentary particulates.

5.3.1.1 Data quality objectives

A formal planning process is required to ensure that project data support project objectives. During this planning process, analytical methods and other related activities are specified. These decisions are based on the data quality objectives, which are developed after the project objectives and expected use of the data are clarified. To best ensure that data quality objectives for a project are met, the laboratory performing the analyses must understand the project requirements in advance of receiving samples.

5.3.1.2 Contamination and low level work

Sample contamination directly affects a laboratory's ability to analyse a sample accurately at low concentrations. In marine water quality monitoring for trace metals, contamination has been known to be the single most difficult barrier to achieving accurate data. Every precaution should be taken to avoid contamination at each stage of sample collection, handling, storage, preparation and analysis. Most of the trace metals analyses to be performed as a part of ASEAN Marine Water Quality Management and Monitoring programs have low limits of detection, making contamination control an essential factor.

Laboratories generating trace level data should conduct quality control (QC) on an ongoing basis. The laboratory's QC program should contain samples such as method blanks, glassware blanks and equipment blanks that allow continual updates in knowledge regarding background levels within the sample processing environment. The laboratory's QC program should assess contamination, identify sources of contamination and eliminate or minimise those sources of contamination. In addition, sample collection methods and the field QC program must be equally rigorous to ensure that the samples are not contaminated during the sampling or transport processes.

Trace metals occur in very low concentrations, as indicated in Table 5-1. Thus, it is necessary to ensure that sample preparation steps such as subsampling, filtration or preconcentration are performed in an area known to be free from contamination, preferably in a clean room or a clean, nonmetal and laminar flow fumehood. Admittance to clean areas should be restricted and personnel should be trained in clean sample-handling techniques. It is recommended to dedicate the clean areas to trace level work and isolate samples with high concentrations of metals to other areas. Personnel must pay strict attention to the work being done. Physical sample handling should be kept to a minimum. Exposure of samples and labware to airborne dust should be minimised during sampling and analysis.

Table 5-1. Examples of concentration range of trace metals in marine waters (adapted from Hickey and Pyle 2001).

Metal	Marine Water ($\mu g\ L^{-1}$)	Estuarine water ($\mu g\ L^{-1}$)	Fresh water ($\mu g\ L^{-1}$)	Country ($\mu g\ L^{-1}$)
Arsenic	1.0-1.6	1.0-3.3	NI	Australia
Cadmium	0.01-0.2	NI	0.002-0.08	USA
	0.001-1.1	NI	0.01, 0.002-0.01, 0.08	World
	0.002-0.7	0.002-0.026	0.001	Australia
		0.51-1.2		
	NI*	NI*	0.008	New Zealand
Copper	0.1-3	NI*	0.4 - 4	USA
	0.003 – 0.37	NI*	1.5	World
	0.025 – 0.38	0.06 – 1.3	0.11	Australia
	0.1 – 0.2	NI*	0.15	New Zealand
Chromium	0.062 – 0.1	0.01 – 0.1	NI*	Australia
Iron	0.006 – 0.14	<0.04 – 13.7	40	World

	NI*	0.76-67	NI*	Australia
Lead	0.01 - 1	NI*	0.01 – 0.19	USA
	<0.006 – 0.03	0.02 – 0.13	NI*	Australia
	NI*	NI*	0.02-0.03	New Zealand
Manganese	0.03 – 0.38	NI	1.5	World
	NI*	0.55 – 3.1	NI*	Australia
Mercury	NI*	0.0007 – 0.003	0.01	World
Nickel	0.3 - 5	NI*	1 - 2	USA
	0.12 – 0.7	NI*	0.5, 3.3	World
	0.13 – 0.5	0.14 – 1.10	0.10	Australia
	0.33	NI*	0.1 – 0.15	New Zealand
Silver	0.006 – 0.2	NI*	NI*	USA
	<0.0005	NI*	NI*	Australia
Zinc	0.1 – 1.5		0.03 - 5	USA
	0.003 – 0.59		0.6, 2.8	World
	<0.022 – 0.1	0.39 – 3.8	0.9	Australia
		0.4 – 1.8		
	0.005 – 0.02		0.15 – 0.2	New Zealand

(NI* = no information found)

Laboratory glassware (Pyrex, Kimax) contains trace metals. Fluoropolymer (PTFE, Teflon) and clear plastic (linear polyethylene) labware are preferred. Ideally, labware would be dedicated according to sample type and anticipated concentration of analytes. Plastic pipet tips may be a source of metals contamination; acid-cleaned pipet tips are commercially available. Other materials known to contain trace levels of metals are rubber, paper cap liners, pigments in marking pens, polyvinyl chloride, nylon, methacrylate, Vycor and talcum powder. Use only clean, powder-free gloves for all sample handling steps.

Always test new products or similar products from a new manufacturer and do not make assumptions about the appropriateness of a product until it has been well tested. For low-level work, reagents should be ultrapure grade or equivalent and should never be returned to their stock containers once removed. Sample carry-over at the instrument must be carefully monitored and rinse times adjusted to eliminate any potential carry-over. The quality of the reagent water used should be >18.2

$M\Omega cm^{-1}$ resistivity and may be generated using an ultrapure water generation system such as Millepore or Barnstead.

Field equipment and labware must be carefully cleaned and cleaning methods must be monitored and verified using field and laboratory blanks. The time between cleaning and use of labware should be kept to a minimum. Labware should be enclosed in polyethylene zip-locked bags for storage or stored in a dilute nitric acid bath until time of use. Labware with tops, such as bottles and volumetric flasks can be filled with dilute nitric acid, closed and stored upright with the nitric acid until time of use. Apparatus can be covered with clean plastic wrap and stored in a clean area. The possibility of contamination will be minimised if labware for the different sample types are kept separate.

The laboratory should have written procedures for labware cleaning methods. All labware should be thoroughly cleaned with a detergent solution (such as Detergent 8™), rinsed with metal-free water, and soaked overnight or longer in a covered acid bath containing a dilute nitric acid solution prepared from reagent grade nitric acid. A 20% nitric acid bath is common practice but other concentrations may be used if verified as adequate by the results of routine blanks. Some laboratories prepare labware for ultraclean work by soaking it overnight in hot concentrated nitric acid and find the use of hot acid particularly important for cleaning PTFE (Teflon) labware. Cleaning of labware for some analytes benefits from the additional step of a dilute hydrochloric acid soak.

Regardless of the strength or type of acid used, it is helpful if labware is stored containing dilute acid or in an acid bath until it is used, to prevent contamination during drying and storage. When labware is removed from the acid bath, it must be rinsed with copious quantities of metal-free water. The rinsing step is critical to minimise contamination. Acid baths should be changed periodically, as the acid becomes contaminated. To avoid contamination with chromium, do not use chromic acid for cleaning any materials.

Acid precleaned plastic bottles and pipet tips are available commercially. Cleanliness of commercially cleaned labware should be monitored by the analysis of blanks.

The USEPA document 821-B-95-0 (EPA 1995) provides more in-depth information on clean room design. EPA Method 1669: (EPA 1996) discusses 'clean' and 'ultraclean' techniques and detailed methods for preventing contamination during sampling.

5.3.1.3 Interferences

Seawater contains approximately 3 percent dissolved salts, which cause problems such as uneven sample transport rates and severe chemical and spectral interferences. The choice of analytical method must be made carefully and must take into account potential interferences. The analyst should be experienced with analysis of marine

samples and resolution of concomitant interference problems. Specific information on minimising interferences from marine samples is found later in this chapter.

5.3.1.4 Safety considerations

Participants in a project should ensure that their activities do not increase the risk to humans or the environment. Laboratories must operate under an active safety program. Laboratory facilities need to have adequate ventilation for labware cleaning, sample preparation and instrumental analysis. Appropriate engineering controls and personal protective equipment must be available and used. Laboratory workers must be trained in safe laboratory techniques.

Health and safety issues need to be considered when choosing methods of analysis. When more than one option exists, the method with fewer hazardous reagents, dangerous procedural steps or toxic by-products should be chosen. For cleaning of labware, care must be taken while using acid baths; acid fumes and potential for acid burns to skin and eyes can pose a risk. Temperatures and concentrations of acids should be kept as low as feasible for decontaminating labware and sampling equipment.

5.3.1.5 Sample acceptance and storage criteria

When samples are received by the laboratory, adherence to the sample acceptance requirements specified in the project planning document should be verified to ensure sample integrity. The following should be considered:

- Technical validity—sample preservation and storage are appropriate for the stability of the analyte; and
- Chain of custody—the personnel handling the sample are properly trained and authorised to do so; tampering with the sample is precluded and all sample handling is documented.

In addition, the following items should be verified: sample identification (between the sample container and the field sheet), sample bottles and sample receipt within holding time. When applicable, any safety hazards associated with the samples should be noted, documented and the appropriate personnel should be notified.

All samples should be preserved and stored according to applicable approved procedures and analysis must start prior to expiration of holding time. Details on the storage of seawater samples for trace metals are given in Chapter 4 (Section 4.3.7).

5.3.2 Method Selection

The selection of analytical methods for a project is influenced by a variety of factors. Some of these factors are client or program specifications, availability of accepted or

standard methods, required detection limits, turn-around time, sample type, available technology, operator expertise and cost-effectiveness. Additional issues to consider include analytes to be measured, expected concentrations and potential interferences. The project manager and the analytical laboratory need to discuss project requirements during the planning stage so that the most appropriate analytical method is selected and documented in the project planning document.

This manual encourages the use of methods that produce comparable data so that data generated for a specific project can be used to support longer-term environmental studies. In addition, project-specific trend analyses require new data sets to compare with historical data sets. The use of USEPA methods is recommended for ASEAN marine water quality monitoring samples. Many laboratories routinely use these methods so that method performance is well-documented. The need for highly sensitive instruments for most trace metals analysis may limit laboratories which are able to provide such services for seawater analysis.

When an appropriate EPA method is not available, a validated standard method from another recognized source, such as Grasshoff et al. (1999) or APHA (2006), may be applied. When the method chosen is not considered as a standard method, the laboratory must document method performance and ability to meet data quality objectives. It is recommended that highly complex methods only be used when essential for meeting project requirements. The preferred approach is to use the most straightforward and standardised method available that meets data quality objectives.

Methods for the determination of trace metals typically fall within the scope of a small number of instrumental methods and variations on those procedures. These include, but are not limited to GFAA and ICP-MS for trace metals including Cd, Cu, Pb, Cr(III) and Zn, hydride generation-atomic absorption method for As, and cold vapour atomic fluorescence (CVAF) for Hg. Laboratories with flame atomic absorption spectrophotometers (AAS) equipped with cold vapour or arsine generation equipment may be able to provide services on As analysis.

5.3.2.1 Determining, defining and verifying detection limits

Environmental analytical chemists have not universally agreed upon terminology for defining or conventions for determining and reporting lower detection limits for analytical procedures. Two 'limits' commonly found in the literature are the method detection limit and the quantification limit.

The USEPA defines *method detection limit* (MDL) as *'the minimum concentration of a substance that can be measured and reported with 99 percent confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the element.'* (US EPA 1996).

Actual detection limits may be affected by instrument sensitivity, bias due to contamination and/or matrix interferences. Common laboratory practice is to calculate MDLs according to the USEPA procedure and subsequently adjust detection limits upward in cases where high instrument precision (i.e., low variability) results in calculated detection limits that are lower than the absolute sensitivity of the analytical instrument. In addition, detection limits may be adjusted upward for some analytes when random contamination or interference is a significant issue for an analytical method.

The *quantification limit* represents a practical and routinely achievable level at which there is relatively good certainty that any reported value is reliable (APHA 1998). The quantification limit for a test is usually about five to ten times the detection limit and always higher than the detection limit. A quantification limit check standard should be analysed to verify quantification limit at the instrument. A spiked method blank fortified with analytes at or near the quantification limit is also recommended as a periodic method check sample to demonstrate method performance near the quantification limit.

Analyte values below the detection limit are not reported. Rather, the result is reported as less than the detection limit, including the numerical value for the detection limit. When an analyte value is between the detection limit and the quantification limit, the value is reported and is qualified as less than the quantification limit.

5.3.2.2 Filtration and pre-treatment

Studies of metals in the water column may require analysis of the whole sample for total metals or separation of dissolved and particulate fractions, depending upon project objectives. Total metals are defined as the concentration of metals determined on an unfiltered sample after digestion. The dissolved fraction of a water sample is defined as the fraction that passes through a 0.4 or 0.45 µm membrane filter when an unpreserved water sample is filtered. The particulate fraction is defined as the material that is retained on a 0.4 or 0.45 µm filter.

Pore size is important in this definition as particulate matter smaller than 0.45 µm exists in the water column. Several types of filters have a nominal pore size of 0.4 µm. In practice, there is probably little difference in the material retained by filters with 0.45 and 0.4 µm pores sizes and subsequent discussion will refer to a 0.45 µm filter, but a 0.4 µm filter may be used as well.

For the AMWQC, concentrations of trace metals in the dissolved form are required. Therefore, only dissolved trace metals analysis may be necessary in those national monitoring programs established to cover the basic set of AMWQC.

5.3.2.3 Sample preparation for dissolved trace metals

There is no detailed standard method available that addresses all the practical issues involved with the preparation of water samples for dissolved trace metals. The most critical concern when preparing samples for analysis of dissolved metals is contamination control (see Section 5.6.1.2). Contamination during the sample collection, splitting and filtering steps is often a major source of bias, resulting in false positive values for samples with low concentrations and limiting the laboratory's ability to accurately measure metals at the low detection limits required for projects driven by water quality or human health criteria. Monitoring each step in the process with QC samples (blanks) is important to verify that analytical data represent sample concentrations and not sample contamination.

When filtering water samples for dissolved metals, it is important to find a method and apparatus that minimises contamination of the sample during the filtering process and that also has the ability to filter adequate volumes of sample in a reasonable amount of time. For reasons of pore size consistency and contamination minimisation, membrane filters are strongly recommended over capsule filters for marine water samples. Membrane filters are available in several sizes, including 47 mm, 90 mm and 142 mm diameter. The 47 mm diameter size is most commonly found in laboratories but larger filters may be necessary when filtering larger volumes of samples. Polycarbonate membrane filters often clog quickly. However, these filters have a lower potential for trace element contamination than alternatives such as cellulose nitrate and cellulose acetate membrane filters.

The USEPA Method 1669 (EPA 1996), Section 6.17.2 method for acid cleaning 0.4 µm, 47 mm polycarbonate Nucleopore (or equivalent) membrane filters is:

1. Fill a 1 L fluoropolymer jar approximately two-thirds full with 1N nitric acid.
2. Using fluoropolymer forceps, place individual filters in the fluoropolymer jar.
3. Allow the filters to soak for 48 hours.
4. Discard the acid, and rinse five times with metal-free water.
5. Fill the jar with metal-free water, and soak the filters for 24 hours.
6. Remove the filters when ready for use, using fluoropolymer forceps, and place them on the filter apparatus.

When using membrane filters, the filter-holding and sample capture equipment are also very important to the process. These must be made of an appropriate material, be acid-cleaned before use and rinsed well between samples. A Teflon in-line filter holder such as Millepore #XX434700 works well and can be opened for filter change without disturbing the attached plumbing. Tubing that contacts the sample should be Teflon. Fritted glass filter holders (use silicon stoppers) are easy to use during

filtering but are difficult to clean well and do not filter samples as quickly as the in-line filter holders.

Other types of filtering equipment are available for dissolved trace metals. Any can be used so long as the final filter is a 0.4 to 0.45 µm membrane and the samples are not contaminated by the filtering process. Filtering may need to be done in a clean room to meet required detection limits. If using a pressure filter device, filter at a pressure of 70 to 130 kPa. Pressure filter units clog less readily than vacuum filters (APHA 1998).

5.3.2.3.1 Filtering samples in the laboratory for dissolved and particulate metals analysis

The following method for filtering may be modified depending upon filter apparatus. Only unacidified samples should be filtered. The volume to be filtered depends upon the tests being run on each sample (remember that mercury is a separate test and that an additional sample is required for duplicates and matrix spikes). If the particulate fraction is to be analysed, the sample must be shaken thoroughly immediately before subsampling to achieve a representative sample. If only the dissolved fraction is to be analysed, allow particulates to settle or centrifuge the sample to minimise filter clogging. If total and dissolved metals samples are to be taken from the same container, take a subsample for total metals before allowing particulates to settle.

1. Conduct filtering in a clean room or on a clean bench **when needed to meet required detection limits.** Set up acid-cleaned filtering apparatus, with filter in place. Use Teflon-coated forceps for handling filters.
2. Rinse the system by filtering at least 1L of metal-free water and discarding the rinse water.
3. Collect a 'before' filtrate blank by filtering 500 mL of metal-free water through the system. Collect the filtrate, transfer it to a 500 mL acid-cleaned sample bottle and label the bottle with date and associated sample batch.
4. Rinse the filtering apparatus with sample water by filtering a portion of the sample and discarding this portion. Filter the required volume of sample and retain the filtrate for dissolved metals analysis. If the filter clogs, change filters. Centrifuging the sample or prefiltering with a 3 µm or 1 µm filter may also minimise filter clogging. Be aware that additional steps or filters used in the filtering process increase the potential for sample contamination.
5. Thoroughly rinse the filtering apparatus with at least 1L of metal-free water between samples. Repeat steps 4 and 5 for additional samples. Decontaminating the apparatus between samples by rinsing with dilute (1 percent) nitric acid may be necessary, depending on sample concentrations and required detection limits.

6. At the end of the sample batch and after decontaminating the filtration apparatus, collect an 'after' filtrate blank as in Step 3.
7. Preserve the filtered samples and blanks with ultrapure nitric acid to pH <2. Dissolved metals samples are now ready for analysis. Place filters for particulate metals analysis in pre-cleaned polystyrene Petri dishes for freezing or to digestion vessels for analysis.

5.3.2.4 Pre-treatment for total trace metals

Seawater contains approximately 3% dissolved salts and pretreatment of samples is often required. The appropriate pretreatment method must separate the matrix from the analytes while maintaining or lowering detection limits. Some of the more sensitive instruments, such as newer ICP-MS models, may be capable of analysing samples of marine water directly, after dilution at a ratio of approximately 1:100. When such instruments are available, a simple technique such as dilution is preferred to complex sample preparation techniques.

When high sensitivity instruments are not available, or when detection limit requirements for marine water samples are very low, other pre-treatment techniques may be necessary. Pre-treatment techniques such as on-line and off-line chelation pre-concentration, chelation/solvent extraction, coprecipitation and reductive precipitation all perform some pre-concentration of trace metals while modifying the sample matrix sufficiently for effective instrumental analysis.

A good deal of attention is currently being devoted to the field of pre-treatment techniques that combine matrix modification with pre-concentration. These procedures are intensive in terms of time, labour, analyst expertise and cleanliness and typically combine the matrix modification/pre-concentration step with the standard determinative methods available in most analytical laboratories. While matrix removal methods usually cite a specific determinative method, this may be flexible, and matrix removal methods may be compatible with other determinative methods. On- and off-line techniques may be interchangeable with appropriate modifications.

While some sample matrix removal/pre-concentration methods have been published by the USEPA, the scope of these procedures is currently less than comprehensive. Other research level methodologies are available through industrial and academic sources. In the absence of standard methods, the use of non-standardised methods and/or performance based methodology may be necessary to meet project requirements.

5.3.3 Methods for trace metal analysis for marine water samples

The suggested methods for the analysis of trace metals in marine water samples for ASEAN applications are:

- On-line chelation matrix removal and pre-concentration and ICP-MS (Cd, Cu, Pb, Zn, Cr(III)).
- Off-line chelation matrix removal and preconcentration and GFAAS (Cd, Cu, Pb, Zn, Cr(III)).
- Cold vapour atomic fluorescence spectrometry (CVAFS) (Hg).
- Hydride generation AAS (HGAAS) (As).
- Ion chromatography (Cr VI).
- Solvent extraction-GFAAS (CrVI, tributyltin).
- GC-PFPD (Organotin compounds including tributyltin).

These are summarised in Table 5-2. The requirements of analytical detection limits of the various methods depend on the expected baseline concentration range of the trace metals in the water body being monitored. AMWQC levels may be significantly higher than the expected baseline concentration range. From a marine water quality management point of view, the baseline concentration range should be used as a guide in setting the requirements of analytical detection limits.

5.3.3.1 On-line chelation matrix removal and pre-concentration and ICP-MS (Cd, Cu, Pb, Zn, Cr(III))

The following USEPA method is recommended for measuring Cd, Cu, Pb, Zn and Cr (III) in marine water:

- **USEPA Method 200.10** *Determination of Trace Metals in Marine Waters by On-Line Chelation Preconcentration and Inductively Coupled Plasma - Mass Spectrometry* (EPA 1997).

  This method is used to preconcentrate trace metals using an iminodiacetate functionalized chelating resin. Acid solubilisation (digestion) is required prior to chelation to break down complexes of colloids that might influence trace element recoveries. Chelation procedures offer the ability to concentrate analytes of interest while at the same time removing undesirable sample constituents from the sample matrix. Pre-assembled iminodiacetate units are commercially available. No single chelation chemistry has been found to be applicable to all of the analytes commonly of interest. The above methods are applicable to Cd, Cr, Cu, and Pb for the purpose of the ASEAN marine water quality monitoring. Zn may be included if satisfactory recovery of the metal can be proven and contamination problems can be eliminated.

  Details on the specific procedure for USEPA Method 200.10 are attached in Appendix 1 (Appendix 5.2.2.4.A).

Table 5-2. Proposed analytical techniques for trace metals analysis in marine water for ASEAN applications.

Parameter	Criterion for Protection of Aquatic Life	Criterion for Protection of Human Health		Analytical Technique Proposed CHECK SECTION NUMBERS BELOW
		Seafood Consumption	Recreational Activities	
Arsenic (As)	120 µg L^{-1} As	3.0 µg L^{-1} As	60 µg L^{-1} As	Hydride generation AAS
Cadmium (Cd)	10.0 µg L Cd	23 µg L^{-1} Cd	35.7 µg L^{-1} Cd	*On-line* chelation matrix removal and pre concentration and ICP-MS *Off-line* chelation matrix removal and pre concentration and GFAAS
Hexavalent Chromium [Cr (VI)]*	48 µg L^{-1} Cr	Not derived	Not derived	Hexavalent chromium by ion chromatography Hexavalent chromium by solvent extraction-GFAAS Total chromium (as III) by *On-line* chelation matrix removal and preconcentration and ICP-MS or *Off-line* chelation matrix removal and preconcentration and GFAAS.
Copper (Cu)*	2.9 µg L^{-1} Cu Check numbers match marine WQ criteria	Not applicable	500 µg L^{-1} Cu	*On-line* chelation matrix removal and preconcentration and ICP-MS *Off-line* chelation matrix removal and preconcentration and GFAAS
Lead (Pb)*	8.5 µg L^{-1} Pb	Not derived	Not applicable	*On-line* chelation matrix removal and preconcentration and ICP-MS *Off-line* chelation matrix removal and preconcentration and GFAAS
Mercury (Hg)*	0.16 µg L^{-1} Hg	0.04 µg L^{-1} Hg	21 µg L^{-1} Hg	Mercury by cold vapour atomic fluorescence spectrometry (CVAFS)
Tributyltin (TBT)*	0.010 µg L^{-1} TBT	Not derived	Not derived	Tributyltin by solvent extraction-GFAAS Organotin compounds by GC-PFPD
Zinc*	50 µg L^{-1} Zn	Not applicable	1,250 µg L^{-1} Zn	*On-line* chelation matrix removal and preconcentration and ICP-MS *Off-line* chelation matrix removal and preconcentration and GFAAS

*identifies those criteria which have been accepted by ASEAN as set out in McPherson et al. 1999.

A commercial automated chelation ion chromatography system is available from Dionex Corporation (Dionex 1992). This chelation system may be interfaced to an ICP-MS for elemental detection. Commercial ICP-MS systems are available from Agilent 7500 series, PerkinElmer Elan series, Varian 820 series, or Thermo Fisher Scientific Elemental X Series.

5.3.3.2 Off-line chelation matrix removal and pre-concentration and GFAAS (Cd, Cu, Pb, Zn, Cr(III))

The following USEPA method is recommended for measuring Cd, Cu, Pb, Zn and Cr (III) in marine water:

- **USEPA Method 200.13** *Determination of Trace metals in Marine Waters by Off-Line Chelation Preconcentration with Graphite Furnace Atomic Absorption* (EPA 1997b).

  This method is similar to section 5.3.4.1 and is applicable to Cd, Cr, Cu, and Pb for the purpose of the ASEAN marine water quality monitoring except that the chelation preconcentration steps are performed off-line. Zn may be included if satisfactory recovery of the metal and contamination problems can be eliminated. Determination of the chelated analytes is determined by GFAAS. To increase sample throughput, determination may also be performed by an ICP-MS system as demonstrated by Tong et al. (1998).

  Details on the specific procedures for USEPA Method 200.13 are attached in Appendix 2 (Appendix 5.2.2.4.B).

  Commercial GFAAS systems are available from Analytik Jena ContrAA, NovAA or Zeenit series, PerkinElmer AAnalyst series, GBC Avanta series or Varian AA series.

5.3.3.3 Mercury by cold vapour atomic fluorescence spectrometry (CVAFS)

The following USEPA method is recommended for measuring Hg in marine water:

- **USEPA Method 1631 (Rev. E)** *Mercury in Water by Oxidation, Purge and Trap, and Cold Vapour Atomic Fluorescence Spectrometry* (EPA 2002).

  The ambient level of total mercury of as low as 0.0001 µg L^{-1} in marine water can be met reproducibly by cold vapour atomic fluorescence spectrometry coupled with a pre-concentration (gold amalgamation) step. The USEPA Method 1631 (Rev. E) describes the gold amalgamation apparatus and procedure and provides information for sample collection, shipping and analysis to prevent contamination.

  Details on the specific procedures for USEPA Method 1631 (Rev. E) are attached in Appendix 3 (Appendix 5.2.2.4.C).

Commercial CVAFS systems are available from Analytik Jena Mercur *plus*, Leeman Labs Hydra AF Gold*plus* or PS Analytical Millenium Merlin. An application of the CVAFS technique in the determination of mercury at the sub-ng/L in seawater is available (Becker 2005).

5.3.3.4 Arsenic by hydride generation atomic absorption spectrometry (HGAAS)

The following USEPA method is recommended for measuring As in marine water:

- **USEPA Method 1632** *Chemical speciation of arsenic in water and tissue by hydride generation quartz furnace atomic absorption spectrometry* (EPA 2001).

  The lower ambient level for arsenic of 1.0 µg L^{-1} in marine water can be met reproducibly by the hydride generation atomic absorption spectrometry. The USEPA Method 1632 (Rev. A) provides for the speciation of As species in water samples. This is performed by converting both the inorganic and organic As species into volatile arsines followed by cryogenic trapping with a gas chromatographic packing. For ASEAN marine water quality monitoring purposes, the cryogenic trapping and heating step is omitted. The generation of volatile arsines is performed under acidic conditions and all volatile arsines are determined directly on a commercial AAS system equipped with a quartz tube atomiser. Alternatively, direct coupling of the volatile arsines to an ICP-OES or ICP-MS system is possible provided equivalent performance is demonstrated.

  Details on the specific procedures for USEPA Method 1632 are attached in Appendix 4 (Appendix 5.2.2.4.D).

  Commercial hydride generation systems are available from Analytik Jena (HS series), GBC (HG series), PerkinElmer (MHS or FIAS series), or Varian (VGA series).

5.3.3.5 Hexavalent chromium by ion chromatography

The following USEPA method is recommended for measuring Cr (VI) in marine water:

- **USEPA Method 1636** *Determination of Hexavalent Chromium by Ion Chromatography* (EPA 1996b).

  This method integrates the earlier USEPA Method 218.6 (EPA 1994) with the quality control and sample handling procedures necessary to avoid contamination and to ensure the validity of analytical results. An aqueous sample is filtered through a 0.45 µm filter and the filtrate is adjusted to a pH of 9-9.5 with a concentrated buffer solution. A measured volume of the sample (50-250 µL) is introduced into the ion chromatograph. A guard column removes

organics from the sample before the Cr(VI), as CrO_4^{2-}, is separated on a high capacity anion exchange separator column. Postcolumn derivatisation of the Cr(VI) with diphenylcarbazide is followed by detection of the colored complex at 530 nm.

Details on the specific procedures for USEPA Method 1636 are attached in Appendix 5 (Appendix 5.2.2.4.E).

A commercial ion chromatography system for this purpose is available from Dionex Corporation (Dionex 1998). Other systems may be used provided equivalent performance is demonstrated.

5.3.3.6 Hexavalent chromium by solvent extraction-GFAAS

The method reported in the following paper is recommended for measuring Cr (VI) in marine water:

- *Determination of Trace Concentrations of Hexavalent Chromium* (Gardner and Comber 2002)

  This method is based on the reaction Cr(VI) with diphenylcarbazide followed by solvent extraction of the derivatised complex and detection by GFAAS. The procedures are relatively simple and highly sensitive with a detection limit of 0.024 µg L^{-1}.

  Details on the specific procedures are attached in Appendix 6 (Appendix 5.2.2.4.F).

5.3.3.7 Tributyltin by solvent extraction-GFAAS

The method reported in the following paper is recommended for measuring tribuyltin in marine water:

- *Tributyltin Distribution in the Coastal Environment of Peninsular Malaysia* (Tong et al. 1996)

  This method is a relatively simple and sensitive methodology to determine TBT in seawater. It is based on the extraction of TBT using n-pentane, an alkaline backwash to remove mono- and di-butyltins, preconcentration by evaporation and reconstitution in methanol-nitric acid and finally determination by GFAAS system. The detection limit of this technique was 1.4 ng L^{-1} (TBT-Sn) or 3.4 ng L^{-1} (TBT).

  Details on the specific procedures are attached in Appendix 7 (Appendix 5.2.2.4.G).

5.3.3.8 Organotin compounds by GC-PFPD

The method reported in the following paper is recommended for measuring tributyltin in marine water:

- *Determination of Organotin Compounds in Environmental Samples by Gas Chromatography Pulse Flame Photometric Detection.*

  This method allows the speciation of the organotin compounds to be obtained. It is based on a one-step ethylation-extraction employing $NaBEt_4$, extraction of the ethylated organotin compounds into isooctane followed by gas chromatography separation and detection using a pulsed flame photometric detector. The detection limit of this technique was 0.3 ng L^{-1} (TBT-Sn) or 0.7 ng L^{-1} (TBT).

  Details on the specific procedures are attached in Appendix 8 (Appendix 5.2.2.4.H).

  A commercial GC-PFPD system is available from Varian (3800 series). Other systems may be used provided equivalent 5.6.4 analytical quality control performance is demonstrated.

All EPA methods include specific recommendations for QC samples, control limits and corrective actions. The approach to analytical QC varies somewhat among the different EPA methods depending upon the data usage that the method was intended to support. In choosing an approach to analytical QC, a laboratory should keep in mind that QC sample results help define both method performance and data quality. The appropriate level of QC for a given set of samples is impacted by the complexity of the analytical method, the sample matrix and the project required detection limits. In addition, the level of QC, limits and corrective actions are impacted by the end use of the data.

Analytical QC for each project must be specified in the project planning document and reflect an agreement between the project manager and the laboratory before the analysis begins. This is particularly important when project specific QC is more stringent than the method QC. In addition, the QC required for a project must take into account any subsequent program-driven data qualification.

All quality control documentation should be maintained and available for easy reference or inspection. Following is a summary of minimum required QC samples and control limits for trace metals analysis. This section is not intended to provide criteria that are more lenient than the reference methods. Rather it provides guidance when reference methods do not include specific QC procedures, as in the case of the experimental methods proposed.

5.3.4 Analytical quality control

All EPA methods include specific recommendations for QC samples, control limits and corrective actions. The approach to analytical QC varies somewhat among the different EPA methods depending upon the data usage that the method was intended

to support. In choosing an approach to analytical QC, a laboratory should keep in mind that QC sample results help define both method performance and data quality. The appropriate level of QC for a given set of samples is impacted by the complexity of the analytical method, the sample matrix and the project required detection limits. In addition, the level of QC, limits and corrective actions are impacted by the end use of the data.

Analytical QC for each project must be specified in the project planning document and reflect an agreement between the project manager and the laboratory before the analysis begins. This is particularly important when project specific QC is more stringent than the method QC. In addition, the QC required for a project must take into account any subsequent program-driven data qualification.

All quality control documentation should be maintained and available for easy reference or inspection. Following is a summary of minimum required QC samples and control limits for trace metals analysis. This section is not intended to provide criteria that are more lenient than the reference methods. Rather it provides guidance when reference methods do not include specific QC procedures, as in the case of the experimental methods proposed.

5.3.5 Instrument quality control

5.3.5.1 Calibration

For trace metals work, it is important to compare daily instrument readings for calibration standards with typical readings for an optimised instrument. If readings for the standards are inconsistent with expected readings, the instrument may need to be optimised and recalibrated. For example, calibration blanks contaminated with analytes could cause a negative bias in the data. This would impact on the accuracy of the data, particularly near the detection limit.

Analytical instruments must be calibrated daily or each time the instrument is run, with a calibration blank and at least three calibration standards for most instruments. Standards should be matrix-matched to the samples, matching acid composition and strength of standards and samples and, in the case of seawater samples, standards may need to be prepared with synthetic seawater.

5.3.5.2 Initial calibration verification (ICV)

Run immediately after calibration, the ICV is an instrument check sample containing all analytes of interest at a concentration above the quantification limit. The ICV must be prepared from a different source (different bottle of stock solution) than calibration standards. Calculated concentration values should not deviate from the actual values by more than 10% for ICP-OES, GFAA and ICP-MS and 20% for mercury (or performance-based intralaboratory control limits, whichever is lower). If values for

the ICV are outside the control limits, the instrument run is stopped, the problem is corrected, the instrument is recalibrated and calibration is verified with another ICV.

5.3.5.3 Initial calibration blank (ICB)

Immediately after calibration verification, a calibration blank should be analysed. If the absolute value of the blank exceeds the detection limit, the analysis should be terminated, the problem corrected, the instrument recalibrated as necessary and the calibration reverified.

5.3.5.4 Continuing calibration verification (CCV)

A CCV check sample containing all analytes of interest should be analysed after every 10 samples, at a concentration above the quantification limit. Calculated concentration values obtained should not deviate from the actual values by more than 10% for ICP-OES and GFAA, 15% for ICP-MS and 20% for mercury. If values for the CCV are outside the control limits, the instrument run should be stopped, the problem corrected, the instrument recalibrated as necessary and the calibration reverified with an ICV. All samples after the last acceptable CCV must be reanalysed.

5.3.5.5 Continuing calibration blank (CCB)

One calibration blank for every 10 samples should be analysed. If the absolute value of the blank exceeds the detection limit, the analysis should be terminated, the problem corrected, the instrument recalibrated as necessary, the calibration reverified and all analytical samples after the last acceptable calibration blank reanalysed.

5.3.6 ICP interference check sample (ICS)

The interference check solution containing known concentrations of interfering elements will provide an adequate test of the interference correction factors. The ICS solutions consists of two parts: solution A contains the interferents at concentrations sufficiently high to be significant (ICSA), while solution AB contains both the interferents and the analytes at approximate concentrations of 10 times the detection limit (ICSAB). The ICSA and ICSAB should be analysed consecutively after the ICV and before the samples. If results for the ICSAB solution fall outside the control limits of +20% of the true value, the analysis should be terminated and the problem corrected. See instrument-specific reference methods for more information on how to prepare interference check samples.

5.3.7 GFAA analytical spike

The GFAA analytical spike is a second aliquot of prepared sample, spiked with the analyte of interest and analysed exactly the same, and immediately after, the

sample. The analytical spike provides information for overcoming matrix problems during analysis by graphite furnace. Most automated GFAA instruments can be programmed to perform this analysis and calculate recoveries. Control limits are 85-115% recovery, if the value of the spiked sample is 2-5 times the original sample concentration. Furnace programs, matrix modifiers or dilutions are adjusted to bring recoveries within these control limits. When recoveries of the instrument spike do not fall within the control limits, the method of standard additions may be necessary to meet the project required detection limits.

5.3.8 Method quality control

5.3.8.1 Method blank (MB)

A method blank is an aliquot of reagent water which is prepared and analysed exactly like, and together with, the samples. Method blanks provide an indication of the response of the measurement system to a sample with zero concentration of analyte. Method blanks also provide an indication of analyte contamination that may occur during sample preparation and analysis. Method blank responses can also be used to estimate the detection limit of the measurement system and, when plotted over time, can be used to monitor the random contamination resulting from the method.

A minimum of one method blank is prepared with each batch of 20 or fewer samples. If the analyte concentration of the method blank is less than the detection limit, no corrective action is necessary. If the analyte concentration of the method blank is greater than or equal to the detection limit and the lowest concentration of the analytes in associated samples is at least ten times the blank concentration, the results of the both the blanks and the samples are reported. If the analyte concentration of the method blank is greater than or equal to the detection limit and the lowest concentration of the analyte in the associated samples is less than 10 times the blank concentration, the source of the contamination is determined and eliminated. Affected samples should be redigested and reanalysed. If insufficient sample is available for redigestion, the results of the blank must be reported with the sample results and the data should be qualified.

5.3.8.2 Laboratory Duplicate (LD)

A laboratory duplicate is a second aliquot of a sample, processed concurrently and identically with the original sample. Analysis of laboratory duplicate samples provides information for the determination of analytical precision for a given sample matrix. In addition, replicate analyses are useful in assessing sample homogeneity. If analytes are present in concentrations that are lower than the quantification limit, results for matrix spike duplicates and replicate check standards may be used to estimate analytical precision. One set of laboratory duplicates should be analysed for

each batch of 20 or fewer samples of the similar matrix. Relative percent difference (RPD), a commonly used means of estimating precision between duplicate analyses, is calculated using the formula:

$$RPD = 100\frac{|x_1 - x_2|}{(x_1 + x_2)/2}$$

The recommended control limit for duplicates is ≤20% RPD if sample concentrations are greater than or equal to the quantification limit. If one sample is above the quantification limit and the other is below, the results are reported and no corrective action is taken. If both samples are less that the quantification limit, the RPD is not calculated from the laboratory duplicate results. If duplicate RPDs do not fall within control limits, the analyst should take into consideration the following: project data quality objectives, regulatory limit for the analyte, the RPD for other analytes, matrix spike and spiked blank recoveries and visual appearance of the sample (sample homogeneity). Appropriate corrective action may involve redigesting and reanalysing the sample if analytical problems are suspected. If sample homogeneity problems are suspected, the project manager should be consulted and the data may be qualified, depending upon specific project requirements as documented in the project planning document.

5.3.8.3 Matrix Spike (MS)

A matrix spike is an aliquot of sample spiked with a known concentration of analyte(s). Spiking occurs prior to sample preparation and analysis. The mean of a significant number of matrix spike results can be used to estimate bias due to matrix interference. One matrix spike should be analysed for each batch of 20 or fewer samples of similar matrix. The spike solution is added to samples prior to digestion. The sample that is chosen for spiking should be the same sample used for laboratory duplicate analysis. A spike blank may be prepared concurrently to check spiking procedure and to provide reference for the matrix spike. The amount of spike added to the sample should be 2 to 5 times the expected sample concentration. Matrix spike recovery is calculated using the formula:

$$\%\,\mathrm{Recov} = \frac{matrix.spike.results - unspiked.sample.results}{calculated.spike.amount} X 100$$

Control limits for spike recovery are usually 75–125%. If the matrix spike recovery falls outside the control limits, the ratio of background concentration to calculated spike amount should be evaluated. If the sample concentration exceeds the spike concentration by a factor of 4, no corrective action is taken and the result is reported. If the factor is less than 4, corrective action is taken. The analyst should take into consideration the following: project data quality objectives, regulatory limit for the

analyte, matrix or physical interferences, the duplicate RPD, matrix spike recoveries for the other analytes, spiked blank recoveries, matrix spike duplicate recoveries, known method limitations (e.g., antimony, silver) and visual appearance of the sample (sample homogeneity). A post-digestion spike should be performed to provide additional information for troubleshooting analytical problems. Appropriate corrective action may involve redigesting and reanalysing the associated samples if analytical problems are suspected. Otherwise, the project manager should be consulted and the data may be qualified, depending upon specific project requirements as documented in the project planning document.

5.3.8.4 Matrix spike duplicate (MSD)

A matrix spike duplicate is an aliquot of sample (same sample as matrix spike) spiked with identical concentrations of analytes as the matrix spike. Results for matrix spike duplicates may be used to estimate analytical precision and may be requested by a project manager when the anticipated analyte concentrations in the samples are too low to be useful for estimating analytical precision. Calculations, control limits and corrective actions for matrix spike duplicates are consistent with those described under the sections Laboratory Duplicate and Matrix Spike, above.

5.3.8.5 Spiked method blank (SB)

A spiked method blank is an aliquot of reagent water spiked at the same time and at the same concentrations as the matrix spike. It is used to check the spiking procedure. It is also useful in evaluating matrix spike results and overall method performance independent of matrix effects. Control limits are 85–115% recovery. Since the spiked method blank does not contain matrix interferences, recoveries should always be within control limits for a proven method. Corrective action for spiked method blanks that are out of control should be to investigate the cause of the problem, correct it and, if necessary, redigest and reanalyse associated samples.

5.3.8.6 Laboratory control sample (LCS)

A laboratory control sample is a known matrix, usually reagent water, which is spiked with analytes and processed through the entire analytical procedure. It is used to document method performance. Replicate LCS results may be used to estimate precision and the difference between the mean of those results and the true value provides an indication of the magnitude of bias due to method error. Analysis of a laboratory control sample is optional but is usually run once per analytical batch. Laboratories that routinely analyse LCSs may develop intralaboratory control limits for each analyte. Control limits should not exceed 80–120% of true value for a proven method. Laboratory control samples are often commercially prepared and control limits may vary, depending upon the supplier.

5.3.8.7 Control limits

Recommended control limits for analytical QC samples are as described above. When appropriate, different control limits may be specified in project planning documents. Project-specific control limits must be developed in consultation with the laboratory. For example, a program may require laboratory results for an analyte that is not routinely measured and best available technology for that analyte may not be well demonstrated or documented.

5.3.9 Corrective actions

The analyst is responsible for monitoring the analysis and troubleshooting problems as they occur. It is important to identify potential analytical problems as soon as possible so that corrective actions can be taken prior to the expiration of holding times. It is the responsibility of the laboratory to communicate analytical problems to the project manager during the analysis so that the project manager can have input into the course of corrective action. This communication is important when the laboratory is experiencing difficulty in meeting any project specific requirements, including detection limits. When reasonable corrective actions do not bring QC sample results into control, resulting data may need to be qualified, depending upon specific project requirements as documented in the project planning document. It is important for the laboratory and the project manager to agree on what constitutes reasonable corrective actions, acceptable data and the appropriate circumstances for data qualification.

5.3.10 Establishing and objectively assessing laboratory performance

5.3.10.1 Applications of reference materials

Standard or Certified Reference Materials are useful for establishing internally the performance of a laboratory in the analysis of trace metals in any specific type of sample materials.

A reference material is a material containing known quantities of analytes in a homogeneous matrix. An aliquot of the material is processed through the entire analytical procedure and used to document bias of the analytical method. When analysed in duplicate, a reference material can also provide both precision and bias information for a particular matrix type.

A certified reference material (CRM) is a material that has one or more property values certified by a technically valid procedure, documented by a certifying body (e.g., National Research Council of Canada (NRCC); National Institute for Standards and Technology (NIST)). A standard reference material (SRM) is a CRM issued by the NIST.

In general, one certified or standard reference material sample is analysed for every batch of 20 or fewer samples of a similar matrix. For mercury, one reference material sample per water bath is sufficient, even if the water bath holds more than 20 samples. A reference material as close as possible to the samples in matrix type and concentration should be used. When evaluating analytical results of the reference material, it is helpful to know the analytical method used to determine the reference values for the analytes. A laboratory can determine intralaboratory control limits for such elements based upon a minimum of seven replicate digestions and analyses.

The following CRMs are recommended for the ASEAN Marine Water Quality Monitoring Program for trace metals:

1. Estuarine water reference material, **SLEW-3;**
2. Nearshore (coastal) seawater reference material, **CASS-4**; and/or
3. Seawater reference material, **NASS-5**.

The above CRMs are available from the National Research Council Canada. Additional information may be obtained from:

National Research Council of Canada, Institute for
National Measurement Standards
M-12, Montreal Road, Ottawa, Ontario, Canada K1A 0R6
Telephone (613) 993-2359 Facsimile (613) 993-2451
Email crm.inms@nrc.ca

For mercury in seawater, CRM 579 (mercury in coastal seawater) may be obtained from the European Commission Institute for Reference Materials and Measurements (IRMM). Additional information may be obtained at: http://www.irmm.jrc.be/html/reference_materials_catalogue/catalogue/index.htm

In the case of Cr(VI) and TBT, no CRMs are currently available for seawater matrix. The assessment of spiked recoveries of seawater samples is required as a quality control of the analytical methodologies adopted.

Control limits for reference materials are often project-specific. It is recommended that laboratories develop intralaboratory control limits for each reference material routinely analysed and that corrective action be based upon these performance-based control limits. In addition, the analyst should take into consideration the project data quality objectives, regulatory limit for the analyte, matrix or physical interferences, duplicate RPDs, matrix spike recoveries, spiked blank recoveries, matrix spike duplicate recoveries and known method limitations when developing corrective actions. When the results for reference materials fall outside the project-specific control limits, the project manager should be consulted and data may be qualified, depending upon specific project requirements as documented in the project planning document.

5.3.11 Participation in inter-comparison or laboratory performance tests

An objective and reliable means for the assessment of the performance of a laboratory in conducting the test using a given method is through the participation in Inter-Comparison Tests schemes or in Laboratory Performance Studies. One of such schemes recommended for consideration by ASEAN laboratories is:

- The **QUASIMEME Laboratory Performance Studies (LPS)**

  QUASIMEME stands for 'Quality Assurance of Information for Marine Environmental Monitoring in Europe'. The current QUASIMEME Project Office is at:

  Wageningen UR, Alterra CWK
  P.O. Box 47, 6700 AA Wageningen, The Netherlands
  Phone: +31 (0)317 486546 Fax: +31 (0)317 419000
  E-mail: quasimeme@wur.nl

ASEAN laboratories participated as a group in the QUASIMEME Laboratory Performance Studies, Round 37 (1 April–30 October 2004) which included AQ-3 for Trace Metals in Seawater; andAQ-4 for Mercury in Seawater. Further details are available in Appendices.

5.4 Chlorophyll

5.4.1 Introduction

Plant pigment concentrations in natural waters provide a semi-quantitative index of phytoplankton biomass. From a practical perspective, the pigment most useful for estimating total phytoplankton biomass is chlorophyll *a*. Concurrent concentrations of chlorophyll *b* and *c* are usually much smaller and vary in response to community floristic composition. All chlorophyll-containing materials are fluorescent. When the organisms are microscopic, such as phytoplankton, this fluorescence may be measured directly in bulk water solutions or extracts of filtered materials. In the method outlined below, the concentration of chlorophyll *a* is estimated using a sensitive photomultiplier for detection of long wavelength light (red) fluoresced from pigment extracts irradiated with short wavelengths (blue) (Yentsch and Menzel 1963).

Direct estimations of chlorophyll *a* concentration from fluorescence can be misleading due to interferences caused by the fluorescence of chlorophyll decomposition products (e.g., phaeophytin). In some circumstances, chlorophyll degradation products can form a significant fraction of the total plant pigment in a seawater sample (Parsons et

al. 1984). The concentration of chlorophyll degradation products can be determined by acidification of the original sample and measurement of the decrease in fluorescence.

***Note**. Other water constituents can also fluoresce which may result in incorrect readings.*

5.4.2 Synopsis of technique

Following collection and filtration at sea the chlorophyll samples filtered though GF/F filter papers are individually wrapped in aluminium foil and stored frozen. Filter papers are ground in 90% acetone(V/V) and centrifuged to extract the chlorophyll pigments. The fluorescence emitted from the chlorophyll is measured directly using a fluorometer. The analogue output is recorded in millivolts (mV) using a digital voltmeter. Phaeophytin readings are measured by taking fluorescence reading before and after acidification of the sample with 6N hydrochloric acid (HCl).

5.4.3 Equipment

- Vacuum pump
- Water trap
- Filter manifold
- High speed tissue grinder
- GF/F Filters (0.45µm)
- Centrifuge tubes
- 90% acetone
- Centrifuge
- 6N HCl

5.4.4 Method

1. Remove filter papers from long-term freezer storage and place them in a lab freezer close to the work bench. Work with one sample at a time.
2. Work in a darkened room to minimise photo-degradation of the pigments. Carry out extraction of the pigment in a well-ventilated fume cupboard. *Working within the fume cupboard will reduce the risk of contamination from outside sources and minimise inhalation of / contact with the acetone.*
3. Record the sample identification number of the wrapped filter paper onto the data sheet.
4. Unwrap the frozen filter paper from the foil, and place it in a glass grinding tube, avoiding hand contact with the paper. Add 4–5 mL of 90% acetone. Homogenise

the filter for 30–60 seconds by grinding the filter paper with the high speed tissue grinder. This is sufficient time for extraction of the chlorophyll pigments (Yentsch and Menzel 1963). Prolonged grinding can cause excessive heat to be generated which can accelerate degradation of the chlorophyll pigments. If no grinder is available, overnight incubation of the filter paper in 10 mL of 90% acetone is sufficient to extract the chlorophyll pigments.

5. Carefully pour the homogenised filter and raw extract into a 12 mL polypropylene screw-cap centrifuge tube designated for use in chlorophyll *a* determination. Rinse the glass grinding tube twice with small amounts of 90% acetone from the squeeze bottle. Add each rinse to the centrifuge tube. Make up the volume of the extract to 10 mL, using graduations on the side of the centrifuge tube. Shake the tube to ensure the extract is well mixed.
6. Place the centrifuge tube in the dark for 30 minutes. This ensures complete extraction of the pigment and allows the sample to come to room temperature.
7. Repeat steps 3–6 until all samples have been extracted and placed in centrifuge tubes. Place the tubes in the centrifuge in the same order as blanks and sample identification numbers have been recorded on the data sheets.
8. Centrifuge the tubes before reading the fluorescence.
9. After centrifuging, pour the contents of each tube into a 10 mL fluorometer quartz cuvette *(available for use with the fluorometer)*. Due care should be taken to avoid resuspension of the centrifuged pellet when it is being transferred into the quartz cuvette.
10. Wipe the cuvette with a tissue to remove any fingerprints or solvent on the outside. Place the cuvette into the fluorometer. Cover with the cap provided and wait 30 seconds for reading to stabilise.
11. Record the range scale on the data sheet. Record the stabilised mV reading under the appropriate column on the data sheet.
12. Remove the cap from the fluorometer and take out the cuvette. Add 2 drops of 6N HCl and carefully invert to ensure adequate mixing of the acid within the cuvette. Rewipe the cuvette with a clean tissue, replace it in the fluorometer and cover it with the cap provided.
13. Wait until the reading has stabilised, then record the mV reading under the appropriate column on the data sheet.
14. Repeat steps 9–13 until all centrifuged samples have been analysed for chlorophyll and phaeophytin fluorescence levels.
15. Conversion of the fluorometer readings into chlorophyll *a* and phaeophytin

levels and integration of the blank data is achieved using a spreadsheet. Values of the fluorescence levels with the specific settings and sample identification numbers are entered directly into the spreadsheet. The spreadsheet converts the digital readings into chlorophyll *a* and phaeophytin readings using the blank value and the difference before and after acidification of the sample.

5.4.5 Quality control

- Analyse a blank filter paper at the start of every eight single samples or every four duplicate samples.
- Treat a clean microfibre filter paper according to the method outlined above.
- Glass and labware to be used for chlorophyll analyses should be kept aside and never used with acids.
- Keep all acids (except the 6N HCL) out of the fume cupboard used for pigment analysis.

The fluorometer is spectrophotometrically standardised against extracts of pigments from exponentially growing cultures of the diatom *Chaetoceros simplex*.

5.5 Suspended Solids

5.5.1 Introduction

Analysis of suspended solids estimates the total amount of particulate matter in a water sample. An increase in the amount of suspended sediment, phytoplankton cells or other solids within the water column can lead to a reduction in penetration of light into ocean waters, which can be detrimental to biota whose survival is dependent on sunlight. Sediment loading can be increased as a result of natural and human disturbances, including river input, storms, strong winds, trawling and dredging.

Extraction of the suspended material from a water sample is necessary step in this procedure to permit easy calculation of total suspended solids. One of the most widely used and popular concentration methods involves filtering the sample onto a pre-weighed filter paper.

5.5.2 Synopsis

Particulate matter is extracted by filtration on a pre-weighed filter paper of nominal pore size. The weight difference between filter papers before and after filtration and drying is used to calculate the amount of suspended solid in the sample. Final suspended solid weight is calculated from the difference in filter paper weight and the volume of water filtered.

5.5.3 Equipment

- Mettler AE 163 analytical balance or equivalent
- Millepore polycarbonate membrane filters, (0.4 µm pore diameter 47 mm filter diameter.)
- Forceps
- Glass vials with screw-top lids
- Oven (temperature set at 60^{o}C)
- Suspended solids data sheets

5.5.4 Method

Preweighing of filter papers (prior to field trip)

1. Turn on the balance 15 minutes before weighing of filter papers, by depressing the bar.
2. Place the date of the analysis on the data sheet.
3. Set balance to zero by depressing the re-zero bar.
4. Separate the polycarbonate membrane filter papers from the surrounding blue protective paper using the forceps. *Do not touch the filter paper with fingers at any stage during the analysis*. Place the filter paper gently on the balance tray.
5. Weigh the filter paper. Record the result on the data sheet under 'Initial Weight'. Place filter paper in a pre-labelled scintillation vial and record this number on the data sheet under the 'Vial number' that corresponds to the Initial Weight value.
6. Weigh a filter paper as a blank after every 14 samples and record the result under 'BLANK'. Place the blank filter paper into the corresponding vial. Repeat with a new filter paper after each 14 samples.
7. Store vials in a box in preparation for field sampling.

Weighing of used filter papers (after field trip)

1. Release the vial cap slightly, place the vials in a clean 60°C oven and leave to dry for 48 hours.
2. After drying, take the vials out of the oven and tighten the lids to seal the vials. Allow the sealed vials to cool to room temperature.
3. Carefully remove the dried filter paper from each vial using forceps and place it on the balance tray. *It is essential that the filter paper is in a horizontal position during this transfer. Particulate matter is not stable on the membrane surface and*

can be dislodged. Weigh the sample. Record the weight on the data sheet under 'Final Weight' next to the corresponding Initial Weight. Record the sample identification number labelled on the scintillation vial in the 'Sample identification number' column.

4. Reweigh the blanks and place the value under 'BLANK final weight'. *The recording of blanks before and after a field trip will account for balance drift and possible contamination of filter papers.*
5. Enter the suspended solids data into a text file.

It is now possible to buy pre-weighed filter papers commercially and these can be used to filter for suspended solids without the need for pre-weighing of the filter papers.

5.6 Bacteria

5.6.1 Introduction

AMWQC for bacteria are set for protection of human health (not for protection of aquatic life) and are expressed as numbers of the indicator organism faecal coliforms per 100 mL for shellfish growing waters and as a combination of faecal coliforms and enterococci criteria for recreational waters.

The following text is adapted from UNEP (1983 Revised 1995). Further details can be found at: http://www.wiolab.org/toolboxes/waterandsedimentquality/

5.6.2 Determination of total coliforms in seawater by membane filtration

5.6.2.1 Introduction

This method is suitable for the determination of total coliforms in coastal bathing waters of temperate and tropical seas. It uses a membrane filter (MF) procedure which allows concentration of the bacteria prior to incubation. It can be employed in alternation with the multiple tube fermentation (MPN) test. In general the MF method is less labour-intensive and, due to the pre-concentration of the bacteria in the sample, it is more suitable in situations where low numbers of coliforms are to be estimated. The MPN test should be given preference when the test sample contains high amounts of particulate matter which will hinder the reading of the MFs after incubation.

Coliform bacteria detectable by this test include *E. coli* Type I which are of faecal origin and irregular types II and VI, which may be not of faecal origin.

5.6.2.2 Synopsis

From seawater samples taken under sterile conditions, a dilution series is set up according to the number of total coliforms expected in the water sample. Aliquots of this dilution series are filtered through 0.45µm pore-size membrane. The membrane filters are placed on the surface of M-endo-agar-MF contained in petri dishes and incubated at 36±1°C for 24 hours. The coliform colonies will appear as pink to dark red spots with a metallic (golden) sheen, which may vary in size from pinhead to complete colony coverage. Residual chlorine, if present, is neutralised by adding thiosulphate to the sampling bottle before sterilisation. Suspect and doubtful colonies can be tested for acid and gas development with a confirmative test such as the MacConkey broth test or the brilliant green broth test.

5.6.2.3 Method

Dilution series

Setup of dilution series can be seen in Figure 5-2. Samples and dilutions must be vigorously shaken before taking aliquots in order to guarantee representative aliquots. Prepare the dilution series with a sterilised pipette after vigorously shaking the sample. Transfer 1 mL of the sample into a culture tube containing 9 mL of phosphate buffer to make the first dilution (D-1). Continue the preparation of the dilution series by taking 1 mL from the first dilution (D-1) and mixing it into a new culture tube containing 9 mL of the phosphate buffer in order to obtain the second dilution (D-2) and so on, up to the required number of dilutions.

Filtration

Begin filtration with the greatest dilution in order to avoid contamination from samples containing bacteria in higher concentrations. Use a sterilised filtration funnel for each dilution series. Place the sterilised membrane filter (MF) with flamed sterilised forceps over the porous plate of the filtration apparatus. Carefully place the matching funnel unit over the receptacle and lock it into place. Add into the funnel about 20 mL of buffer solution. With a sterilised pipette add 1 mL of the dilution in to the buffer solution in the funnel. Filter with a partial vacuum. Wash the funnel with approximately 20 mL of buffer solution. Filter with a partial vacuum. Wash the funnel walls two more times with 20 mL of buffer solution and filter again. Unlock and remove the funnel, immediately remove the MF with flamed sterilised forceps and place the MF on the agar surface of the medium contained in the petri dish with a rolling motion to avoid the entrapment of air. Before filtering the next dilution in the same manner pass 20 mL of buffer solution through the assembled filtration unit.

Incubation

The petri dishes containing the MF on agar are sealed and incubated immediately for 24 hours at 36°C. As a sterility check, incubate also one blank without MF (i.e., a petri dish containing agar only).

Interpretation

Count with a stereomicroscope (or similar) only colonies that appear as pink to dark red spots with a metallic (golden) sheen, and which may vary in size from pinhead to complete colony coverage. If the number of dubious colonies is greater than 10% of the total number of colonies, test dubious colonies either by the MacConkey broth test or the brilliant green bile broth test.

Expression of results

Report the number of coliform colonies on individual MFs after the incubation is complete and adjust this count after confirmatory tests, if necessary, have been made. Use only MFs with a total number of colonies (i.e., coliforms plus non-coliforms) between 20 and 200. Retain only two significant digits of the counted number of coliform colonies per filter. Indicate the results obtained for each filter separately in a test report. Express the results in terms of total coliforms per 100 mL of sample, using the following equation:

Total coliform per 100 ml sample = number of coliform colonies/ml sample filters **x** 100

Indicate the results obtained for each dilution separately in the test report. Report also the results obtained on MFs with less than 20 coliform colonies per filter. Compute the number of total coliforms per 100 mL sample and report it as the final test result. If there are MFs containing between 20 and 200 characteristic colonies in two consecutive dilutions calculate the mean of the dilutions and report it as final test result.

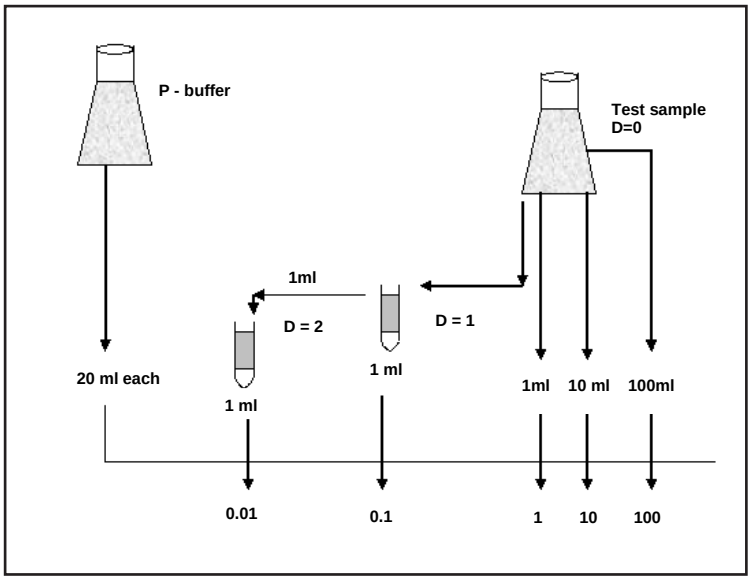


Figure 5-2. Example of dilution series for the incubation of total and faecal coliforms in seawater.

5.6.3 Determination of faecal coliforms in seawater by membrane filtration

5.6.3.1 Introduction

This method is suitable for the determination of faecal coliforms in coastal bathing waters of temperate and tropical seas. It uses a membrane filtration procedure which allows concentrations of the bacteria prior to incubation.

Faecal coliforms exhibit a highly specific positive correlation with faecal contamination from warm-blooded animals and therefore are good indicators for the sanitary quality of coastal waters. Since faecal coliforms die within hours when exposed to sunlight in seawaters at temperatures above 40°C, their presence in seawater indicates only recent contamination by faecal material. Die-away rate depends on salinity, temperature, solar radiation etc and these must be taken into consideration when interpreting results.

5.6.3.2 Synopsis

From seawater samples taken under sterile conditions, a dilution series is set up accordingly to the number of faecal coliforms expected in the water sample. Aliquots of this dilution series are filtered through 0.45µm pore-size membrane filters. The membrane filters are placed on the surface of m-FC agar contained in petri dishes and incubated at 44.5+0.20°C for 24 hours. Lactose fermentation will cause colonies of faecal coliforms to exhibit a characteristic blue colour. Residual chlorine, if present, is neutralised by adding thiosulphate to the sampling bottle before sterilisation.

Suspect and doubtful colonies can be tested for acid and gas development with confirmative test using the MacConkey broth test or the brilliant green bile broth test.

5.6.3.3 Method

Dilution series

See 5.6.2.3.

Filtration procedure

See 5.6.2.3.

Incubation

The petri dishes containing the MFs on agar are sealed and immediately placed horizontally inside clean metal boxes. These metal boxes are then placed in a water bath and incubated immediately for 24 hours at 44.5+/-0.20°C As a sterility check also incubate one blank, (without MF).

Interpretation

Count with a stereomicroscope (or similar) only colonies which appear as blue coloured. If the number of dubious colonies is greater than 10% of the total number of colonies, test dubious colonies by the MacConkey broth test or the brilliant green bile broth test. The colonies produced by faecal coliform bacteria are blue in colour. The non-faecal coliform colonies are grey to cream coloured. Background colours on the membrane filter will vary from a yellowish cream to a faint blue.

Expression of results

Report the number of faecal coliform colonies on individual MFs after the incubation has been completed and adjust this count after the confirmatory testS, if necessary, have been made. Use only MFs with a total number of colonies between 20 and 200. Retain only two significant digits of the counted number of faecal coliform colonies per filter. Indicate the results obtained for each filter separately in a test report. Express the results in terms of total coliforms per 100 ml of sample, using the

Total faecal coliform per 100 ml sample=number of faecal coliform colonies/ml sample filters x 100

Indicate the results obtained for each dilution separately in the test report. Report also the results obtained on MFs with less than 20 faecal coliform colonies per filter. Compute the number of total faecal coliforms per 100 mL sample and report it as the final test result. If there are MFs containing between 20 and 200 characteristic colonies in two consecutive dilutions calculate the mean of the dilutions and report it as final test result.

5.7 Oil and Grease and Phenols

5.7.1 Oil and Grease

5.7.1.1 Introduction

Oil and Grease (OG) is a term encompassing a variety of chemical compounds including fatty material of biogenic origin (vegetable oils, animal fats) and petroleum hydrocarbon oils. As the individual components in this 'mixture' will vary from sample to sample and from place to place the parameter thus falls into the group of parameters known as 'method-defined' (see Section 5.1.2 for a description of this term).

5.8.1.2 Standard methods

The publication *Standard Methods for the Examination of Water and Wastewater* (SMWW 2000) lists three possible methods for Oil and Grease (OG). These are:

(a) a partition-gravimetric method (5520B) which involves extraction into n-hexane, removal of the n-hexane by evaporation and weighing the residue. This is a conceptually and practically simple method but with poor sensitivity and thus detection limits of the order of 5–10 mg L^{-1}.

(b) a partition-infrared (IR) method (5520C) which involves extraction into Freon 113 (1,1,2-trichloro-1,2,2-trifluoroethane) and measurement of IR absorption at a number of wavelengths characteristic of C-H bonds in aliphatic and aromatic substances. This is a more sensitive method with detection limits near 1 mg L^{-1} but requires an IR spectrophotometer. Unfortunately, Freon 113 is no longer recommended for use due to efforts to reduce the production of ozone-depleting substances. Freon 113 may be replaced with other solvents which do not contain C-H bonds such as tetrachloroethylene but a complete standardisation of the method is then required (Farmaki et al. 2007).

(c) a Soxhlet extraction method (5520D) is recommended when relatively polar, heavy petroleum fractions are present, or when the levels of nonvolatile greases are high and a single solvent extraction is not sufficient to extract all of the materials. It is not normally necessary for natural waters which are 'lightly' polluted but is more suitable for heavily polluted waters, industrial wastewaters, sludges and sediments.

As the AMWQC for Oil and Grease is 0.14 mg L^{-1}, none of these methods satisfies the levels set by the criterion. More sensitive methods for specific analytes such as petroleum hydrocarbons may be necessary to measure 'Oil' at these levels. Tong et al. (1999) discussed methods for separating the biogenic hydrocarbons from petroleum hydrocarbons followed by fluorescence detection of the petroleum hydrocarbon fraction. Detection limits using fluorescence are satisfactory—in the order of 0.1 mg L^{-1}. However, should this is not a method for 'Oil and Grease' but only the petroleum fraction.

5.8.1.3 Recommended method

The method described in Farmaki et al. (2007) meets the sensitivity requirements of the AMWQC (0.14 mg L^{-1}), uses commonly available instrumentation (IR spectrophotometer) and also uses safe and acceptable solvents. For full details see Farmaki et al. 2007 (and references to SMWW and ASTM therein). In summary the method is as follows:

1. Acidify the sample with 5 mL of 1:1 sulphuric acid per litre.
2. Extract a 1 L sample with 3**x**30 mL volumes of tetrachloroethylene.

3. Filter the combined extracts through 10 g sodium sulphate.
4. Measure the absorbance at the maximum near 2930 cm^{-1} using 50 mm cells.
5. Calibrate with standards made from equal volumes of isooctane and n-hexadecane.

5.8.2 Phenol

5.8.2.1 Introduction

Phenol is the chemical C_6H_6O but the pure substance is only one of a large number of substituted phenolic compounds known collectively as phenols. Phenols, defined as hydroxy derivatives of benzene and its condensed nuclei, may occur in domestic and industrial wastewater, natural waters and potable water supplies and thus as pollutants of coastal waters. The AMWQC list a criterion for phenol itself (0.12 mg L^{-1}) but standards may also be set for 'total phenols' or 'phenolic substances as phenols' thus including all phenols and it is thus important to distinguish between the analysis of 'phenol' versus 'total phenols'.

5.8.2.2 Methods

Total phenols are best analysed by the 4-aminoantipyrine colorimetric method (APHA 2006, Method 5530) but this method cannot distinguish phenol itself from other phenols. It is thus not directly suitable for analysis of the parameter as specified in the AMWQC. As not all phenols produce the same colour intensity in the colorimetric method, the method can best be described as producing an estimate of the minimum concentration of phenolic substances in the sample. A cleanup procedure is given in APHA Method 5530B. Phenols are distilled from nonvolatile impurities. Because the volatilisation of phenols is gradual, the distillate volume must ultimately equal that of the original sample. The analytical procedures offered use the 4-aminoantipyrine colorimetric method that determines phenol, ortho- and meta-substituted phenols and, under proper pH conditions, those para-substituted phenols in which the substitution is a carboxyl, halogen, methoxyl or sulfonic acid group. Method 5530C describes chloroform extraction, while 5530D describes the direct photometric method.

Phenol itself is best determined by gas-liquid chromatography (APHA 2006). This method is intended for the determination of individual phenolic compounds. For specific compounds covered, see each method. Method 6420B is a gas chromatographic (GC) method using liquid-liquid extraction and either flame ionisation detection (FID) or derivatisation and electron capture detection to determine a wide variety of phenols at relatively low concentrations. In addition, Method 6420C, a liquid-liquid extraction gas chromatographic/mass spectrometric (GC/MS) method, can be used to determine the phenols at slightly higher concentrations.

5.9 Cyanide

5.9.1 Introduction

Cyanide in water can be measured in three different forms: free cyanide, total cyanide and cyanide amenable to chlorination. Free cyanide is a measure of the cyanide present as HCN or CN, and total cyanide is a measure of all cyanides, including iron cyanide complexes. Methods for determining cyanide amenable to chlorination measure simple metal cyanides and the more complex cyanides with the exception of iron cyanides. The ASEAN criterion specifies 'free cyanide' only and the criterion is 7 µg L^{-1}.

Cyanide can be analysed using volumetric titration or colorimetry, with detection limits of 1 mg L^{-1} and 20 µg L^{-1}, respectively. Other methods include absorption spectrophotometry, ion-selective electrodes, indirect atomic absorption spectrophotometry, fluorometry and gas chromatography, with detection limits of 0.5 µg $^{-1}$L, 25 µg L^{-1}, 60 µg L^{-1} (iron complex)/30 µg L^{-1} (silver cyanide), 1 µg L^{-1} and 0.2 µg L^{-1} respectively.

5.9.2 Method

This method is drawn from ASTM D7237-06 Standard Test Method for Aquatic Free Cyanide with Flow Injection Analysis (www.astm.org). The full method can be purchased from the above website.

This method is used to establish the concentration of aquatic 'free' cyanide in an aqueous wastewater or effluent. The test conditions of this method are used to measure free cyanide (HCN and CN^-) and cyanide bound in the metal-cyanide complexes that are easily dissociated into free cyanide ions at the pH of the aquatic environment ranging from pH 6 to pH 8. The extent of HCN formation is less dependent on temperature than the pH; however, the temperature can be regulated if deemed necessary to further simulate the actual aquatic environment.

The aquatic free cyanide measured by this procedure should be similar to actual levels of HCN in the original aquatic environment. This in turn may give a reliable index of toxicity to aquatic organisms.

This procedure is applicable over a range of approximately 2–500 µg L^{-1} (parts per billion) aquatic free cyanide. Sample dilution may increase cyanide recoveries depending on the cyanide speciation, so dilution is not recommended. Higher concentrations can be analysed by increasing the range of calibration standards or by using a lower injection volume.

This standard does not purport to address all of the safety concerns associated with its

use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and to determine the applicability of regulatory limitations prior to use.

5.10 Salinity

5.10.1 Introduction

Salinity in seawater samples is determined through the precise measurement of conductivity using a salinometer. The conductivity of individual samples is expressed as a ratio to the conductivity of a sample of standard seawater. The electrical conductivity measured by the salinometer is proportional to the salinity of the sample. Electrical conductivity values are transformed to a salinity value.

5.10.2 Equipment

- Portable laboratory salinometer
- Reference seawater (for calibration)
- Standardised seawater (sub-standard)
- Salinity data sheets

5.10.3 Procedure

1. Collect approximately 20 L of seawater to be used as analytical substandard.

   The sub-standard should have a nominal salinity close to that of the actual samples, so the seawater should be collected during a sampling trip.

2. Before analysis, store samples in a cold room (10°C) to prevent evaporation.

3. At least 24 hours before analysis of the sample, move the samples and the seawater standard to the analysis site to allow the salinity samples, a working sub-standard and the seawater standard to reach room temperature.

4. Switch on the salinity meter, by turning power switch to the ON position, **one hour** before commencement of analysis and allow it to stabilise.

5. Record the date of analysis and initials of the user on each new data sheet used in the analysis.

6. Draw portions of the unknown sample through the salinometer at least three times, ensuring all connecting hoses are well rinsed with the sample. After each rinse, turn off the flow to the cell and note the conductivity reading. If the reading is constant after three rinses, record the conductivity value on the data

sheet next to the corresponding sample identification number. Check the cell for air bubbles before taking the final reading. If air has entered the cell, empty the cell and refill it with the sample.

7. Rinse the salinometer and all connecting hoses several times with freshwater after completion of sample analysis. *Washing with freshwater will minimise corrosion.*
8. Enter the salinity data into a text file.

6 Data analysis and interpretation

6.1 Introduction

Correct statistical analysis of the data collected in a monitoring program is essential to fully utilise all available information and to provide adequate, confident direction in the outcomes of the program.

Studies may be designed for reconnaissance, to detect impacts, measure change through space or time, for operational decisions or for modelling and prediction. Statistically we are faced with determining the level (or numbers) of something (estimate), or with comparing two measurements to see if they are different (comparison). In both cases some knowledge of the precision of the mean is needed. Environmental variability can only be accounted for by replicating measurements at some part of the study design.

Before starting any monitoring program, decisions are needed on:

(a) Whether data is collected for estimates or comparisons

(b) The desired precision for estimates

(c) The specifications of direction of change for comparisons

(d) The probability of acceptance of a difference

(e) The probability that the test will detect a difference

These decisions will have been a consideration when designing the monitoring program, in the context of the system model.

6.2 Selection of indicators

The selection of valid indicators that can be measured reliably is an important component of any monitoring program. Is it appropriate to measure factors which drive change in the ecosystem (such as phosphorus in a study of eutrophication) or outputs such as chlorophyll? Or are both needed? If so, why? How will the two

data streams be used? If a correlation is being sought, how many data pairs are appropriate? Is there a time lag which could cloud any relationship? There is often a trade-off required between the exactitude of some measure and its cost and difficulty of measuring. In selecting appropriate indicators, some thought should be given to the resolution required of each indicator. For example, is the chlorophyll level needed to the nearest milligram, or is it satisfactory to have bands perhaps 5 mg wide? Is the full range of interest, or only if it is below some threshold?

When selecting indicators the following considerations may be helpful:

- **Relevance.** Does the indicator reflect directly on the issue of concern?
- **Validity.** Does the indicator respond to changes in the environment and have some explanatory power?
- **Trends.** Does the indicator have long term significance? The indicator must be able to detect changes that occur slowly but consistently, and detect trends over reasonable time periods.
- **Diagnostic value**. Does the indicator have short-term significance? Indicators must be able to detect changes in conditions that occur within any particular year or defined period.
- **Responsiveness.** Does the indicator detect changes early enough to enable a management response and will it reflect changes due the manipulation by management?
- **Quantitative.** The indicator should be measurable and allow the amount of change to be assessed quantitatively.
- **Reliable**. The indicator should be measurable in a reliable and cost effective way.
- **Appropriate.** Is the indicator appropriate for the time and spatial scales of this study?

6.3 Data analysis and interpretation

The recommended steps on the development of data analysis and interpretation procedures are shown in Figure 6-1.

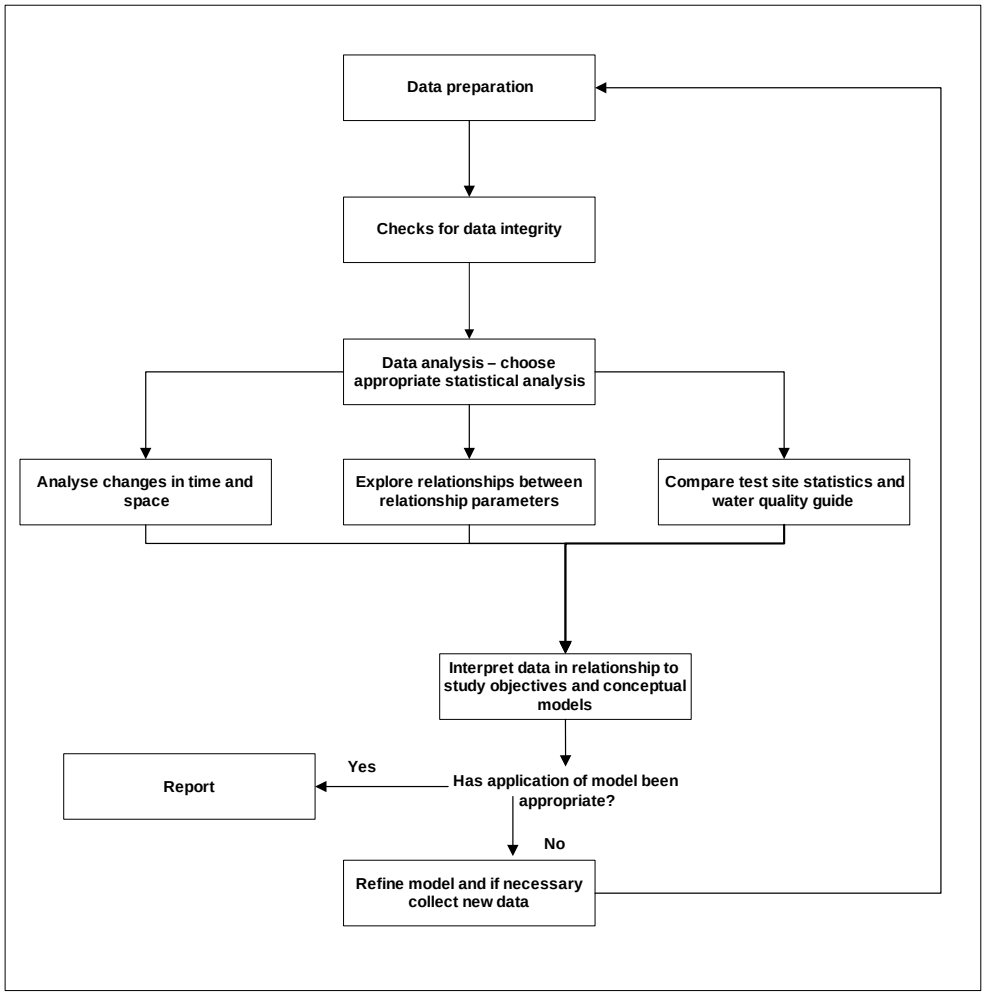


Figure 6-1. Framework for data analysis and interpretation.

6.4 Quality control

For the purpose of statistical design, the quality control measures include *precision*, *accuracy* and *limit of detection*. For definitions of these terms, see Section 4.2.5.

6.4.1 Sampling errors

The two types of sampling errors are *systematic (bias)* and *random* errors. For definitions of these terms see Section 4.2.4.

6.4.2 Precision and Bias

Precision is an indication of the agreement among the results of replicate measurements. To estimate precision, the results for the replicate samples must be at or above the detection limit. If they are not, precision can be checked by analysing replicates of check standards or matrix spikes. The best measure of precision is the relative standard deviation (RSD) or coefficient of variation (CV):

$$RSD = CV = 100S_x \rangle x$$

where $\boldsymbol{x}$ is the arithmetic mean of the x_i measurements and s_x is the standard deviation.

The relative percent difference (RPD) is used when only two samples are available:

$$RPD = 100\frac{|x_1 - x_2|}{(x_1 + x_2)/2}$$

The standard deviation can be calculated as follows:

$$S_x = \sqrt{\frac{1}{n-1}} \int \sum_{i=1}^{n} (x_1 -$$

where n is the number of measurements.

Bias is described as the deviation due to a systematic error (i.e., a consistent tendency for results to be either greater or smaller than the true value), such as calibration error, matrix interference, inability to measure all forms of the analyte, analyte contamination, etc. Deviation due to matrix effects is assessed by comparing a measured value to an accepted reference value in a sample of known concentration (such as a standard reference material) or by determining recovery of a known amount of analyte spiked into a sample (matrix spike). Bias due to matrix effects based on a matrix spike is indicated by:

Bias = (X_s - X_u) - K,

where X_s is the measured value for the spiked sample, X_u is the measured value for the unspiked sample and K is the known (calculated) spike amount.

The percent recovery (%R) for check standard or matrix spikes is given by:

$$\%R = 100(R_s / R_t)$$

where R_s is the result for the check standard or the difference between the results for the spiked and the unspiked samples and R_t is the known value for the check standard or the amount of the analyte added to the matrix spike.

6.5 Methods for analysis of data

6.5.1 Introduction

The statistical analysis of data is a huge subject, covered extensively by a wide range of standard texts in the literature (e.g., Emery and Thomson 2001; McBride 2005). In addition, many statistical software packages now include their own handbooks or supporting explanatory notes (e.g., MATLAB, NAG, SAS, SPSS, StatSoft and many others). Analysing marine water quality data often involves the analysis of time-series data, the manipulation of geographic information, or a combination of the two. Time-series analysis is well covered in the literature (e.g., Young 1999; McBride 2005). Spatial analysis is also well developed and with the rapid proliferation of GIS for environmental studies there is now a growing literature (e.g., Burrough and McDonnell 1998; Raper 2001).

This type of analysis seeks to identify underlying trends or cycles (in time or space), associations between different parameters or the probability distribution of the data (statistically or in time or space). In any such analysis, the recognition of uncertainty and use of error analysis is an essential component of helping to avoid spurious conclusions (Mayo 1996; Taylor 1997; Bevington and Robinson 2002).

A variety of data are generated during a marine water quality monitoring program. As noted in Section 3.4, concentrations of water quality parameters are influenced by many highly variable factors such as wind, tide, season and the magnitude and frequency of pollutant discharges from a catchment area. It is nearly impossible to assess in a statistical sense (i.e., with some level of error) interactions among all factors. The tools of statistical analysis are used to infer, with a predictable level of error, generalities about average conditions (or trends over time) and the variability from the limited information obtained from monitoring programs.

6.5.2 Data quality control and validation

Prior to conducting a statistical test, data should be screened to eliminate potentially biased or non-representative values. Biased and non-representative values may arise due to equipment malfunctions, field or laboratory protocol errors, weather problems,

human error and similar events. In addition, there are procedures for addressing data below laboratory detection values, and estimation of particulate fractions of metals. Data may be transferred to a normal distribution if parametric statistical tests are to be used, because they rely on normality of the data as one of their assumptions.

QA/QC Qualifiers: Based on the results of the QA/QC evaluation, laboratory data considered suspect due to the contamination of blanks, exceeded holding times or low surrogate recoveries should be qualified or rejected. Ideally, statistical tests will be performed only on data that have passed this screening process. Although it is possible to use data that have been qualified as estimated values, a higher level of uncertainty is associated with the test results. It is up to the data user to make an educated decision whether to include estimated values. Prior to conducting statistical tests, the data set should be examined to determine the percentage of points that are below the method detection limit MDL. If a large proportion of the data is below the MDL, statistical testing may not be appropriate.

6.5.3 Statistical methods

***Averaging of Duplicates*:** Data from duplicate samples (laboratory or field) should be averaged prior to statistical analysis. That is, the average value should be used in place of either of the two duplicate values.

***Treatment of Non-detects*:** Water quality data sets normally include some results below the limit of detection (LOD; non-detects) and separate data analysis techniques are required to accurately estimate sample statistics. When below-LOD data exist in a data set, they will affect statistical parameters computed from that set. For example, when below-LOD data are set to the detection limit (often cited as a conservative approach), it causes an overestimation of central tendency measures and an underestimation of dispersion measures, as opposed to what would have been obtained had the true values of the below-detection-limit data been known. A traditional practice has been simply to set the values equal to one half of the detection limit. When a significant percentage of a data set is at or below the detection limit, the treatment method can seriously affect analytical results and their interpretation.

***Distributional Tests*:** Many commonly used statistical tests (e.g., parametric Analysis of Variance) are based on the assumption that the data were sampled at random from a population with a normal distribution. Therefore, another attribute of the data that should be investigated is its apparent probability distribution. It is important to determine whether the probability distribution is normal or log-normal. Often the log-normal distribution provides the best fit to water quality data. If the data are not normally distributed, or if the data set contains a very high proportion of non-detects, a nonparametric statistical procedure should be utilised for testing trends. Non-parametric techniques examine the data based on rank rather than distribution.

6.5.4 Descriptive statistics

The purpose of calculating general descriptive statistics is to gain an overview of the data and to prepare for more formal statistical hypothesis testing. The data are displayed in a variety of ways and summary statistics are generated. These exploratory techniques can provide clues as to the presence of major treatment effects (e.g., station, year, land-use type) that can be tested for statistical significance. Boxplots and cumulative distribution curves can provide concise, but rich, visual summaries. Non-linear correlation can detect associations that might be missed with linear methods.

Box plots. First, calculate simple descriptive statistics, characterising the central tendency, variability and distribution of the data set. Central tendency is measured by the sample mean (if normal, the arithmetic average of the data), the median (the 50th percentile of the distribution) and the mode (the most probable value). The variability of the data set is represented by the sample standard deviation and by its squared value, the variance. For non-parametric tests, data variability is measured by the interquartile difference, the difference between the values of the 1st (25th percentile) and 3rd quartile (75th percentile) values. Any statistical software program and most hand calculators can be used to calculate these parameters. These statistics can be conveniently displayed as box and whisker plots as shown in Figure 6-2. The Box and Whisker plot is a graphical method of displaying the variability, spread, and distribution of the data set.

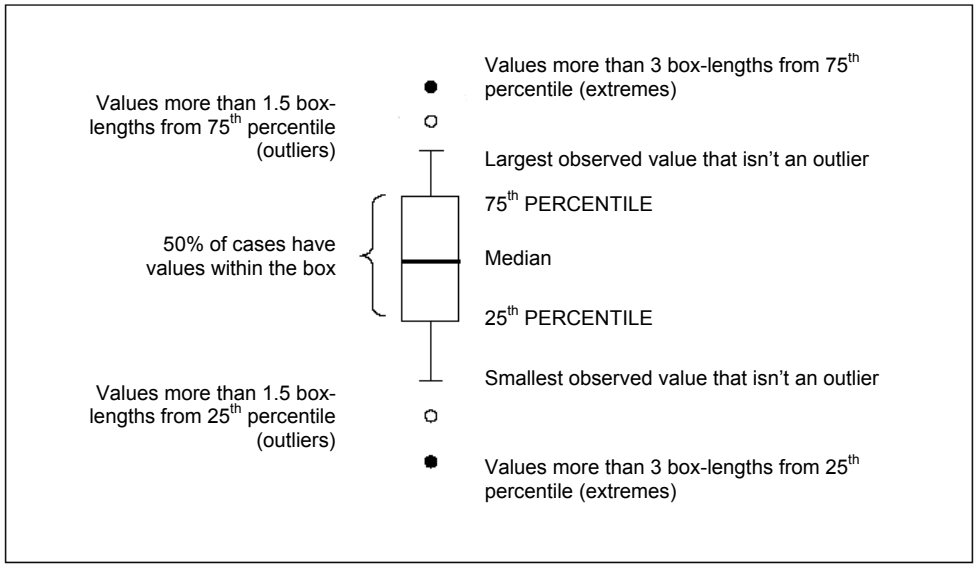


Figure 6-2. Features of a Box and Whisker plot.

6.5.5 Hypothesis testing

Hypothesis testing is performed using statistical procedures to measure the significance of a particular effect e.g., TSS concentration, station location. Statistical analysis is used to determine whether a particular mathematical model describes the pattern of variability in the data set better than a 'random' model. Two types of models are commonly used. Respectively, they state that:

1. There is a significant, mathematical relationship between a change in the magnitude of one variable to that of another variable (e.g., total suspended solids and wind speed). OR
2. There is a significant effect of a treatment on the magnitude of a variable (e.g., an effect of station location or monitoring year on total suspended solids concentrations).

These hypotheses are tested using the tools of Correlation Analysis and Analysis of Variance (ANOVA) respectively.

***Correlation Analysis*.** Correlation analysis considers the linear relationship between two variables. Correlation analysis can be used to identify parameters, which may explain or reduce some of the variability inherent in the process of statistical hypothesis testing, but doesn't necessarily imply a cause and effect relationship. Correlation is expressed on a scale from -1 to +1, with +1 representing perfect correlation; -1 representing perfect inverse correlation; and 0 representing no correlation.

***Analysis of Variance*:** ANOVA is a statistical technique used to assess the effects of different treatments on a particular water quality parameter and to determine whether the effects of different levels of each treatment are significantly different from each other. ANOVAs may be parametric (two-way) or non-parametric.

A two way ANOVA can be used to determine the relationship between effects of two treatments, e.g., station location and monitoring year, on the total concentration of a parameter of interest. The ANOVA model tests whether:

- Stations differ from each other across all monitoring years; and
- Monitoring years differ from each other across all stations.

In addition, by testing for interactions in the station and year combinations, the model tests whether monitoring year influences the parameter concentration at each station equally. In this approach, the null hypothesis states that there are no significant effects of station location or monitoring year on parameter concentrations in samples. The two-way ANOVA is used to determine whether the null hypothesis can be rejected, indicating that significant differences between treatment effects were observed. If the null hypothesis is rejected, additional analyses are conducted to identify which of the stations or monitoring years were significantly different from each other.

Nonparametric ANOVA: If the assumptions of a parametric ANOVA cannot be met or if the proportion of non-detects in the data set exceeds approximately 15%, a Kruskal Wallis non-parametric ANOVA can be used to examine hypotheses regarding significant differences, e.g., in constituent concentrations between stations and between years. The non-parametric ANOVA evaluates the ranks of the observed concentrations within each treatment. 'Non-detects' are treated as tied values and are assigned an average rank. If a significant difference between treatments is detected, a nonparametric multiple comparison procedure can be used to determine which treatments are heterogeneous. It should be noted that in general, nonparametric methods are less powerful than their parametric counterparts, reducing the likelihood that a (true) significant difference between treatments will be detected. Typical applications of statistical testing procedures to water quality quality data include determining whether any of the following are significant:

- Differences between stations;
- Differences between monitoring years; and
- Correlations between different water quality parameters.

The following steps are common to both procedures:

1. Formulate the hypothesis to be tested, called the null hypothesis (H_0).
2. Determine the test statistic.
3. Define the rejection criterion for the test statistic.
4. Determine whether the calculated value of the test statistic falls above or below the rejection criterion.

***Test Statistics*:** The sum of squares (of the deviations of the measurements from the mean) is used as a measure of the amount of variability in the data set that is explained by the statistical model. The total sum of squares can be decomposed into a portion due to variation among treatment groups ('sum of squares for treatments') and a portion due to variation within groups ('sum of squares for error'). The 'mean square for error' is calculated by dividing the sum of squares for an effect source (treatment, error or total) by the number of degrees of freedom for that effect. This 'normalises' the variability from one source for comparison with the variability from another. The 'F ratio' is then calculated as the ratio of the mean square for treatments to the unexplained variability mean square for error. If treatments have only a small effect on the variable of interest, then the portion of the total mean square due to variation within groups will be small relative to the portion between groups.

The probability that a given F ratio could be generated by chance alone using a random model (i.e., by chance alone) is measured by the parameter 'P>F.' 'F' is called the statistic of interest. A P value of '0.10>F,' for example, would mean that the observed F ratio could have been generated 10% of the time by chance alone. The

effect of treatments is said to be 'significant' if this probability is less than the chosen significance level, which is commonly set at 0.05.

***Significance Levels and Rejection Criterion*:** Statistical tests are not absolutely conclusive. There is always some degree of risk that one of two types of error will be committed:

- Rejection of a true hypothesis (Type I error); or
- Failure to reject a false hypothesis (Type II error).

If a calculated test statistic meets the rejection criterion, then reject the null hypothesis; otherwise, continue to assume that the null hypothesis is correct. The probability of committing a Type I error is denoted by the Greek symbol alpha (α), that of committing a Type II error by beta (β). α is also called the 'significance level of the test' (i.e., the probability of rejecting a true hypothesis). Common values for α are 0.10, 0.05, and 0.01. As the value of α decreases, the confidence in the test increases. However, at the same time, the probability of committing a Type II error (β) also increases. Therefore, setting α too low will result in too strict a test, which will reduce the chance of rejecting a true hypothesis, but fail to reject many false ones. Statistical tests of runoff data generally use a target α of 0.05 or a 95% level of confidence.

6.5.6 Comparison with water quality criteria

The analytical results for samples from water bodies being tested can be compared with water quality criteria for the protection of aquatic life. For most parameters, this will entail a simple comparison of the observed concentration and the corresponding criterion. If the initial statistical analysis indicates that the data set is adequate, statistical testing can be conducted to assess the probability that a water quality criterion will be exceeded at a given location. A minimum of seven samples is generally required to achieve a meaningful result.

6.5.7 Probability of misclassification

Any statistical analysis should also be able to calculate the probability of wrongly assigning a site. Estimating the probability of misclassification looks beyond the face value summary statistics to the whole spread of data characterising a water body or marine area. It considers the uncertainty surrounding the water body score and, in some cases, the uncertainty surrounding the final statistical analysis. For example, using class boundaries (the values which determine the upper and lower limits of a class) is, in effect, an administrative tool. Class boundaries divide a gradient of quality and may reflect expert consensus, but they will probably not relate to ecological expression.

6.5.7.1 Burden of proof

When placing a site in a class there are two types of errors that need to be considered and, where possible, controlled. (They cannot both be *avoided* unless error-free continuous monitoring is available.) These are:

1. Mistakenly placing a site in a class denoting poorer ecological quality than it is actually achieving. If this occurs there is a risk that resources will be spent improving a site that does not need to be improved.
2. Mistakenly placing a site in a class denoting better ecological quality than it is actually achieving. If this occurs there is a risk that ecological degradation will proceed, and a poor quality site may not be improved.

Deciding on which of these errors dominates depends upon the where the burden of proof should lie. Several approaches can be used to estimate the probability of wrongly assigning a site. They vary in how they account for the uncertainty surrounding class boundaries. Approaches include:

- Confidence of compliance
- Bayesian estimation
- Randomised methods, e.g. bootstrapping.

Confidence of compliance only considers the uncertainty relating to the water body measurements. The class boundaries themselves are considered to be set without error. The approach keeps the setting of class boundaries completely independent from estimating the probability of misclassifying any single water body.

The Bayesian approach considers both the uncertainty surrounding the water body measurements and the uncertainty surrounding the class boundaries.

6.5.7.2 Confidence of Compliance

The essence of the confidence of compliance approach is to calculate a confidence interval around the statistic in question, and to 'tune' this by varying the confidence coefficient until the lower confidence limit just touch the lower boundary of the face-value class. The confidence interval is similarly tuned until its upper limit coincides with the upper boundary of the face-value class. From these two intervals the confidence of compliance can be calculated. Thus, the process addresses the natural variability and sampling error associated with data from a water body, but it assumes that the class boundary is a fixed standard with no associated uncertainty.

The approach is illustrated in Figure 6-3. In this example the water body ecological quality ratio (EQR) score was 87, the 'moderate-good' class boundary was set at 80 and the 'good-high' class boundary was set at 90. EQR is described as the ratio representing the relationship between the values of the biological parameters observed

for a given body of surface water and values for these parameters in the reference conditions applicable to that body. The ratio shall be represented as a numerical value between zero and one, with high ecological status represented by values close to one and bad ecological status by values close to zero.

At face value the water body has been classified as 'good'. On closer examination of the situation, it is apparent that:

1. The 75% confidence interval (running from 84 to 90) just touches the *upper* class limit. As the interval is calculated symmetrically, there is 12.5% confidence that the true EQR is actually in the 'high' class (and also 12.5% confidence that it is worse than 84).
2. The 94% confidence interval (running from 80 to 94) just touches the lower class limit. Thus there is 3% confidence that the true EQR is actually in the 'moderate' class (and also 3% confidence that it is better than 94).

From these two statements, the confidence is 100 – 12.5 – 3 = **84.5%** that the true EQR is in the face-value class. And, as already noted, there is 3% confidence that the water body should actually have been classified as 'high', and 12.5% confidence that it should have been classified as 'moderate'.

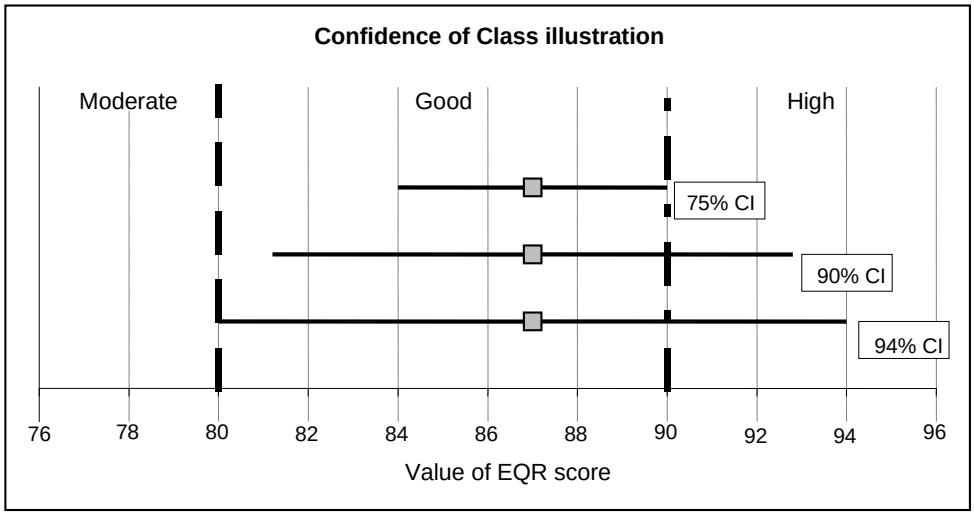


Figure 6-3. Estimating confidence of compliance by comparing water body statistical output with class boundaries.

6.5.7.3 Bayesian estimation

Bayesian statistics provide a conceptually simple process for updating uncertainty in the light of evidence. Bayesian methodology has been used for hundreds of years, but until recently the heavy computational burden often required for its application

has restricted its use. Now, however, many simple applications can be handled with readily available software such as Excel, while more advanced exercises can be analysed by the flexible and authoritative BUGS software package.

The essence of the Bayesian approach is best explained with an example. Suppose some monitoring data is provided for a new site. The site needs to be classified, on the basis of its observed mean quality, into the 'high' or the 'good' class. First quantify the prior belief by setting up a 'prior distribution' for each of the two hypotheses. These are depicted in Figure 6-4 by the two faint-lined distributions. Since there is no particular reason to say whether the site is 'good' or 'high' the two hypotheses are given prior probabilities of 0.5 each. Accordingly the two prior distributions in the figure each have areas of 0.5 – *and their spread reflects the scatter shown by the two sets of reference sites when the limits were being set*. Thus the two classes can be defined by probability distributions reflecting the spread of quality seen in their membership, rather than by a single good-high boundary value. This is the first big strength of the Bayesian approach.

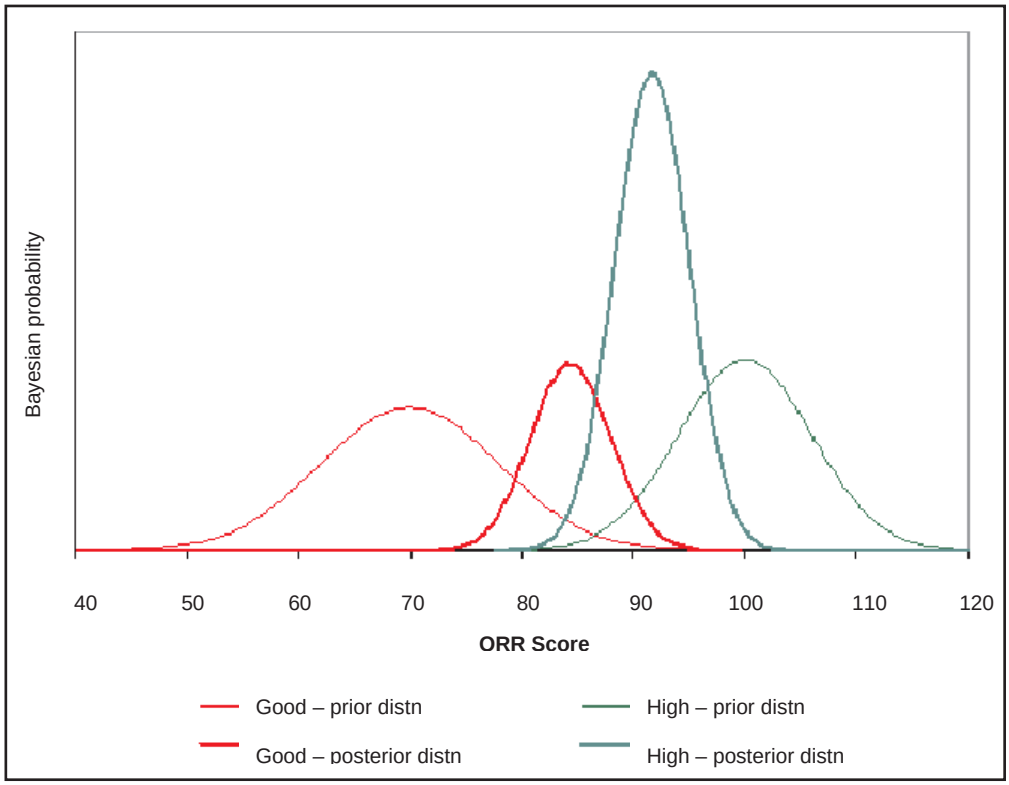


Figure 6-4. Illustration of a Bayesian approach to confidence of compliance. (www,ukwfd.org).

Next, consider the available data. The example shown in Figure 6-4 supposes 25 sample values with a mean of 88 and a standard deviation of 20. The standard error of the mean is therefore $20/\sqrt{25} = 4.0$, which implies that the true mean might lie anywhere between 84 and 92 with 95% confidence. In the light of this new information, the Bayesian approach provides an objective method to revise the prior belief by calculating 'posterior distributions' for the two hypotheses. These are shown in the figure by the two strong-lined distributions. The areas of the two distributions again sum to unity, but now the right-hand one is substantially the larger of the two; the two areas are actually 0.28 and 0.72. These are the posterior probabilities that the site is respectively 'good' and 'high'. The fact that these numbers fall naturally out of the calculations, and may be interpreted as probabilities of class membership, is the second major benefit conferred by the Bayesian approach.

6.5.8 Assessing trends in water quality data

***Time Trends*:** Several statistical methods, both parametric and non-parametric, are available for detecting trends. They include graphical methods, regression methods, the Mann Kendall test, Sen's non-parametric estimator of slope and the Seasonal Kendall test. Preliminary evaluations of data correlations and seasonal effects should be made prior to trend analysis. Data correlations are likely if data are taken close together in time or space. Close data can be influenced by each other and do not provide unique information. Seasonal effects should be removed, or a procedure that is unaffected by data cycles should be selected (Seasonal Kendall test).

Graphical methods: Plots of trends in constituent concentrations over time can be examined for seasonal or annual patterns:

1. Sort the data set by station and sampling date (i.e., first station and oldest sampling data are the first line of data);
2. For each station, select 'date' as the x variable and plot the parameter of interest on the y axis; and
3. Visually inspect the data for upward or downward trends and note any large 'peaks' or 'valleys.'

Regression methods: Linear least squares regression on water quality versus time, with a t test to determine if the true slope is not different from zero, can be used if the data are not cyclic or correlated and are normally distributed.

Mann Kendall Test: This test is useful when data are missing. It can consider multiple data observations per time period, and enables examinations of trends at multiple stations and comparisons of trends between stations. Seasonal cycles and other data relationships (such as flow versus concentration correlation) affect this test and must be corrected. If data are highly correlated, the test can be applied to median values in discrete time groupings.

Sen's Non-parametric Estimator of Slope: This is a non-parametric test based on rank. It is not sensitive to extreme values, gross data errors, or missing data (Gilbert 1987).

Seasonal Kendall Test: This method is preferred to most regression methods if the data are skewed, serially correlated or cyclic (Gilbert 1987). It can be used for data sets having missing values, tied values, censored values (below detection limits) and single or multiple data observations in each time period. Data correlations and dependence must be considered in the analysis.

6.5.9 Multivariate methods

Multivariate analysis techniques are very useful in the analysis of data with a large number of variables. Analysis using these techniques produces easily interpretable results. Multivariate data consists of observations on several variables for a number of samples. A wide variety of multivariate analysis techniques is available. The choice of the most appropriate technique depends on the nature of the data, problem and objectives. The underlying theme of many multivariate analysis techniques is simplification where it is desired to summarise a large body of data by means of relatively few parameters. Cluster analysis, factor analysis, multiple regression analysis, principal component analysis, MANOVA and multidimensional scaling are some of the multivariate statistical techniques that can be used to characterise water quality and assist in water quality monitoring data interpretation.

Cluster analysis is data reduction method that is used to classify entities with similar properties. The method divides a large number of objects into a smaller number of homogeneous groups on the basis of their correlation structure. The objective of cluster analysis is to identify the complex nature of multivariate relationships (by searching for natural groupings or types) among the data under investigation, so as to foster further hypothesis development about the phenomena being studied. Cluster analysis imposes a characteristic structure on the data analysis for exploratory purposes.

Factor analysis is used to understand the correlation structure of collected data and identify the most important factors contributing to the data structure. In factor analysis, the relationship among a number of observed quantitative variables are represented in terms of a few underlying, independent variables called factors, which may not be directly measured or even measurable. Factor analysis can be used to find associations between parameters so that the number of measured parameters can be reduced. Known associations are then used to predict unmeasured water quality parameters.

Multivariate regression analysis is recommended in cases requiring the analysis of dependence between variables if the variables do not arise on an equal footing. It should be noted that the term `equal footing' does not imply that some variables are more important than others, though they may be. Rather it indicates that there are dependent and explanatory variables. In multiple regression, the variation in

one dependent variable is explained by means of the variation in several explanatory variables. In multivariate regression, more than two dependent variables are in question.

Principal component analysis (PCA) (Mardia et al. 1979) is a method for reducing the dimensionality of multivariate data sets such as water quality parameters. Since many water quality parameters are positively or negatively correlated, some of the variables essentially contain the same information. PCA finds a new orthogonal coordinate system of uncorrelated variables to represent the original chemical data. Each coordinate direction (principal vector) is expressed as a linear combination of the original variables. The first principal vector is in the direction of greatest variance in the original data set. The eigenvalues associated with each direction are a measure of the variance accounted for by that direction. Each succeeding principal vector is orthogonal to the preceding vectors and is in the direction of the greatest variance not accounted for by the previous vectors. The dimensionality of the data can be reduced by ignoring the vectors associated with small eigenvalues that account for the least amount of variance. This procedure is also useful for identifying which variables are correlated. Consequently, PCA offers a powerful tool for identifying process and grouping water quality types. The data vectors for each chemical sample are rotated into the new coordinate system by taking a dot product between a weighting matrix and the vector of chemical analysis data. Only the significant components are retained during the subsequent analysis.

Multivariate ordination models.

A biological assessment aims to determine whether a test site is a member of an unimpaired reference population, and if it is not, the amount by which it deviates. Multivariate ordination can help answer these questions. Ordination analysis reduces the complexity of many variables (e.g. abundance of 100 species from 50 sites) into fewer variables; i.e. ordering the sites and species on new variables called the principal axis of analysis. A site is degraded if it is outside the area on an ordination diagram defined by reference sites. The distance (in ordination space) between the site and the reference centroid determines its degree of impairment. An example of this is shown in Figure 6-5.

Solid circles are reference sites, known impacted sites (triangles) deviate from the reference group, primarily on the first axis. Impairment may be judged by whether a site is outside the region bounding reference sites (ellipse), or by the distance between a site and the reference centroid (arrow).

An ordination model may be developed with the following steps:

- Reference sites are clustered according to their biological similarity to create reference classes. This might be done using tools such as cluster analysis or TWINSPAN. GLOSSARY

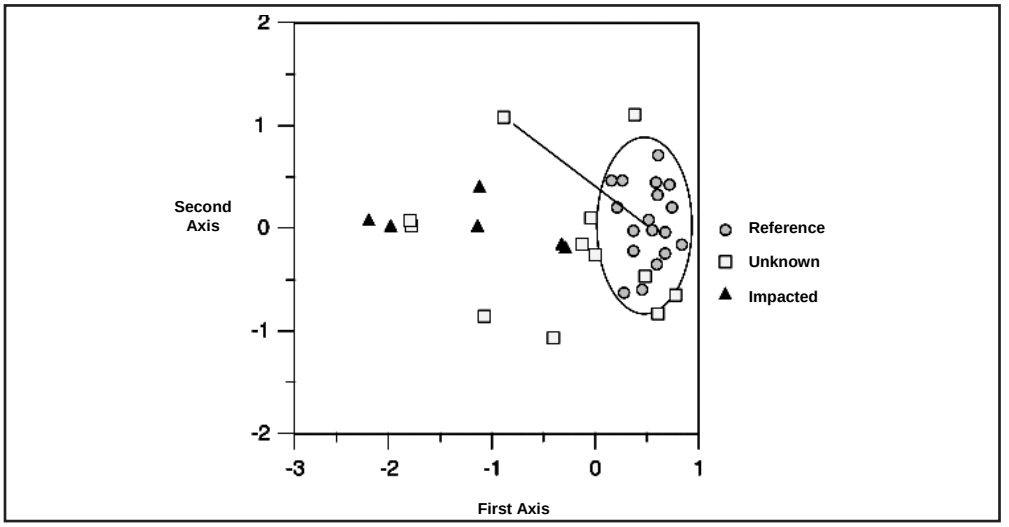


Figure 6-5. Example of assessment by ordination (from US EPA 1998).

- After determining clusters, their separation in ordination space can be determined. This might be done using tools such as correspondence analysis or Non-metric Multidimensional Scaling (NMDS). Both species abundance and environmental variables are related to the principal axis.
- A discriminant model is then developed to predict community composition using non-biological (physico-chemical) data from reference sites /classes.
- Deviation from reference classes is assessed by first applying physico-chemical data from the test sites to the discriminant model to assign each test site to a reference class. The biological assemblage structure of the test site can then be compared to the assemblage structure of the reference sites in ordination space (USEPA 1998).

The ordination approach requires the entire reference data set to be reanalysed for each new batch of monitoring sites. However, it may be the most cost effective approach if the biological survey is a single event, i.e. a large number of samples surveyed at once with no plans to continue monitoring or to survey new sites (USEPA 1998).

6.6 Interpretation of ASEAN marine water quality criteria

6.6.1 Bacteria

For the comparison against the threshold of faecal coliform counts should be expressed as a geometric mean value of 90th percentile values. (See McPherson et al. 1999).

6.6.2 Suspended solids

Assessment of compliance of suspended solids is taken against a maximum 10% increase above the seasonal average concentration, i.e., the maximum permissible increase is 10%. This criterion is dependent on which season is most appropriate under local conditions. Once the season is identified, sampling should be taken during this time-frame. The geometric mean of suspended solids for the seasons then calculated. Compliance is assessed by the calculation of a 10% increase in the geometric mean and values above this number are taken as non-compliance.

7 Reporting and Disseminating Information

7.1 Introduction

One of the final components of any successful monitoring program is the reporting and dissemination of the data collected and analysed in the program. It is important to work out who will use the information, the time-frames for reporting and in what form the information is best presented. Some users will need to know when a measurement falls outside a particular range, other might not care about an odd outlier but are concerned by trends or regular failure to stay within limits. Information technology should enable delivery of information from monitoring programs in a variety of forms to suit a range of users.

The recommended steps on the development of the reporting and information dissemination procedures are shown in Figure 7-1.

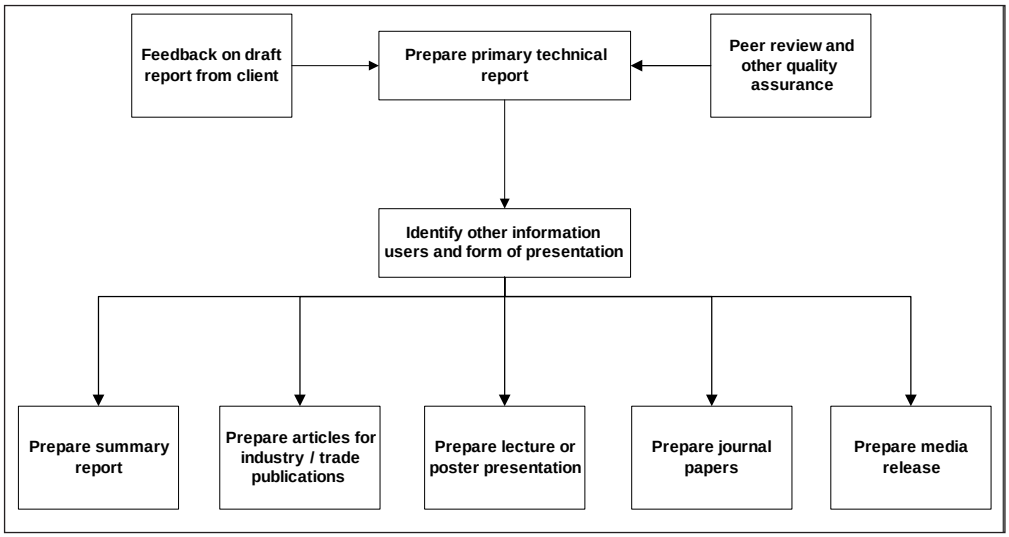


Figure 7-1. A framework for designing a reporting system.

The reporting process is one that should

- ensure that the monitoring has provided meaningful information for decision-making.
- Be based on good quality, robust data and a interpretative data management system.
- show cause and effect relationships where possible – what has changed, and how does this relate to the policy or plan, and the environment? Accept that you will often be making decisions on the basis of some uncertainty.
- Illustrate how indicators can help focus monitoring effort.
- Once you have developed indicators, it is important to re-check that the information generated will be directly useful for measuring the outcomes of your policy or plan, and the quality of the environment.

There are many different types of reporting of the monitoring process. Specific examples include:

- Report Card;
- State of the environment monitoring /reporting;
- Policy and plan monitoring /reporting; and
- Resource consent, compliance and complaint monitoring

Before beginning any report, specific deliverable requirements should be outlined in the project planning document. Care must be taken to ensure that deliverable requirements meet project data objectives. At a minimum, the laboratory should provide a data report that includes analytical results, a tabular summary of associated quality control results and control ranges, and a cover letter that references or describes the analytical procedure(s) and discusses any analytical problems. For the final report, it is important to identify the audience and, if there is a range of users, there might be a number of publications to deal with different perceptions e.g., technical report, scientific paper, reports for environmental managers, government policy-makers and government ministers, the general public and school children.

7.2 Report card

Steps to be considered in the preparation of a 'report card' reporting process:

1. What is your audience?
2. What are the parameters that you are reporting?
3. Have you defined measurable indicators in your report?

All laboratories are required to submit results that are supported by sufficient quality control results and backup documentation (maintained at the laboratory) to enable independent quality assurance reviewers to evaluate data quality and reconstruct final results from the raw data. Legible photocopies of original data sheets should be available from the laboratory with sufficient information to unequivocally identify the following items:

- Calibration results.
- Method blanks.
- Samples, sample sizes and dilution factors.
- Replicates and spikes, including amount spiked.
- Control or reference samples.
- Chain of custody and sampling records .
- Any anomalies in instrument performance or unusual instrument adjustments.

7.3 State of the Environment monitoring and reporting

State of the Environment monitoring helps with policy development and informs decision-makers of the consequences of actions and changes in the environment. It involves setting targets, monitoring, analysing and reviewing data, then reporting findings and continuing this process over time. SOE reporting provides managers and communities with access to information on the condition of the environment, the key pressures on it and the effectiveness of measures taken to address those issues. Such reporting should provide an early warning of environmental problems and possible solutions. It informs decision-makers by indicating where high-level environmental outcomes and results can be improved or have been successful, and where environmental management has been effective. It allows councils and communities to access information on the state or condition of the environment, to identify key environmental pressures, and to assess possible and actual responses. SOE monitoring and reporting can also help determine whether these requirements are being met.

Figure 7-2 shows the cycle of steps involved in monitoring and reporting the state of the environment. The first step is to report. This feeds the development of action plans and the setting of targets. The plans are implemented and monitored. Analysis of the data gathered through monitoring and review of the implementation of action plans feeds into the next report, completing the circle.

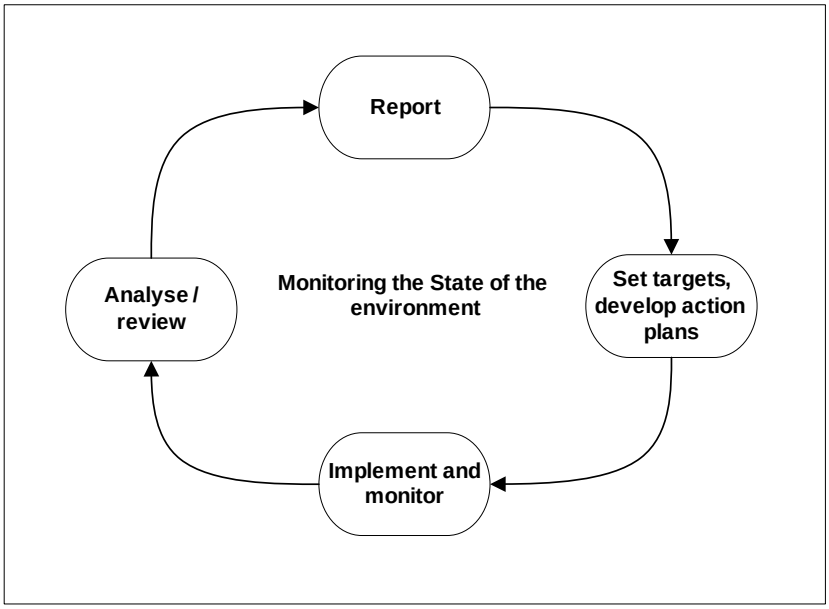


Figure 7-2. Steps involved in state of the environment reporting.

The purpose of SOE reports is to:

- Compile and assess information regularly on the condition of the environment, the key pressures on it, and what is and can be done to address pressures (the responses).
- Provide information to answer basic questions, including:
  - What's happening in the environment?
  - Why is it happening?
  - Where are the gaps?
  - What is/can be done about it (to make a difference)?
  - How do this situation compare (over time and space and with others)?
- Help monitoring programs report on how well they are achieving their stated goals (information and input into policy and plan monitoring).
- Educate people about their local environment and inspire community action. This may require links to education strategies.
- Provide some accountability in terms of expenditure.
- Develop data into useful information for decision making.
- Provide information for other reporting and review.

7.4 Policy and plan effectiveness reporting

Policy and plan effectiveness reporting helps determine whether the regional policy and management decisions are effective as a means of achieving objectives and anticipated outcomes. This type of report can signal the need for future action and provides information on possible improvements to policy and plan content and implementation.

Policy and plan monitoring and reporting is more than a statutory requirement. It is a useful management tool to evaluate and review the effectiveness of policy provisions and plans.

It is important to have a clear purpose for policy and plan monitoring. Is it for:

- Accountability to the community (to show that you have provided a means of managing what you said you would manage and achieved the plan's environmental goals?
- Continuous improvement of the organisation/institution involved?
- Both—which is likely to be a useful approach?

Assessing if the environmental outcomes have been achieved has strong links to SOE monitoring and reporting

7.5 Resource consents, compliance and complaints reporting

Monitoring and reporting of resource consents, compliance and complaints indicates performance in relation to a number of issues, highlights areas that may require further action by managers and providers and provides feedback on policies, regulations and processes in plans (including plan implementation and process) and the state of the environment.

Be clear on the purpose of resource consent, compliance and complaint monitoring and reporting. Is it to:

- Check that consent holders are meeting the conditions of consent? (administrative/process monitoring)
- Check on predicted environmental effects of consented activities, including links to anticipated environmental results (AERs) in policy statements and plans (environmental performance/outcomes monitoring)
- Provide information for state of the environment monitoring and reporting?
- Assist in assessing the effectiveness of policy and plan provisions?

A combination of all these purposes may be a desirable approach.

Reporting of resource consents involves checking compliance with consent decisions, the effectiveness of consent conditions and monitoring the impact of activities on the environment. Complaints can also provide useful information on compliance or areas where policies and plans are not meeting the desired and anticipated environmental outcomes. State of the Environment and complaints monitoring can provide useful information for monitoring the effects of permitted activities

7.6 Summary

Reporting of any monitoring programs should be integrated with identifiable goals. For a clear and concise reporting structure that is useful to both scientists and managers, remember:

- Establish protocols and systems for gathering monitoring information.
- Develop criteria to prioritise monitoring and make it cost-effective eg prioritisation table. Be focused and monitor the most important things first. This will involve establishing priorities with your key audiences.
- Ensure there is quality, robust data to show key trends—have a long-term perspective and a strategic approach.
- Be clear about data quality and reliability and be mindful of the context within which data is collected, eg., what does it mean?
- Don't monitor for the sake of monitoring.
- Integrate different types of monitoring.
- New issues will emerge so be flexible.
- Monitor and report on key indicators.
- Review indicators to allow for improving performance.
- Ensure that feedback is provided to the council (to inform policy development) and community (for environmental education).
- Obtain senior management and political support and on-going resourcing.

7.7 Who to involve

- Have the senior management and politicians on board for on-going resourcing and action:
    - Link reporting to issues that are relevant to functions.

- Show how reporting information is useful.
- Demonstrate the benefits of being a data provider and manager.

- Have an integrated team throughout the science and management process—include planners, scientists, managers, compliance and resource consent staff, IT and data management people, communications and education staff, as appropriate;
  - Consider using a neutral editor.
  - Consider having a data review process—quality of data is critical, particularly scientific data.
- Consider community viewpoints on key issues and whether to incorporate stakeholder/public perceptions i.e., satisfaction surveys.
- Establish links between the monitoring programmes of other agencies and encourage partnership and integration.

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Appendix 1

USEPA Method 200.10

Determination of Trace Elements in Marine Waters by On-Line Chelation Preconcentration and Inductively Coupled Plasma-Mass Spectrometry

Revision 1.6

September 1997

Method 200.10

Determination of Trace Elements in Marine Waters by On-Line Chelation Preconcentration and Inductively Coupled Plasma - Mass Spectrometry

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Method 200.10

Determination of Trace Elements in Marine Waters by On-Line Chelation Preconcentration and Inductively Coupled Plasma - Mass Spectrometry

1.0 Scope and Application

1.1 This method describes procedures for preconcentration and determination of total recoverable trace elements in marine waters, including estuarine water, seawater, and brines.

1.2 Acid solubilization is required prior to the determination of total recoverable elements to facilitate breakdown of complexes or colloids that might influence trace element recoveries. This method should only be used for preconcentration and determination of trace elements in aqueous samples.

1.3 This method is applicable to the following elements:

Element		Chemical Abstracts Service Registry Numbers (CASRN)
Cadmium	(Cd)	7440-43-9
Cobalt	(Co)	7440-48-4
Copper	(Cu)	7440-50-8
Lead	(Pb)	7439-92-1
Nickel	(Ni)	7440-02-0
Uranium	(U)	7440-61-1
Vanadium	(V)	7440-62-2

1.4 Method detection limits (MDLs) for these elements will be dependent on the specific instrumentation employed and the selected operating conditions. However, the MDLs should be essentially independent of the matrix because elimination of the matrix is a feature of the method. Reagent water MDLs, which were determined using the procedure described in Section 9.2.4, are listed in Table 1.

1.5 A minimum of 6-months experience in the use of commercial instrumentation for inductively coupled plasma mass spectrometry (ICP-MS) is recommended.

2.0 Summary of Method

2.1 This method is used to preconcentrate trace elements using an iminodiacetate functionalized chelating resin.[1,2] Following acid solubilization, the sample is buffered prior to the chelating column using an on-line system. Groups I and II metals, as well as most anions, are selectively separated from the analytes by elution with ammonium acetate at pH 5.5. The analytes are subsequently eluted into a simplified matrix consisting of dilute nitric acid and are determined by ICP-MS using a directly coupled on-line configuration.

2.2 The determinative step in this method is ICP-MS.[3-5] Sample material in solution is introduced by pneumatic nebulization into a radio frequency plasma where energy transfer processes cause desolvation, atomization and ionization. The ions are extracted from the plasma through a differentially pumped vacuum interface and separated on the basis of their mass-to-charge ratio by a quadrupole mass spectrometer having a minimum resolution capability of 1 amu peak width at 5% peak height. The ions transmitted through the quadrupole are registered by a continuous dynode electron multiplier or Faraday detector and the ion information is processed by a data handling system. Interferences relating to the technique (Section 4) must be recognized and corrected. Such corrections must include compensation for isobaric elemental interferences and interferences from polyatomic ions derived from the plasma gas, reagents or sample matrix. Instrumental drift must be corrected for by the use of internal standardization.

3.0 Definitions

3.1 Calibration Blank (CB) -- A volume of reagent water fortified with the same matrix as the calibration standards but without the analytes, internal standards, or surrogate analytes.

3.2 Calibration Standard (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

3.3 Instrument Detection Limit (IDL) -- The minimum quantity of analyte or the concentration equivalent that gives an analyte signal equal to three times the standard deviation of the background signal at the selected wavelength, mass, retention time, absorbance line, etc.

3.4 Instrument Performance Check Solution (IPC) -- A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.5 Internal Standard (IS) -- A pure analyte(s) added to a sample, extract, or standard solution in known amount(s) and used to measure the relative responses

of other method analytes and surrogates that are components of the same sample or solution. The internal standard must be an analyte that is not a sample component.

3.6 Laboratory Fortified Blank (LFB) -- An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control and whether the laboratory is capable of making accurate and precise measurements.

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

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

3.9 Linear Dynamic Range (LDR) -- The absolute quantity or concentration range over which the instrument response to an analyte is linear.

3.10 Material Safety Data Sheet (MSDS) -- Written information provided by vendors concerning a chemical's toxicity, health hazards, physical properties, fire, and reactivity data including storage, spill, and handling precautions.

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

3.12 Quality Control Sample (QCS) -- A solution of method analytes of known concentrations that is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

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

3.14 Total Recoverable Analyte (TRA) -- The concentration of analyte determined to be in either a solid sample or an unfiltered aqueous sample following treatment by refluxing with hot dilute mineral acid(s) as specified in the method.

3.15 Tuning Solution (TS) -- A solution that is used to adjust instrument performance prior to calibration and sample analyses.

4.0 Interferences

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

4.1.1 Isobaric elemental interferences -- Are caused by isotopes of different elements that form singly or doubly charged ions of the same nominal mass-to-charge ratio and that cannot be resolved by the mass spectrometer in use. All elements determined by this method have, at a minimum, one isotope free of isobaric elemental interference. The analytical isotopes recommended for use with this method are listed in Table 1.

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

4.1.3 Isobaric polyatomic ion interferences -- Are caused by ions consisting of more than one atom that have the same nominal mass-to-charge ratio as the isotope of interest and that cannot be resolved by the mass spectrometer in use. These ions are commonly formed in the plasma or interface system from support gases or sample components. Such interferences must be recognized, and when they cannot be avoided by the selection of alternative analytical isotopes, appropriate corrections must be made to the data. Equations for the correction of data should be established at the time of the analytical run sequence as the polyatomic ion interferences will be highly dependent on the sample matrix and chosen instrument conditions.

4.1.4 Physical interferences -- Are associated with the physical processes that govern the transport of sample into the plasma, sample conversion processes in the plasma, and the transmission of ions through the plasma mass spectrometer interface. These interferences may result in differences between instrument responses for

the sample and the calibration standards. Physical interferences may occur in the transfer of solution to the nebulizer (e.g., viscosity effects), at the point of aerosol formation and transport to the plasma (e.g., surface tension), or during excitation and ionization processes within the plasma itself. Internal standardization may be effectively used to compensate for many physical interference effects.[6] Internal standards ideally should have similar analytical behavior to the elements being determined.

4.1.5 Memory interferences -- Result when isotopes of elements in a previous sample contribute to the signals measured in a new sample. Memory effects can result from sample deposition on the sampler and skimmer cones and from the buildup of sample material in the plasma torch and spray chamber. The site where these effects occur is dependent on the element and can be minimized by flushing the system with a rinse blank between samples. Memory interferences from the chelating system may be encountered especially after analyzing a sample containing high concentrations of the analytes. A thorough column rinsing sequence following elution of the analytes is necessary to minimize such interferences.

4.2 A principal advantage of this method is the selective elimination of species giving rise to polyatomic spectral interferences on certain transition metals (e.g., removal of the chloride interference on vanadium). As the majority of the sample matrix is removed, matrix induced physical interferences are also substantially reduced.

4.3 Low recoveries may be encountered in the preconcentration cycle if the trace elements are complexed by competing chelators in the sample or are present as colloidal material. Acid solubilization pretreatment is employed to improve analyte recovery and to minimize adsorption, hydrolysis, and precipitation effects.

5.0 Safety

5.1 Each chemical reagent used in this method should be regarded as a potential health hazard and exposure to these reagents should be as low as reasonably achievable. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.[7,8] A reference file of material data handling sheets should also be available to all personnel involved in the chemical analysis.

5.2 Analytical plasma sources emit radio frequency radiation in addition to intense UV radiation. Suitable precautions should be taken to protect personnel from such hazards.

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

5.4 All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease causative agents.

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

6.0 Equipment and Supplies

6.1 Preconcentration System -- System containing no metal parts in the analyte flow path, configured as shown in Figure 1.

6.1.1 Column -- Macroporous iminodiacetate chelating resin (Dionex Metpac CC-1 or equivalent).

6.1.2 Sample loop -- 10-mL loop constructed from narrow bore, high-pressure inert tubing, Tefzel ethylene tetra-fluoroethylene (ETFE) or equivalent.

6.1.3 Eluent pumping system (PI) -- Programmable flow, high pressure pumping system, capable of delivering either one of two eluents at a pressure up to 2000 psi and a flow rate of 1-5 mL/min.

6.1.4 Auxiliary pumps -- On line buffer pump (P2), piston pump (Dionex QIC pump or equivalent) for delivering 2M ammonium acetate buffer solution; *carrier pump (P3),* peristaltic pump (Gilson Minipuls or equivalent) for delivering 1% nitric acid carrier solution; *sample pump (P4),* peristaltic pump for loading sample loop.

6.1.5 Control valves -- Inert double stack, pneumatically operated four-way slider valves with connectors.

6.1.5.1 Argon gas supply regulated at 80-100 psi.

6.1.6 Solution reservoirs -- Inert containers, e.g., high density polyethylene (HDPE), for holding eluent and carrier reagents.

6.1.7 Tubing -- High pressure, narrow bore, inert tubing (e.g., Tefzel ETFE or equivalent) for interconnection of pumps/valve assemblies and a minimum length for connection of the preconcentration system to the ICP-MS instrument.

6.2 Inductively Coupled Plasma - Mass Spectrometer

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

6.2.2 Argon gas supply (high-purity grade, 99.99%).

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

6.2.4 Operating conditions -- Because of the diversity of instrument hardware, no detailed instrument operating conditions are provided. The analyst is advised to follow the recommended operating conditions provided by the manufacturer.

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

6.3 Labware -- For the determination of trace elements, contamination and loss are of **critical** concern. Potential contamination sources include improperly cleaned laboratory apparatus and general contamination within the laboratory environment. A clean laboratory work area, designated for trace element sample handling, must be used. Sample containers can introduce positive and negative errors in the determination of trace elements by (1) contributing contaminants through surface desorption or leaching or (2) depleting element concentrations through adsorption processes. For these reasons, borosilicate glass is *not* recommended for use with this method. All labware in contact with the sample should be cleaned prior to use. Labware may be soaked overnight and thoroughly washed with laboratory-grade detergent and water, rinsed with water, and soaked for 4 hr in a mixture of dilute nitric and hydrochloric acids, followed by rinsing with ASTM type I water and oven drying.

6.3.1 Griffin beakers, 250-mL, polytetrafluoroethylene (PTFE) or quartz.

6.3.2 Storage bottles -- Narrow mouth bottles, Teflon FEP (fluorinated ethylene propylene), or HDPE, 125-mL and 250-mL capacities.

6.4 Sample Processing Equipment

6.4.1 Air displacement pipetter -- Digital pipet system capable of delivering volumes from 10 to 2500 • Ł with an assortment of metal-free, disposable pipet tips.

6.4.2 Balances -- Analytical balance, capable of accurately weighing to ±0.1 mg; top pan balance, accurate to ± 0.01g.

6.4.3 Hot plate -- Corning PC100 or equivalent.

6.4.4 Centrifuge -- Steel cabinet with guard bowl, electric timer and brake.

6.4.5 Drying oven -- Gravity convection oven with thermostatic control capable of maintaining 105• €±5• €.

6.4.6 pH meter -- Bench mounted or hand-held electrode system with a resolution of ± 0.1 pH units.

7.0 Reagents and Standards

7.1 Water -- For all sample preparation and dilutions, ASTM type I water (ASTM D1193) is required.

7.2 Reagents may contain elemental impurities that might affect the integrity of analytical data. Because of the high sensitivity of this method, ultra high-purity reagents must be used unless otherwise specified. To minimize contamination, reagents should be prepared directly in their designated containers where possible.

7.2.1 Acetic acid, glacial (sp. gr. 1.05).

7.2.2 Ammonium hydroxide (20%).

7.2.3 Ammonium acetate buffer 1M, pH 5.5 -- Add 58-mL (60.5 g) of glacial acetic acid to 600-mL of ASTM type water. Add 65 mL (60 g) of 20% ammonium hydroxide and mix. Check the pH of the resulting solution by withdrawing a small aliquot and testing with a calibrated pH meter, adjusting the solution to pH 5.5±0.1 with small volumes of acetic acid or ammonium hydroxide as necessary. Cool and dilute to 1 L with ASTM type I water.

7.2.4 Ammonium acetate buffer 2M, pH 5.5 -- Prepare as for Section 7.2.3 using 116 mL (121g) glacial acetic acid and 130 mL (120 g) 20% ammonium hydroxide, diluted to 1000 mL with ASTM type I water.

Note: The ammonium acetate buffer solutions may be further purified by passing them through the chelating column at a flow rate of 5.0-mL/min. With reference to Figure 1, pump the buffer solution through the column using pump P1, with valves A and B off and valve C on. Collect the purified solution in a container at the waste outlet. Following this, elute the collected contaminants from the column using 1.25M nitric acid for 5 min at a flow rate of 4.0 mL/min.

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

7.2.5.1 Nitric acid 1.25M -- Dilute 79 mL (112 g) conc. nitric acid to 1000-mL with ASTM type I water.

7.2.5.2 Nitric acid 1% -- Dilute 10 mL conc. nitric acid to 1000 mL with ASTM type I water.

7.2.5.3 Nitric acid (1+1) -- Dilute 500 mL conc. nitric acid to 1000-mL with ASTM type I water.

7.2.5.4 Nitric acid (1+9) -- Dilute 100 mL conc. nitric acid to 1000-mL with ASTM type I water.

7.2.6 Oxalic acid dihydrate (CASRN 6153-56-6), 0.2M -- Dissolve 25.2 g reagent grade $C_2H_2O_4 \cdot 2H_2O$ in 250-mL ASTM type I water and dilute to 1000 mL with ASTM type I water. **Caution** - Oxalic acid is toxic; handle with care.

7.3 Standard Stock Solutions -- May be purchased from a reputable commercial source or prepared from ultra high-purity grade chemicals or metals (99.99-99.999% pure). All salts should be dried for 1 h at 105• €, unless otherwise specified. (**Caution-** Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.) Stock solutions should be stored in plastic bottles. The following procedures may be used for preparing standard stock solutions:

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

7.3.1 Cadmium solution, stock 1 mL = 1000 • g Cd: Pickle cadmium metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5-mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100-mL with ASTM type I water.

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

7.3.3 Copper solution, stock 1 mL = 1000 • g Cu: Pickle copper metal in (1+9) nitric acid to an exact weight 0.100 g. Dissolve in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.3.4 Indium solution, stock 1 mL = 1000 • g In: Pickle indium metal in (1+1) nitric acid to an exact weight 0.100 g. Dissolve in 10 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.3.5 Lead solution, stock 1 mL = 1000 • g Pb: Dissolve 0.1599 g $PbNO_3$ in 5 mL (1+1) nitric acid. Dilute to 100 mL with ASTM type I water.

7.3.6 Nickel solution, stock 1 mL = 1000 • g Ni: Dissolve 0.100 g nickel powder in 5 mL conc. nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.3.7 Scandium solution, stock 1 mL = 1000 • g Sc: Dissolve 0.1534 g Sc_2O_3 in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.3.8 Terbium solution, stock 1 mL = 1000 • g Tb: Dissolve 0.1176 g Tb_4O_7 in 5 mL conc. nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.3.9 Uranium solution, stock 1 mL = 1000 • g U: Dissolve 0.2110 g $UO_2(NO_3)_2 \cdot 6H_2O$ (Do Not Dry) in 20 mL ASTM type I water. Add 2-mL (1+1) nitric acid and dilute to 100-mL with ASTM type I water.

7.3.10 Vanadium solution, stock 1 mL = 1000 • g V: Pickle vanadium metal in (1+9) nitric acid to an exact weight of 0.100 g. Dissolve in 5-mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.3.11 Yttrium solution, stock 1 mL = 1000 • g Y: Dissolve 0.1270 g Y_2O_3 in 5 mL (1+1) nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.4 Multielement Stock Standard Solution -- Care must be taken in the preparation of multielement stock standards that the elements are compatible and stable. Originating element stocks should be checked for impurities that might influence the accuracy of the standard. Freshly prepared standards should be transferred to acid cleaned, new FEP or HDPE bottles for storage and monitored periodically for stability. A multielement stock standard solution containing the elements, cadmium, cobalt, copper, lead, nickel, uranium, and vanadium (1 mL = 10 • g) may be prepared by diluting 1 mL of each single element stock in the list to 100 mL with ASTM type I water containing 1% (v/v) nitric acid.

7.4.1 Preparation of calibration standards -- Fresh multielement calibration standards should be prepared weekly. Dilute the stock multielement standard solution in 1% (v/v) nitric acid to levels appropriate to the required operating range. The element concentrations in the standards should be sufficiently high to produce good measurement precision and to accurately define the slope of the response curve. A suggested mid-range concentration is 10 • g /L.

7.5 Blanks -- Four types of blanks are required for this method. A calibration blank is used to establish the analytical calibration curve, and the laboratory reagent blank is used to assess possible contamination from the sample preparation procedure. The laboratory fortified blank is used to assess the recovery of the method

analytes and the rinse blank is used between samples to minimize memory from the nebulizer/spray chamber surfaces.

7.5.1 Calibration blank -- Consists of 1% (v/v) nitric acid in ASTM type I water (Section 7.2.5.2).

7.5.2 Laboratory reagent blank (LRB) -- Must contain all the reagents in the same volumes as used in processing the samples. The LRB must be carried through the entire sample digestion and preparation scheme.

7.5.3 Laboratory Fortified Blank (LFB) -- To an aliquot of LRB, add aliquots from the multielement stock standard (Section 7.4) to produce a final concentration of 10 • g/L for each analyte. The fortified blank must be carried through the entire sample pretreatment and analytical scheme.

7.5.4 Rinse Blank (RB) -- Is a 1% (v/v) nitric acid solution that is delivered to the ICP-MS between samples (Section 7.2.5.2).

7.6 Tuning Solution -- This solution is used for instrument tuning and mass calibration prior to analysis (Section 10.2). The solution is prepared by mixing nickel, yttrium, indium, terbium, and lead stock solutions (Section 7.3) in 1% (v/v) nitric acid to produce a concentration of 100 • g/L of each element.

7.7 Quality Control Sample (QCS) -- A quality control sample having certified concentrations of the analytes of interest should be obtained from a source outside the laboratory. Dilute the QCS if necessary with 1% nitric acid, such that the analyte concentrations fall within the proposed instrument calibration range.

7.8 Instrument Performance Check (IPC) Solution -- The IPC solution is used to periodically verify instrument performance during analysis. It should be prepared by combining method analytes at appropriate concentrations to approximate the midpoint of the calibration curve. The IPC solution should be prepared from the same standard stock solutions used to prepare the calibration standards and stored in a FEP bottle. Agency programs may specify or request that additional instrument performance check solutions be prepared at specified concentrations in order to meet particular program needs.

7.9 Internal Standards Stock Solution, 1 mL = 100 • g -- Dilute 10-mL of scandium, yttrium, indium, terbium, and bismuth stock standards (Section 7.3) to 100-mL with ASTM type I water, and store in a Teflon bottle. Use this solution concentrate for addition to blanks, calibration standards and samples (Method A, Section 10.5), or dilute by an appropriate amount using 1% (v/v) nitric acid, if the internal standards are being added by peristaltic pump (Method B, Section 10.5).

Note: Bismuth should not be used as an internal standard using the direct addition method (Method A, Section 10.5) as it is not efficiently concentrated on the iminodiacetate column.

8.0 Sample Collection, Preservation, and Storage

8.1 Prior to the collection of an aqueous sample, consideration should be given to the type of data required, so that appropriate preservation and pretreatment steps can be taken. Acid preservation should be performed at the time of sample collection or as soon thereafter as practically possible. The pH of all aqueous samples must be tested immediately prior to aliquoting for analysis to ensure the sample has been properly preserved. If properly acid preserved, the sample can be held up to 6 months before analysis.

8.2 For the determination of total recoverable elements in aqueous samples, acidify with (1+1) nitric acid (high purity) at the time of collection to pH<2; normally, 3 mL of (1+1) acid per liter of sample is sufficient for most samples. The sample should not be filtered prior to analysis.

Note: Samples that cannot be acid preserved at the time of collection because of sampling limitations or transport restrictions, or are >pH2 because of high alkalinity should be acidified with nitric acid to pH<2 upon receipt in the laboratory. Following acidification, the sample should be held for 16 h and the pH verified to be <2 before withdrawing an aliquot for sample processing.

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

9.0 Quality Control

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

9.2 Initial Demonstration of Performance (Mandatory)

9.2.1 The initial demonstration of performance is used to characterize instrument performance (determination of

linear dynamic ranges and analysis of quality control samples) and laboratory performance (determination of method detection limits) prior to samples being analyzed by this method.

9.2.2 Linear calibration ranges -- The upper limit of the linear calibration range should be established for each analyte. Linear calibration ranges should be determined every six months or whenever a significant change in instrument response is expected.

9.2.3 Quality control sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS (Section 7.7). If the determined concentrations are not within ± 10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding with the initial determination of method detection limits or continuing with ongoing analyses.

9.2.4 Method detection limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit.[9] To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

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

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

S = standard deviation of the replicate analyses.

Note: If the relative standard deviation (RSD) from the analyses of the seven aliquots is <15%, the concentration used to determine the analyte MDL may have been inappropriately high for the determination. If so, this could result in the calculation of an unrealistically low MDL. If additional confirmation of the MDL is desired, reanalyze the seven replicate aliquots on two more nonconsecutive days and again calculate the MDL values for each day. An average of the three MDL values for each analyte may provide for a more appropriate MDL estimate. Concurrently, determination of MDL in reagent water represents a best case situation and does not reflect possible matrix effects of real world samples. However, successful analyses of LFMs (Section 9.4) can give confidence to the MDL value determined in reagent water. Typical single laboratory MDL values using this method are given in Table 1.

MDLs should be determined every six months, when a new operator begins work or whenever there is a significant change in the background or instrument response.

9.3 Assessing Laboratory Performance (Mandatory)

9.3.1 Laboratory reagent blank (LRB) -- The laboratory must analyze at least one LRB (Section 7.5.2) with each batch of 20 or fewer samples. LRB data are used to assess contamination from the laboratory environment. LRB values that exceed the MDL indicate laboratory or reagent contamination should be suspected. Any determined source of contamination must be corrected and the samples reanalyzed for the affected analytes after acceptable LRB values have been obtained.

9.3.2 Laboratory fortified blank (LFB) -- The laboratory must analyze at least one LFB (Section 7.5.3) with each batch of samples. Calculate accuracy as percent recovery (Section 9.4.3). If the recovery of any analyte falls outside the required control limits of 85-115%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

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

$$\text{Upper Control Limit} = x + 3S$$

$$\text{Lower Control Limit} = x - 3S$$

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

9.3.4 Instrument performance check (IPC) solution -- For all determinations the laboratory must analyze the IPC solution (Section 7.8) and a calibration blank immediately following daily calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within ±10% of calibration. Subse-

quent analyses of the IPC solution must verify the calibration within ±15%. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with the sample analyses data.

9.3.5 The overall sensitivity and precision of this method are strongly influenced by a laboratory's ability to control the method blank. Therefore, it is recommended that the calibration blank response be recorded for each set of samples. This record will aid the laboratory in assessing both its long- and short-term ability to control the method blank.

9.4 Assessing Analyte Recovery and Data Quality

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

9.4.2 The laboratory must add a known amount of each analyte to a minimum of 10% of the routine samples. In each case the LFM aliquot must be a duplicate of the aliquot used for sample analysis and for total recoverable determinations added prior to sample preparation. For water samples, the added analyte concentration must be the same as that used in the laboratory fortified blank (Section 9.3.2).

9.4.3 Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range of 75-125%. Recovery calculations are not required if the concentration added is less than 25% of the unfortified sample concentration. Percent recovery may be calculated in units appropriate to the matrix, using the following equation:

$$R = \frac{(C_s - C)}{S} \times 100$$

where, R = percent recovery.
C_S = fortified sample concentration.
C = sample background concentration.
S = concentration equivalent of analyte added to sample.

9.4.4 If the recovery of any analyte falls outside the designated LFM recovery range and the laboratory performance for that analyte is shown to be in control (Section 9.3), the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related.

9.4.5 If analysis of LFM sample(s) and the test routines above indicate an operative interference and the LFMs are typical of the other samples in the batch, those samples that are similar must be analyzed in the same manner as the LFMs. Also, the data user must be informed when a matrix interference is so severe that it prevents the successful analysis of the analyte or when the heterogeneous nature of the sample precludes the use of duplicate analyses.

9.4.6 Where reference materials are available, they should be analyzed to provide additional performance data. The analysis of reference samples is a valuable tool for demonstrating the ability to perform the method acceptably.

10.0 Calibration and Standardization

10.1 Initiate proper operating configuration of ICP-MS instrument and data system. Allow a period of not less than 30 min for the instrument to warm up. During this process conduct mass calibration and resolution checks using the tuning solution. Resolution at low mass is indicated by nickel isotopes 60, 61, 62. Resolution at high mass is indicated by lead isotopes 206, 207, 208. For good performance adjust spectrometer resolution to produce a peak width of approximately 0.75 amu at 5% peak height. Adjust mass calibration if it has shifted by more than 0.1 amu from unit mass.

10.2 Instrument stability must be demonstrated by analyzing the tuning solution (Section 7.6) a minimum of five times with resulting relative standard deviations of absolute signals for all analytes of less than 5%.

10.3 Prior to initial calibration, setup proper instrument software routines for quantitative analysis and connect the ICP-MS instrument to the preconcentration apparatus. The instrument must be calibrated for the analytes of interest using the calibration blank (Section 7.5.1) and calibration standard (Section 7.4.1) prepared at one or more concentration levels. The calibration solutions should be processed through the preconcentration system using the procedures described in Section 11.

10.4 Demonstration and documentation of acceptable initial calibration is required before any samples are analyzed. After initial calibration is successful, a calibration check is required at the beginning and end of each period during which analyses are performed and at requisite intervals.

10.4.1 After the calibration has been established, it must be initially verified for all analytes by analyzing the IPC (Section 7.8). If the initial calibration verification exceeds ±10% of the established IPC value, the analysis should be terminated, the source of the problem identified and corrected, the instrument recalibrated, and the new calibration verified before continuing analyses.

10.4.2 To verify that the instrument is properly calibrated on a continuing basis, analyze the calibration blank (Section 7.5.1) and IPC (Section 7.8) after every 10 analyses. The results of the analyses of the standards will indicate whether the calibration remains valid. If the indicated concentration of any analyte deviates from the true concentration by more than 15%, reanalyze the standard. If the analyte is again outside the 15% limit, the instrument must be recalibrated and the previous 10 samples reanalyzed. The instrument responses from the calibration check may be used for recalibration purposes.

10.5 Internal Standardization -- Internal standardization must be used in all analyses to correct for instrument drift and physical interferences. For full mass range scans, a minimum of three internal standards must be used. Internal standards must be present in all samples, standards and blanks at identical levels. This may be achieved by directly adding an aliquot of the internal standards to the CAL standard, blank or sample solution (Method A), or alternatively by mixing with the solution prior to nebulization using a second channel of the peristaltic pump and a mixing coil (Method B). The concentration of the internal standard should be sufficiently high that good precision is obtained in the measurement of the isotope used for data correction and to minimize the possibility of correction errors if the internal standard is naturally present in the sample. Internal standards should be added to blanks, samples and standards in a like manner, so that dilution effects resulting from the addition may be disregarded.

Note: Bismuth should not be used as an internal standard using the direct addition method (Method A, Section 10.5) because it is not efficiently concentrated on the iminodiacetate column.

11.0 Procedure

11.1 Sample Preparation -- Total Recoverable Elements

11.1.1 Add 2-mL(1+1) nitric acid to the beaker containing 100-mL of sample. Place the beaker on the hot plate for solution evaporation. The hot plate should be located in a fume hood and previously adjusted to provide evaporation at a temperature of approximately but no higher than 85•C. (See the following note.) The beaker should be covered with an elevated watch glass or other necessary steps should be taken to prevent sample contamination from the fume hood environment.

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

11.1.2 Reduce the volume of the sample aliquot to about 20-mL by gentle heating at 85•C. **Do Not Boil.** This step takes about 2 h for a 100-mL aliquot with the rate of evaporation rapidly increasing as the sample volume approaches 20 mL. (A spare beaker containing 20-mL of water can be used as a gauge.)

11.1.3 Cover the lip of the beaker with a watch glass to reduce additional evaporation and gently reflux the sample for 30 min. (Slight boiling may occur, but vigorous boiling must be avoided.)

11.1.4 Allow the beaker to cool. Quantitatively transfer the sample solution to a 100-mL volumetric flask, dilute to volume with reagent water, stopper and mix.

11.1.5 Allow any undissolved material to settle overnight, or centrifuge a portion of the prepared sample until clear. (If after centrifuging or standing overnight, the sample contains suspended solids, a portion of the sample may be filtered prior to analysis. However, care should be exercised to avoid potential contamination from filtration.) The sample is now ready for analysis. Because the effects of various matrices on the stability of diluted samples cannot be characterized, all analyses should be performed as soon as possible after the completed preparation.

11.2 Prior to first use, the preconcentration system should be thoroughly cleaned and decontaminated using 0.2M oxalic acid.

11.2.1 Place approximately 500-mL 0.2M oxalic acid in all the eluent/solution containers and fill the sample loop with 0.2M oxalic acid using the sample pump (P4) at a flow rate of 3-5 mL/min. With the preconcentration system disconnected from the ICP-MS instrument, use the pump program sequence listed in Table 2 to flush the complete system with oxalic acid. Repeat the flush sequence three times.

11.2.2 Repeat the sequence described in Section 11.2.1 using 1.25M nitric acid and again using ASTM type I water in place of the 0.2M oxalic acid.

11.2.3 Rinse the containers thoroughly with ASTM type I water, fill them with their designated reagents (see Figure 1) and run through the sequence in Table 2 once to prime the pump and all eluent lines with the correct reagents.

11.3 Initiate ICP-MS instrument operating configuration. Tune the instrument for the analytes of interest (Section 10).

11.4 Establish instrument software run procedures for quantitative analysis. Because the analytes are eluted from the preconcentration column in a transient manner, it is recommended that the instrument software is configured in a rapid scan/peak hopping mode. The instrument is now ready to be calibrated.

11.5 Reconnect the preconcentration system to the ICP-MS instrument. With valves A and B in the off position and valve C in the on position, load sample through the sample loop to waste using pump P4 for 4 min at 4 mL/min. Switch on the carrier pump (P3) and pump 1% nitric acid to the nebulizer of the ICP-MS instrument at a flow rate of 0.8-1.0-mL/min.

11.6 Switch on the buffer pump (P2), and pump 2M ammonium acetate at a flow rate of 1.0 mL/min.

11.7 Preconcentration of the sample may be achieved by running through an eluent pump program (P1) sequence similar to that illustrated in Table 2. The exact timing of this sequence should be modified according to the internal volume of the connecting tubing and the specific hardware configuration used.

11.7.1 Inject sample -- With valves A, B, and C on, load sample from the loop onto the column using 1M ammonium acetate for 4.5 min at 4.0 mL/min. The analytes are retained on the column, while the majority of the matrix is passed through to waste.

11.7.2 Elute analytes -- Turn off valves A and B and begin eluting the analytes by pumping 1.25M nitric acid through the column at 4.0 mL/min, then turn off valve C and pump the eluted analytes into the ICP-MS instrument at 1.0 mL/min. Initiate ICP-MS software data acquisition and integrate the eluted analyte profiles.

11.7.3 Column Reconditioning -- Turn on valve C to direct column effluent to waste, and pump 1.25M nitric acid, 1M ammonium acetate, 1.25M nitric acid and 1M ammonium acetate alternately through the column at 4.0 mL/min. During this process, the next sample can be loaded into the sample loop using the sample pump (P4).

11.8 Repeat the sequence described in Section 11.7 for each sample to be analyzed. At the end of the analytical run leave the column filled with 1M ammonium acetate buffer until it is next used.

11.9 Samples having concentrations higher than the established linear dynamic range should be diluted into range with 1% HNO_3 (v/v) and reanalyzed.

12.0 Data Analysis and Calculations

12.1 Analytical isotopes and elemental equations recommended for sample data calculations are listed in Table 3. Sample data should be reported in units of μg/L. Do not report element concentrations below the determined MDL.

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

12.3 Reported values should be calibration blank subtracted. If additional dilutions were made to any samples, the appropriate factor should be applied to the calculated sample concentrations.

12.4 Data values should be corrected for instrument drift by the application of internal standardization. Corrections for characterized spectral interferences should be applied to the data.

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

13.0 Method Performance

13.1 Experimental conditions used for single laboratory testing of the method are summarized in Table 4.

13.2 Data obtained from single laboratory testing of the method are summarized in Tables 5 and 6 for two reference water samples consisting of National Research Council Canada (NRCC) Estuarine Water (SLEW-1) and Seawater (NASS-2). The samples were prepared using the procedure described in Section 11.1.1. For each matrix, three replicates were analyzed and the average of the replicates was used to determine the sample concentration for each analyte. Two further sets of three replicates were fortified at different concentration levels, one set at 0.5 • g/L, the other at 10 μg/L. The sample concentration, mean percent recovery, and the relative standard deviation of the fortified replicates are listed for each method analyte. The reference material certificate values are also listed for comparison.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that place pollution pre-

vention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation (e.g., Section 7.8). When wastes cannot be feasibly reduced at the source, the Agency recommends recy--cling as the next best option.

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

15.0 Waste Management

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

16.0 References

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7. OSHA Safety and Health Standards, General Industry, (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).

8. Safety in Academic Chemistry Laboratories, American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

9. Code of Federal Regulations 40, Ch. 1, Pt. 136 Appendix B.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

Table 1. Total Recoverable Method Detection Limits for Reagent Water

Element	Recommended Analytical Mass	MDL[1] • g/L
Cadmium	111	0.041
Cobalt	59	0.021
Copper	63	0.023
Lead	206, 207,208	0.074
Nickel	60	0.081
Uranium	238	0.031
Vanadium	51	0.014

[1] Determined using 10-mL sample loop.

Table 2. Eluent Pump Programming Sequence for Preconcentration of Trace Elements

Time (min)	Flow (mL/min)	Eluent	Valve A,B	Valve C
0.0	4.0	1M ammonium acetate	ON	ON
4.5	4.0	1.25M nitric acid	ON	ON
5.1	1.0	1.25M nitric acid	OFF	ON
5.5	1.0	1.25M nitric acid	OFF	OFF
7.5	4.0	1.25M nitric acid	OFF	ON
8.0	4.0	1M ammonium acetate	OFF	ON
10.0	4.0	1.25M nitric acid	OFF	ON
11.0	4.0	1M ammonium acetate	OFF	ON
12.5	0.0		OFF	ON

Table 3. Recommended Analytical Isotopes and Elemental Equations for Data Calculations

Element	Isotope	Elemental Equation	Note
Cd	106, 108, *111*, 114	$(1.000)(^{111}C)-(1.073)[(^{108}C)-(0.712)(^{106}C)]$	(1)
Co	59	$(1.000)(^{59}C)$	
Cu	*63*, 65	$(1.000)(^{63}C)$	
Pb	*206, 207, 208*	$(1.000)(^{206}C)+(1.000)(^{207}C)+(1.000)(^{208}C)$	(2)
Ni	60	$(1.000)(^{60}C)$	
U	238	$(1.000)(^{238}C)$	
V	51	$(1.000)(^{51}C)$	

C - calibration blank subtracted counts at specified mass.
(1) - correction for MoO interference. An additional isobaric elemental correction should be made if palladium is present.
(2) - allowance for isotopic variability of lead isotopes.
NOTE: As a minimum, all isotopes listed should be monitored. Isotopes recommended for analytical determination are italized.

Table 4. Experimental Conditions for Single Laboratory Validation

Chromatography	
Instrument	Dionex chelation system
Preconcentration column	Dionex MetPac CC-1
ICP-MS Instrument Conditions	
Instrument	VG PlasmaQuad Type I
Plasma forward power	1.35 kW
Coolant flow rate	13.5 L/min
Auxiliary flow rate	0.6 L/min
Nebulizer flow rate	0.78 L/min
Internal standards	Sc, Y, In, Tb
Data Acquisition	
Detector mode	Pulse counting
Mass range	45-240 amu
Dwell time	160 • s
Number of MCA channels	2048
Number of scan sweeps	250

Table 5. Precision and Recovery Data for Estuarine Water (SLEW-1)

Analyte	Certificate (• g/L)	Sample Conc. (• g/L)	Spike Addition (• g/L)	Average Recovery (%)	RSD (%)	Spike Addition (• g/L)	Average Recovery (%)	RSD (%)
Cd	0.018	<0.041	0.5	94.8	9.8	10	99.6	1.1
Co	0.046	0.078	0.5	102.8	4.0	10	96.6	1.4
Cu	1.76	1.6	0.5	106.0	2.7	10	96.0	4.8
Pb	0.028	<0.074	0.5	100.2	4.0	10	106.9	5.8
Ni	0.743	0.83	0.5	100.0	1.5	10	102.0	2.1
U	--	1.1	0.5	96.7	7.4	10	98.1	3.6
V	--	1.4	0.5	100.0	3.2	10	97.0	4.5

-- No certificate value

Table 6. Precision and Recovery Data for Seawater (NASS-2)

Analyte	Certificate (• g/L)	Sample Conc. (• g/L)	Spike Addition (• g/L)	Average Recovery (%)	RSD (%)	Spike Addition (• g/L)	Average Recovery (%)	RSD (%)
Cd	0.029	<0.041	0.5	101.8	1.0	10	96.4	3.7
Co	0.004	<0.021	0.5	98.9	3.0	10	99.2	1.7
Cu	0.109	0.12	0.5	95.8	2.3	10	93.1	0.9
Pb	0.039	<0.074	0.5	100.6	8.5	10	92.1	2.6
Ni	0.257	0.23	0.5	102.2	2.3	10	98.2	1.2
U	3.00	3.0	0.5	94.0	0.7	10	98.4	1.7
V	--	1.7	0.5	104.0	3.4	10	109.2	3.7

--No certificate value

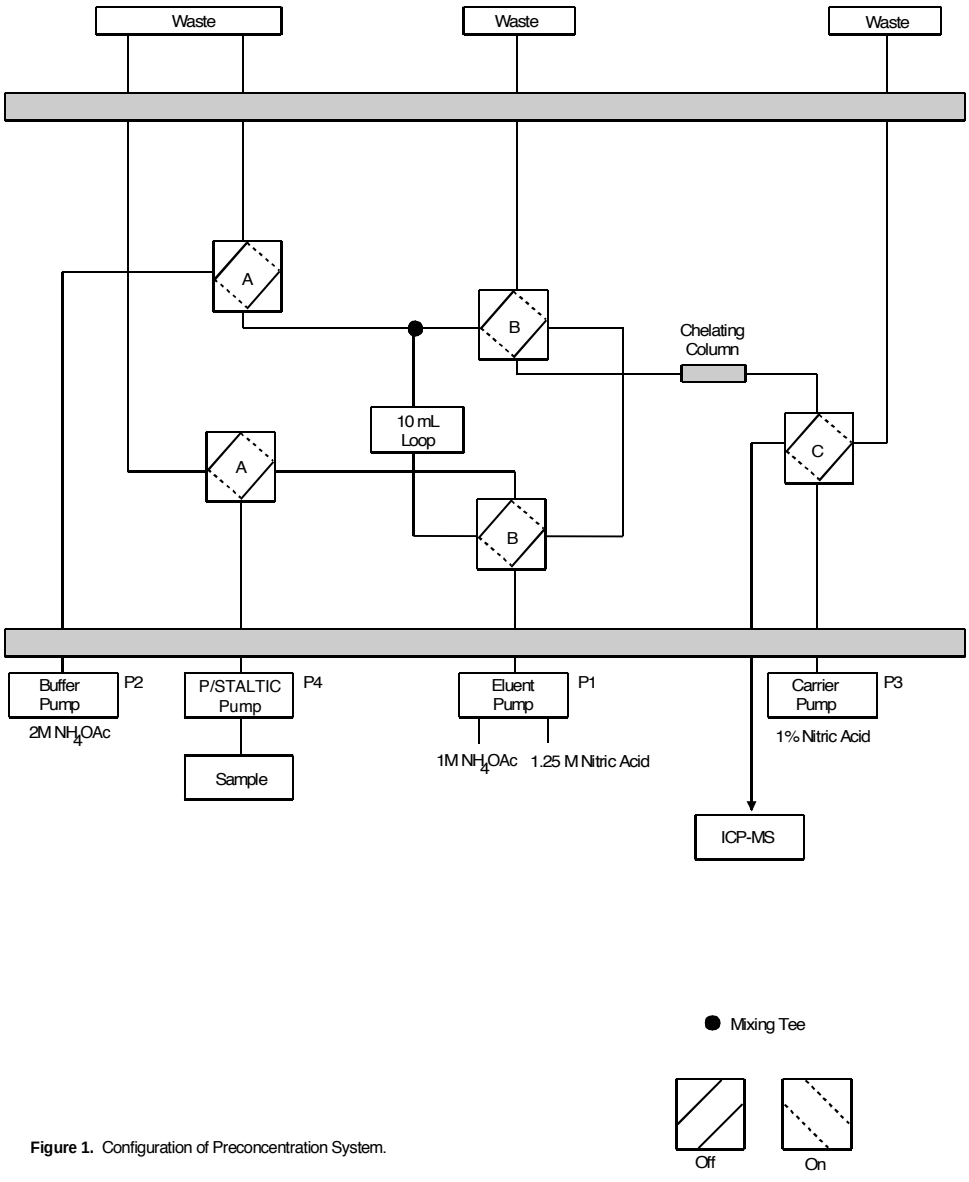

Figure 1. Configuration of Preconcentration System.

Appendix 2

USEPA Method 200.13

Determination of Trace Elements in Marine Waters by Off-Line Chelation Preconcentration with Graphite Furnace Atomic Absorption

Revision 1.0

September 1997

Method 200.13

Determination of Trace Elements in Marine Waters by Off-Line Chelation Preconcentration with Graphite Furnace Atomic Absorption

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Revision 1.0
September 1997

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Method 200.13

Determination of Trace Elements in Marine Waters by Off-Line Chelation Preconcentration with Graphite Furnace Atomic Absorption

1.0 Scope and Application

1.1 This method describes procedures for preconcentration and determination of total recoverable trace elements in marine waters, including estuarine water, seawater and brines.

1.2 Acid solubilization is required prior to determination of total recoverable elements to facilitate breakdown of complexes or colloids which might influence trace element recoveries. This method should only be used for preconcentration and determination of trace elements in aqueous samples.

1.3 This method is applicable to the following elements:

Element		Chemical Abstracts Service Registry Numbers (CASRN)
Cadmium	(Cd)	7440-43-9
Cobalt	(Co)	7440-48-4
Copper	(Cu)	7440-50-8
Lead	(Pb)	7439-92-1
Nickel	(Ni)	7440-02-0

1.4 Method detection limits (MDLs) for these elements will be dependent on the specific instrumentation employed and the selected operating conditions. MDLs in NASS-3 (Reference Material, National Research Council of Canada) were determined using the procedure described in Section 9.2.4 and are listed in Table 1.

1.5 A minimum of 6-months experience in graphite furnace atomic absorption (GFAA) is recommended.

2.0 Summary of Method

2.1 Nitric acid is dispensed into a beaker containing an accurately weighed or measured, well-mixed, homogeneous aqueous sample. The sample volume is reduced to approximately 20 mL and then covered and allowed to reflux. The resulting solution is diluted to volume and is ready for analysis.

2.2 This method is used to preconcentrate trace elements using an iminodiacetate functionalized chelating resin.[1,2] Following acid solubilization, the sample is buffered using an on-line system prior to entering the chelating column. Group I and II metals, as well as most anions, are selectively separated from the analytes by elution with ammonium acetate at pH 5.5. The analytes are subsequently eluted into a simplified matrix consisting of 0.75 M nitric acid and are determined by GFAA.

3.0 Definitions

3.1 Calibration Blank (CB) -- A volume of reagent water fortified with the same matrix as the calibration standards, but without the analytes, internal standards, or surrogate analytes.

3.2 Calibration Standard (CAL) -- A solution prepared from the primary dilution standard solution or stock standard solutions and the internal standards and surrogate analytes. The CAL solutions are used to calibrate the instrument response with respect to analyte concentration.

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

3.4 Instrument Performance Check Solution (IPC) -- A solution of one or more method analytes, surrogates, internal standards, or other test substances used to evaluate the performance of the instrument system with respect to a defined set of criteria.

3.5 Laboratory Fortified Blank (LFB) -- An aliquot of reagent water or other blank matrices to which known quantities of the method analytes are added in the laboratory. The LFB is analyzed exactly like a sample, and its purpose is to determine whether the methodology

is in control, and whether the laboratory is capable of making accurate and precise measurements.

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

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

3.8 Linear Dynamic Range (LDR) -- The absolute quantity or concentration range over which the instrument response to an analyte is linear.

3.9 Matrix Modifier (MM) -- A substance added to the instrument along with the sample in order to minimize the interference effects by selective volatilization of either analyte or matrix components.

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

3.11 Quality Control Sample -- A solution of method analytes of known concentrations which is used to fortify an aliquot of LRB or sample matrix. The QCS is obtained from a source external to the laboratory and different from the source of calibration standards. It is used to check laboratory performance with externally prepared test materials.

3.12 Standard Addition -- The addition of a known amount of analyte to the sample in order to determine the relative response of the detector to an analyte within the sample matrix. The relative response is then used to assess either an operative matrix effect or the sample analyte concentration.

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

3.14 Total Recoverable Analyte (TRA) -- The concentration of analyte determined to be in either a solid sample or an unfiltered aqueous sample following treatment by refluxing with hot dilute mineral acid(s) as specified in the method.

4.0 Interferences

4.1 Several interference sources may cause inaccuracies in the determination of trace elements by GFAA. These interferences can be classified into three major subdivisions: spectral, matrix, and memory. Some of these interferences can be minimized via the preconcentration step, which reduces the Ca, Mg, Na and Cl concentration in the sample prior to GFAA analysis.

4.2 Spectral interferences are caused by absorbance of light by a molecule or atom which is not the analyte of interest or emission from black body radiation.

4.2.1 Spectral interferences caused by an element only occur if there is a spectral overlap between the wavelength of the interfering element and the analyte of interest. Fortunately, this type of interference is relatively uncommon in STPGFAA (Stabilized Temperature Platform Graphite Furnace Atomic Absorption) because of the narrow atomic line widths associated with STPGFAA.

In addition, the use of appropriate furnace temperature programs and high spectral purity lamps as light sources can minimize the possibility of this type of interference. However, molecular absorbances can span several hundred manometers, producing broadband spectral interferences. This type of interference is far more common in STPGFAA. The use of matrix modifiers, selective volatilization, and background correctors are all attempts to eliminate unwanted nonspecific absorbance. Because the nonspecific component of the total absorbance can vary considerably from sample type to sample type, to provide effective background correction and eliminate the elemental spectral interference of palladium on copper and iron on selenium, the exclusive use of Zeeman background correction is specified in this method.

4.2.2 Spectral interferences are also caused by emissions from black body radiation produced during the atomization furnace cycle. This black body emission

reaches the photomultiplier tube, producing erroneous results. The magnitude of this interference can be minimized by proper furnace tube alignment and monochromator design. In addition, atomization temperatures which adequately volatilize the analyte of interest without producing unnecessary black body radiation can help reduce unwanted background emission produced during atomization.

4.3 Matrix interferences are caused by sample cornponents which inhibit formation of free atomic analyte atoms during the atomization cycle. In this method the use of a delayed atomization device which provides warmer gas phase temperatures is required. These devices provide an environment which is more conducive to the formation of free analyte atoms and thereby minimize this type of interference. This type of interference can be detected by analyzing the sample plus a sample aliquot fortified with a known concentration of the analyte. If the determined concentration of the analyte addition is outside a designated range, a possible matrix effect should be suspected (Section 9.4).

4.4 Memory interferences result from analyzing a sample containing a high concentration of an element (typically a high atomization temperature element) which cannot be removed quantitatively in one complete set of furnace steps. The analyte which remains in the furnace can produce false positive signals on subsequent sample(s). Therefore, the analyst should establish the analyte concentration which can be injected into the furnace and adequately removed in one complete set of furnace cycles. If this concentration is exceeded, the sample should be diluted and a blank analyzed to assure the memory effect has been eliminated before reanalyzing the diluted sample.

4.5 Low recoveries may be encountered in the preconcentration cycle if the trace elements are complexed by competing chelators (humic/fulvic) in the sample or are present as colloidal material. Acid solubilization pretreatment is employed to improve analyte recovery and to minimize adsorption, hydrolysis and precipitation effects.

4.6 Memory interferences from the chelating system may be encountered, especially after analyzing a sample containing high analyte concentrations. A thorough column rinsing sequence following elution of the analytes is necessary to minimize such interferences.

5.0 Safety

5.1 The toxicity or carcinogenicity of each reagent used in this method has not been fully established. Each chemical should be regarded as a potential health hazard and exposure to these compounds should be as low as reasonably achievable. Each laboratory is responsible for maintaining a current awareness file of OSHA regulations regarding the safe handling of the chemicals specified in this method.[3-6] A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Specifically, concentrated nitric and hydrochloric acids present various hazards and are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear safety glasses or a shield for eye protection, protective clothing and observe proper mixing when working with these reagents.

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

5.3 All personnel handling environmental samples known to contain or to have been in contact with human waste should be immunized against known disease causative agents.

5.4 The graphite tube during atomization emits intense UV radiation. Suitable precautions should be taken to protect personnel from such a hazard.

5.5 The use of the argon/hydrogen gas mixture during the dry and char steps may evolve a considerable amount of HCI gas. Therefore, adequate ventilation is required.

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

6.0 Equipment and Supplies

6.1 Graphite Furnace Atomic Absorption Spectrometer

6.1.1 The GFAA spectrometer must be capable of programmed heating of the graphite tube and the

associated delayed atomization device. The instrument should be equipped with an adequate background correction device capable of removing undesirable non-specific absorbance over the spectral region of interest. The capability to record relatively fast (< 1 sec) transient signals and evaluate data on a peak area basis is preferred. In addition, a recirculating refrigeration unit is recommended for improved reproducibility of furnace temperatures. The data shown in the tables were obtained using the stabilized temperature platform and Zeeman background correction.

6.1.2 Single element hollow cathode lamps or single element electrodeless discharge lamps along with the associated power supplies.

6.1.3 Argon gas supply (high-purity grade, 99.99%).

6.1.4 A 5% hydrogen in argon gas mix and the necessary hardware to use this gas mixture during specific furnace cycles.

6.1.5 Autosampler-- Although not specifically required, the use of an autosampler is highly recommended.

6.1.6 Graphite Furnace 0perating Conditions -- A guide to experimental conditions for the applicable elements is provided in Table 1.

6.2 Preconcentration System -- System containing no metal parts in the analyte flow path, configured as shown with a sample loop in Figure 1 and without a sample loop in Figure 2.

6.2.1 Column -- Macroporous iminodiacetate chelating resin (Dionex Metpac CC-1 or equivalent).

6.2.2 Control valves -- Inert double stack, pneumatically operated four-way slider valves with connectors.

6.2.2.1 Argon gas supply regulated at 80-100 psi.

6.2.3 Solution reservoirs -- Inert containers, e.g., high density polyethylene (HDPE), for holding eluent and carrier reagents.

6.2.4 Tubing -- High pressure, narrow bore, inert tubing such as Tefzel ETFE (ethylene tetra-fluoro ethylene) or equivalent for interconnection of pumps/ valve assemblies and a minimum length for connection of the pre-concentration system with the sample collection vessel.

6.2.5 Eluent pumping system (Gradient Pump) -- Programmable flow, high-pressure pumping system, capable of delivering either one of three eluents at a pressure up to 2000 psi and a flow rate of 1-5 mL/min.

6.2.6 System setup, including sample loop (See Figure 1).

6.2.6.1 Sample loop -- 10-mL loop constructed from narrow bore, high-pressure inert tubing, Tefzel ETFE or equivalent.

6.2.6.2 Auxiliary pumps -- On-line buffer pump, piston pump (Dionex QIC pump or equivalent) for delivering 2M ammonium acetate buffer solution; carrier pump, peristaltic pump (Gilson Minipuls or equivalent) for delivering 1% nitric acid carrier solution; sample pump, peristaltic pump for loading sample loop.

6.2.7 System setup without sample loop (See Figure 2).

6.2.7.1 Auxiliary Pumps - Sample pump (Dionex QIC Pump or equivalent) for loading sample on the column. Carrier pump (Dionex QIC Pump or equivalent) used to flush collection line between samples.

6.3 Labware -- For determination of trace elements, contamination and loss are of **critical** consideration. Potential contamination sources include improperly cleaned laboratory apparatus and general contamination within the laboratory environment. A clean laboratory work area, designated for trace element sample handling must be used. Sample containers can introduce positive and negative errors in determination of trace elements by (1) contributing contaminants through surface desorption or leaching and (2) depleting element concentrations through adsorption processes. For these reasons, borosilicate glass is <u>not</u> recommended for use with this method. All labware in contact with the sample should be cleaned prior to use. Labware may be soaked overnight and thoroughly washed with laboratory-grade detergent and water, rinsed with water, and soaked for 4 h in a mixture of dilute nitric and hydrochloric acids, followed by rinsing with ASTM type I water and oven drying.

6.3.1 Griffin beakers, 250 mL, polytetrafluoroethylene (PTFE) or quartz.

6.3.2 Storage bottles -- Narrow mouth bottles, Teflon FEP (fluorinated ethylene propylene), or HDPE, 125-mL and 250-mL capacities.

6.4 Sample Processing Equipment

6.4.1 Air displacement pipetter -- Digital pipet system capable of delivering volumes from 100 to 2500 µL with an assortment of metal-free, disposable pipet tips.

6.4.2 Balances -- Analytical balance, capable of accurately weighing to ± 0.1 mg; top pan balance, accurate to ± 0.01 g.

6.4.3 Hot plate -- Corning PC100 or equivalent.

6.4.4 Centrifuge -- Steel cabinet with guard bowl, electric timer and brake.

6.4.5 Drying oven -- Gravity convection oven with thermostatic control capable of maintaining 105•C ± 5•C.

6.4.6 pH meter -- Bench mounted or hand-held electrode system with a resolution of ± 0.1 pH units.

6.4.7 Class 100 hoods are recommended for all sample handling.

7.0 Reagents and Standards

7.1 Reagents may contain elemental impurities which might affect analytical data. Only high-purity reagents that conform to the American Chemical Society specifications[7] should be used whenever possible. If the purity of a reagent is in question, analyze for contamination. All acids used for this method must be of ultra high-purity grade or equivalent. Suitable acids are available from a number of manufacturers. Redistilled acids prepared by sub-boiling distillation are acceptable.

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

7.1.1.1 Nitric acid 0.75M -- Dilute 47.7 mL (67.3g) conc. nitric acid to 1000 mL with ASTM type I water.

7.1.1.2 Nitric acid (1+1) -- Dilute 500 mL conc. nitric acid to 1000 mL with ASTM type I water.

7.1.1.3 Nitric acid (1+9) -- Dilute 100 mL conc. nitric acid to 1000 mL with ASTM type I water.

7.1.2 Matrix Modifier, dissolve 300 mg Palladium (Pd) powder in a minimum amount of concentrated $HN0_3$ (1 mL of HNO_3, adding concentrated HCl only if necessary). Dissolve 200 mg of $Mg(NO_3)_2$•$6H_2O$ in ASTM type I water. Pour the two solutions together and dilute to 100 mL with ASTM type I water.

Note: It is recommended that the matrix modifier be analyzed separately in order to assess the contribution of the modifier to the overall laboratory blank.

7.1.3 Acetic acid, glacial (sp.gr. 1.05). High purity acetic acid is recommended.

7.1.4 Ammonium hydroxide (20%). High purity ammonium hydroxide is recommended.

7.1.5 Ammonium acetate buffer 1M, pH 5.5 -- Add 58 mL (60.5 g) of glacial acetic acid to 600 mL of ASTM type I water. Add 65 mL (60 g) of 20% ammonium hydroxide and mix. Check the pH of the resulting solution by withdrawing a small aliquot and testing with a calibrated pH meter, adjusting the solution to pH 5.5 ± 0.1 with small volumes of acetic acid or ammonium hydroxide as necessary. Cool and dilute to 1 L with ASTM type I water.

7.1.6 Ammonium acetate buffer 2M, pH 5.5 -- Prepare as for Section 7.1.5 using 116 mL (121 g) glacial acetic acid and 130 mL (120 g) 20% ammonium hydroxide, diluted to 1000 mL with ASTM type I water.

Note: If the system is configured as shown in Figure 1, the ammonium acetate buffer solutions may be further purified by passing them through the chelating column at a flow rate of 5.0 mL/min. Collect the purified solution in a container. Following this, elute the collected contaminants from the column using 0.75M nitric acid for 5 min at a flow rate of 4.0 mL/min. If the system is configured as shown in Figure 2, the majority of the buffer is being purified in an on-line configuration via the clean-up column.

7.1.7 Oxalic acid dihydrate (CASRN 6153-56-6), 0.2M -- Dissolve 25.2 g reagent grade $C_2H_2O_4$•$2H_2O$ in 250 mL ASTM type I water and dilute to 1000 mL with ASTM type I water. **CAUTION -** Oxalic acid is toxic; handle with care.

7.2 Water -- For all sample preparation and dilutions, ASTM type I water (ASTM D1193) is required.

7.3 Standard Stock Solutions -- May be purchased from a reputable commercial source or prepared from ultra high-purity grade chemicals or metals (99.99 - 99.999% pure). All salts should be dried for one hour at 105•C, unless otherwise specified. (CAUTION - Many metal salts are extremely toxic if inhaled or swallowed. Wash hands thoroughly after handling.) Stock solutions should be stored in plastic bottles. The following procedures may be used for preparing standard stock solutions:

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

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

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

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

7.3.4 Lead solution, stock 1 mL = 1000 µg Pb -- Dissolve 0.1599 g $PbNO_3$ in 5 mL (1+1) nitric acid. Dilute to 100 mL with ASTM type I water.

7.3.5 Nickel solution, stock 1 mL = 1000 µg Ni -- Dissolve 0.100 g nickel powder in 5 mL conc. nitric acid, heating to effect solution. Cool and dilute to 100 mL with ASTM type I water.

7.4 Multielement Stock Standard Solution -- Care must be taken in the preparation of multielement stock standards that the elements are compatible and stable. Originating element stocks should be checked for the presence of impurities which might influence the accuracy of the standard. Freshly prepared standards should be transferred to acid cleaned, new FEP or HDPE bottles for storage and monitored periodically for stability. A multielement stock standard solution containing cadmium, cobalt, copper, lead, and nickel may be prepared by diluting an appropriate aliquot of each single element stock in the list to 100 mL with ASTM type I water containing 1% (v/v) nitric acid.

7.4.1 Preparation of calibration standards -- Fresh multielement calibration standards should be prepared weekly. Dilute the stock multielement standard solution in 1% (v/v) nitric acid to levels appropriate to the required operating range. The element concentrations in the standards should be sufficiently high to produce good measurement precision and to accurately define the slope of the response curve.

7.5 Blanks -- Four types of blanks are required for this method. A calibration blank is used to establish the analytical calibration curve, the laboratory reagent blank (LRB) is used to assess possible contamination from the sample preparation procedure and to assess spectral background. The laboratory fortified blank is used to assess routine laboratory performance, and a rinse blank is used to flush the instrument autosampler uptake system. All diluent acids should be made from concentrated acids (Section 7.1) and ASTM type I water.

7.5.1 The calibration blank consists of the appropriate acid diluent in ASTM type I water. The calibration blank should be stored in a FEP bottle.

7.5.2 The laboratory reagent blanks must contain all the reagents in the same volumes as used in processing the samples. The preparation blank must be carried through the entire sample digestion and preparation scheme.

7.5.3 The laboratory fortified blank (LFB) is prepared by fortifying an aliquot of the laboratory reagent blank with all analytes to provide a final concentration which will produce an absorbance of approximately 0.1 for each analyte. The LFB must be carried through the complete procedure as used for the samples.

7.5.4 The rinse blank is prepared as needed by adding 1.0 mL of conc. HNO_3 and 1.0 mL conc. HCl to 1 L of ASTM Type I water and stored in a convenient manner.

7.6 Instrument Performance Check (IPC) Solution -- The IPC solution is used to periodically verify instrument performance during analysis. The IPC solution should be a fortified seawater prepared in the same acid mixture as the calibration standards and should contain method analytes such that the resulting absorbances are near the midpoint of the calibration curve. The IPC solution should be prepared from the same standard stock solutions used to prepare the calibration standards and stored in a FEP bottle. Agency programs may specify or request that additional instrument performance check solutions be prepared at specified concentrations in order to meet particular program needs.

7.7 Quality Control Sample (QCS) -- A quality control sample having certified concentrations of the analytes of interest should be obtained from a source outside the

laboratory. Dilute the QCS if necessary with 1% nitric acid, such that the analyte concentrations fall within the proposed instrument calibration range.

8.0 Sample Collection, Preservation and Storage

8.1 Prior to collection of an aqueous sample, consideration should be given to the type of data required, so that appropriate preservation and pretreatment steps can be taken. Acid preservation, etc., should be performed at the time of sample collection or as soon thereafter as practically possible. The pH of all aqueous samples must be tested immediately prior to aliquoting for analysis to ensure the sample has been properly preserved. If properly acid-preserved, the sample can be held up to 6 months before analysis.

8.2 For determination of total recoverable elements in aqueous samples, acidify with (1+1) nitric acid at the time of collection to pH < 2. Normally 3 mL of (1+1) acid per liter of sample is sufficient. The sample should not be filtered prior to analysis.

Note: Samples that cannot be acid-preserved at the time of collection because of sampling limitations or transport restrictions, or have pH > 2 because of high alkalinity should be acidified with nitric acid to pH < 2 upon receipt in the laboratory. Following acidification, the sample should be held for 16 h and the pH verified to be <2 before withdrawing an aliquot for sample processing.

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

9.0 Quality Control

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

9.2 Initial Demonstration of Performance (Mandatory)

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

9.2.2 Linear dynamic range (LDR) -- The upper limit of the LDR. must be established for the wavelength utilized for each analyte by determining the signal responses from a minimum of 6 different concentration standards across the range, two of which are close to the upper limit of the LDR. Determined LDRs must be documented and kept on file. The linear calibration range which may be used for analysis of samples should be judged by the analyst from the resulting data. The upper LDR. limit should be an observed signal no more than 10% below the level extrapolated from the four lower standards. New LDRs should be determined whenever there is a significant change in instrument response, a change in instrument analytical hardware or operating conditions.

Note: Multiple cleanout furnace cycles may be necessary in order to fully define or utilize the LDR. for certain elements such as nickel. For this reason, the upper limit of the linear calibration range may not correspond to the upper LDR limit.

Measured sample analyte concentrations that exceed the upper limit of the linear calibration range must either be diluted and reanalyzed with concern for memory effects (Section 4.4) or analyzed by another approved method.

9.2.3 Quality control sample (QCS) -- When beginning the use of this method, on a quarterly basis or as required to meet data-quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS (Section 7.7). If the determined concentrations are not within ± 10% of the stated values, performance of the determinative step of the method is unacceptable. The source of the problem must be identified and corrected before either proceeding on with the initial determination of method detection limits or continuing with ongoing analyses.

9.2.4 Method detection limit (MDL) -- MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit.[8] To determine MDL values, take seven replicate aliquots of the fortified

reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

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

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

Note: If the relative standard deviation (RSD) from the analyses of the seven aliquots is < 15%, the concentration used to determine the analyte MDL may have been in appropriately high for the determination. If so, this could result in the calculation of an unrealistically low MDL. If additional confirmation of the MDL is desired, reanalyze the seven replicate aliquots on two more nonconsecutive days and again calculate the MDL values for each day. An average of the three MDL values for each analyte may provide for a more appropriate MDL estimate. Determination of MDL in reagent water represents a best case situation and does not reflect possible matrix effects of real world samples. However, successful analyses of LFMs (Section 9.4) can give confidence to the MDL value determined in reagent water. Typical single laboratory MDL values using this method are given in Table 1. MDLs should be determined every 6 months, when a new operator begins work, or whenever there is a significant change in the background or instrument response.

9.3 Assessing Laboratory Performance (Mandatory)

9.3.1 Laboratory reagent blank (LRB) - The laboratory must analyze at least one LRB (Section 7.5.2) with each batch of 20 or fewer samples. LRB data are used to assess contamination from the laboratory environment. LRB values that exceed the MDL indicate laboratory or reagent contamination should be suspected. Any determined source of contamination must be corrected and the samples reanalyzed for the affected analytes after acceptable LRB values have been obtained.

9.3.2 Laboratory fortified blank (LFB) -- The laboratory must analyze at least one LFB (Section 7.5.3) with each batch of samples. Calculate accuracy as percent recovery (Section 9.4.3). If the recovery of any analyte falls outside the required control limits of 85-115%, that analyte is judged out of control, and the source of the problem should be identified and resolved before continuing analyses.

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

$$\text{Upper Control Limit} = x + 3S$$

$$\text{Lower Control Limit} = x - 3S$$

The optional control limits must be equal to or better than the required control limits of 85-115%. After each 5-10 new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an ongoing precision statement for the level of concentrations included in the LFB. These data must be kept on file and be available for review.

9.3.4 Instrument Performance Check (IPC) Solution -- For all determinations the laboratory must analyze the IPC solution (Section 7.6) and a calibration blank immediately following each calibration, after every tenth sample (or more frequently, if required) and at the end of the sample run. The IPC solution should be a fortified seawater matrix. Analysis of the IPC solution and calibration blank immediately following calibration must verify that the instrument is within ±10% of calibration. Subsequent analyses of the IPC solution must be within ±10% of calibration. If the calibration cannot be verified within the specified limits, reanalyze the IPC solution. If the second analysis of the IPC solution confirms calibration to be outside the limits, sample analysis must be discontinued, the cause determined and/or in the case of drift the instrument recalibrated. All samples following the last acceptable IPC solution must be reanalyzed. The analysis data of the calibration blank and IPC solution must be kept on file with the sample analyses data.

9.3.5 The overall sensitivity and precision of this method are strongly influenced by a laboratory's ability to control the method blank. Therefore, it is recommended that the calibration blank response be recorded for each set of samples. This record will aid the laboratory in assessing both its long- and short-term ability to control the method blank.

9.4 Assessing Analyte Recovery and Data Quality

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

9.4.2 The laboratory must add a known amount of each analyte to a minimum of 10% of routine samples. In each case, the LFM aliquot must be a duplicate of the aliquot used for sample analysis and for total recoverable determinations added prior to sample preparation. For water samples, the added analyte concentration must be the same as that used in the laboratory fortified blank (Section 7.5.3). Over time, samples from all routine sample sources should be fortified.

9.4.3 Calculate the percent recovery for each analyte, corrected for concentrations measured in the unfortified sample, and compare these values to the designated LFM recovery range of 75-125%. Recovery calculations are not required if the concentration added is <25% of the unfortified sample concentration. Percent recovery may be calculated in units appropriate to the matrix, using the following equation:

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

where, R = percent recovery.
C_s = fortified sample concentration.
C = sample background concentration.
s = concentration equivalent of analyte added to sample.

9.4.4 If the recovery of any analyte falls outside the designated LFM recovery range (but is still within the range of calibration and the background absorbance is < 1 abs.) and the laboratory performance for that analyte is shown to be in control (Section 9.3), the recovery problem encountered with the LFM is judged to be either matrix or solution related, not system related. This situation should be rare given the matrix elimination preconcentration step prior to analysis. If a low recovery is found, check the pH of the sample plus the buffer mixture. The resulting pH should be about 5.5. The pH of the sample strongly influences the column's ability to preconcentrate the metals; therefore, a low recovery may be caused by a low pH. If the pH for the LFM/buffer mixture is about 5.5, the analyst is advised to make an in furnace analyte addition to the LFM using the preconcentrated standard solution. If recovery of the in furnace analyte addition is shown to be out of control, a matrix interference is confirmed and the sample must be analyzed by MSA.

9.5 Utilizing Reference Materials

9.5.1 It is recommended that a reference material such as NASS-3 (from the Research Council of Canada) be fortified and used as an Instrument Performance Check Solution.

10.0 Calibration and Standardization

10.1 The preconcentration system can be configured utilizing a sample loop to define the sample volume (Figure 1) or the system can be configured such that a sample pump rate and a pumping time defines the sample volume (Figure 2). The system illustrated in Figure 1 is recommended for sample sizes of <10 mL. A thorough rinsing of the sample loop between samples with HNO_3 is required. This rinsing will minimize the cross-contamination which may be caused by the sample loop. The system in Figure 2 should be used for sample volumes of >10 mL. The sample pump used in Figure 2 must be calibrated to assure that a reproducible/defined volume is being delivered.

10.2 Specific wavelengths and instrument operating conditions are listed in Table 1. However, because of differences among makes and models of spectrophotometers and electrothermal furnace devices, the actual instrument conditions selected may vary from those listed.

10.3 Prior to the use of this method, instrument operating conditions must be optimized. The analyst should follow the instructions provided by the manufacturer while using the conditions listed in Table 1 as a guide. Of particular importance is the determination of the charring temperature limit for each analyte. This limit is the furnace temperature setting where a loss in analyte will occur prior to atomization. This limit should be determined by conducting char temperature profiles for each analyte and when necessary, in the matrix of question. The charring temperature selected should minimize background absorbance while providing some furnace temperature variation without loss of analyte. For routine analytical operation the charring temperature is usually

set at least 100•C below this limit. The optimum conditions selected should provide the lowest reliable MDLs and be similar to those listed in Table 1. Once the optimum operating conditions are determined, they should be recorded and available for daily reference.

10.4 Prior to an initial calibration, the linear dynamic range of the analyte must be determined (Section 9.2.2) using the optimized instrument operating conditions. For all determinations allow an instrument and hollow cathode lamp warm-up period of not less than 15 min. If an EDL is to be used, allow 30 min for warm-up.

10.5 Before using the procedure (Section 11.0) to analyze samples, data must be available to document initial demonstration of performance. The required data and procedure are described in Section 9.2. This data must be generated using the same instrument operating conditions and calibration routine (Section 11.4) to be used for sample analysis. These documented data must be kept on file and be available for review by the data user.

11.0 Procedure

11.1 Sample Preparation -- Total Recoverable Elements

11.1.1 Add 2 mL (1+1) nitric acid to the beaker containing 100 mL of sample. Place the beaker on the hot plate for solution evaporation. The hot plate should be located in a fume hood and previously adjusted to provide evaporation at a temperature of approximately but no higher than 85•C. (See the following note.) The beaker should be covered with an elevated (ribbed) watch glass or other necessary steps should be taken to prevent sample contamination from the fume hood environment.

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

11.1.2 Reduce the volume of the sample aliquot to about 20 mL by gentle heating at 85•C. DO NOT BOIL. This step takes about 2 hr for a 100-mL aliquot with the rate of evaporation rapidly increasing as the sample volume approaches 20 mL. (A spare beaker containing 20 mL of water can be used as a gauge.)

11.1.3 Cover the lip of the beaker with a watch glass to reduce additional evaporation and gently reflux the sample for 30 min. Slight boiling may occur, but vigorous boiling must be avoided.

11.1.4 Allow the beaker to cool. Quantitatively transfer the sample solution to a 100-mL volumetric flask, dilute to volume with reagent water, stopper and mix.

11.1.5 Allow any undissolved material to settle overnight, or centrifuge a portion of the prepared sample until clear. (If after centrifuging or standing overnight the sample contains suspended solids that would clog or affect the sample introduction system, a portion of the sample may be filtered prior to analysis. However, care should be exercised to avoid potential contamination from filtration.) The sample is now ready for analysis. Because the effects of various matrices on the stability of diluted samples cannot be characterized, all analyses should be performed as soon as possible after the completed preparation.

11.2 Prior to first use, the preconcentration system should be thoroughly cleaned and decontaminated using 0.2M - oxalic acid.

11.2.1 Precleaning the Preconcentration System

11.2.1.1 Place approximately 500 mL 0.2M - oxalic acid in each of the sample/eluent containers. Flush the entire system by running the program used for sample analysis 3 times.

11.2.1.2 Rinse the containers with ASTM type I water and repeat the sequence described in Section 11.2.1.1 using 0.75M nitric acid and again using ASTM type I water in place of the 0.2M - oxalic acid.

11.2.1.3 Rinse the containers thoroughly with ASTM type I water, fill them with their designated reagents and run through the program used for sample analysis in order to prime the pump and all eluent lines with the correct reagents.

11.2.2 Peak Profile Determination

11.2.2.1 The peak elution time or the collection window should be determined using an ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometer) or Flame AA. Figure 3 is a plot of time vs. emission intensity for Cd, Pb, Ni, and Cu. The collection window is marked in Figure 3 and should provide about 30 sec buffer on

either side of the peak. If an ICP-AES is not available, it is recommended that the peak profile be determined by collecting 200-μL samples during the elution part of the preconcentration cycle and then reconstructing the peak profile from the analysis of the 200-μL samples.

11.3 **Sample Preconcentration**

11.3.1 Preconcentration utilizing a sample loop.

11.3.1.1 Loading Sample Loop -- With valve 1 in the off position and valve 2 in the on position, load sample through the sample loop to waste using the sample pump for 4 min at 4 mL/min. Switch on the carrier pump and pump 1 % nitric acid to flush the sample collection line.

11.3.1.2 Column Loading -- With valve 1 in the on position, load sample from the loop onto the column using 1 M ammonium acetate for 4.5 min at 4.0 mL/min. Switch on the buffer pump, and pump 2M ammonium acetate at a flow rate of 1 mL/min. The analytes are retained on the column, while the majority of the matrix is passed through to waste.

11.3.1.3 Elution Matrix -- With valve 1 in the on position the gradient pump is allowed to elute the matrix using the 1M ammonium acetate. During which time the carrier, buffer and the sample pumps are all off.

11.3.1.4 Elute Analytes -- Turn off valve 1 and begin eluting the analytes by pumping 0.75M nitric acid through the column and turn off valve 2 and pump the eluted analytes into the collection flask. The analytes should be eluted into a 2-mL sample volume.

11.3.1.5 Column Reconditioning -- Turn on valve 2 to direct column effluent to waste, and pump 0.75M nitric acid, 1M ammonium acetate, 0.75M nitric acid and 1M ammonium acetate alternately through the column at 4.0 mL/min. Each solvent should be pumped through the column for 2 min. During this process, the next sample can be loaded into the sample loop using the sample pump.

11.3.1.6 Preconcentration of the sample may be achieved by running through an eluent pump program. The exact timing of this sequence should be modified according to the internal volume of the connecting tubing and the specific hardware configuration used.

11.3.2 Preconcentration utilizing an auxiliary pump to determine sample volume.

11.3.2.1 Sample Loading -- With the valves 1 and 2 on and the sample pump on, load the sample on the column buffering the sample utilizing the gradient pump and the 2M buffer. The actual sample volume is determined by knowing the sample pump rate and the time. While the sample is being loaded the carrier pump can be used to flush the collection line.

11.3.2.2 Elution Matrix -- With valve 1 in the off position the gradient pump is allowed to elute the matrix using the 1M ammonium acetate. During which time the carrier, buffer and the sample pumps are all off.

11.3.2.3 Elution of Analytes -- With valves l and 2 in the off position the gradient pump is switched to 0.75M HNO_3 and the analytes are eluted into the collection vessel. The analytes should be eluted into a 2 mL sample volume.

11.3.2.4 Column Reconditioning -- Turn on valve 2 to direct column effluent to waste, and pump 0.75M nitric acid, 1M ammonium acetate, 0.75M nitric acid and 1M ammonium acetate alternately through the column at 4.0 mL/min.

Note: When switching the gradient pump from nitric acid back to the ammonium acetate it is necessary to flush the line connecting the gradient pump to valve 2 with the ammonium acetate prior to switching the valve. If the line contains nitric acid it will elute the metals from the cleanup column.

11.3.2.5 Preconcentration of the sample may be achieved by running through an eluent pump program. The exact timing of this sequence should be modified according to the internal volume of the connecting tubing and the specific hardware configuration used.

11.4 Repeat the sequence described in Section 11.3.1 or 11.3.2 for each sample to be analyzed. At the end of the analytical run leave the column filled with 1M ammonium acetate buffer until it is next used.

11.5 Samples having concentrations higher than the established linear dynamic range should be diluted into range and reanalyzed.

11.6 Sample Analysis

11.6.1 Prior to daily instrument calibration, inspect the graphite furnace, the sample uptake system and autosampler injector for any change that would affect instrument performance. Clean the system and replace

the graphite tube and/or platform when needed or on a daily basis. A cotton swab dipped in a 50/50 mixture of isopropyl alcohol (IPA) and H_2O (such that it is damp but not dripping) can be used to remove the majority of the salt buildup. A second cotton swab is dipped in IPA and the contact rings are wiped down to assure they are clean. The rings are then allowed to thoroughly dry and then a new tube is placed in the furnace and conditioned according to instrument manufacturers specifications.

11.6.2 Configure the instrument system to the selected optimized operating conditions as determined in Sections 10.1 and 10.2.

11.6.3 Before beginning daily calibration the instrument should be reconfigured to the optimized conditions. Initiate data system and allow a period of not less than 15 min for instrument and hollow cathode lamp warm-up. If an EDL is to be used, allow 30 min for warm-up.

11.6.4 After the warm-up period but before calibration, instrument stability must be demonstrated by analyzing a standard solution with a concentration 20 times the IDL a minimum of five times. The resulting relative standard deviation of absorbance signals must be <5%. If the relative standard deviation is >5%, determine and correct the cause before calibrating the instrument.

11.6.5 For initial and daily operation calibrate the instrument according to the instrument manufacturer's recommended procedures using the calibration blank (Section 7.5.1) and calibration standards (Section 7.4) prepared at three or more concentrations within the usable linear dynamic range of the analyte (Sections 4.4 & 9.2.2).

11.6.6 An autosampler must be used to introduce all solutions into the graphite furnace. Once the standard, sample or QC solution plus the matrix modifier is injected, the furnace controller completes furnace cycles and cleanout period as programmed. Analyte signals must be integrated and collected as peak area measurements. Background absorbances, background corrected analyte signals, and determined analyte concentrations on all solutions must be able to be displayed on a CRT for immediate review by the analyst and be available as hard copy for documentation to be kept on file. Flush the autosampler solution uptake system with the rinse blank (Section 7.5.4) between each solution injected.

11.6.7 After completion of the initial requirements of this method (Section 9.2), samples should be analyzed in the same operational manner used in the calibration routine.

11.6.8 During sample analyses, the laboratory must comply with the required quality control described in Sections 9.3 and 9.4.

11.6.9 Determined sample analyte concentrations that are $\geq$90% of the upper limit of calibration must either be diluted with acidified reagent water and reanalyzed with concern for memory effects (Section 4.4), or determined by another approved test procedure that is less sensitive. Samples with a background absorbance > 1.0 must be appropriately diluted with acidified reagent water and reanalyzed (Section 9.4.6). If the method of standard additions is required, follow the instructions described in Section 11.5.

11.6.10 Report data as directed in Section 12.

11.7 Standard Additions -- If the method of standard addition is required, the following procedure is recommended:

11.7.1 The standard addition technique[9] involves preparing new standards in the sample matrix by adding known amounts of standard to one or more aliquots of the processed sample solution. This technique compensates for a sample constituent that enhances or depresses the analyte signal, thus producing a different slope from that of the calibration standards. It will not correct for additive interference, which causes a baseline shift. The simplest version of this technique is the single addition method. The procedure is as follows: Two identical aliquots of the sample solution, each of volume V_x, are taken. To the first (labeled A) is added a small volume V_S of a standard analyte solution of concentration C_s. To the second (labeled B) is added the same volume V_s of the solvent. The analytical signals of A and B are measured and corrected for nonanalyte signals. The unknown sample concentration C_x is calculated:

$$C_X = \frac{S_B V_S C_S}{(S_A - S_B) V_X}$$

where, S_A and S_B are the analytical signals (corrected for the blank) of solutions A and B, respectively. V_S and C_S should be chosen so that S_A is roughly twice S_B on the average. It is best if V_S is made much less than V_X, and thus C_S is much greater than C_X, to avoid excess dilution of the sample matrix. If a separation or concentration step is used, the additions are best made first and carried through the entire procedure. For the results from this technique to be valid, the following limitations must be taken into consideration:

1. The analytical curve must be linear.

2. The chemical form of the analyte added must respond in the same manner as the analyte in the sample.

3. The interference effect must be constant over the working range of concern.

4. The signal must be corrected for any additive interference.

12.0 Data Analysis and Calculations

12.1 Sample data should be reported in units of µg/L for aqueous samples.

12.2 For total recoverable aqueous analytes (Section 11.1), when 100-mL aliquot is used to produce the 100 mL final solution, round the data to the tenths place and report the data in µg/L up to three significant figures. If an aliquot volume other than 100 mL is used for sample preparation, adjust the dilution factor accordingly. Also, account for any additional dilution of the prepared sample solution needed to complete the determination of analytes exceeding the upper limit of the calibration curve. Do not report data below the determined analyte MDL concentration or below an adjusted detection limit reflecting smaller sample aliquots used in processing or additional dilutions required to complete the analysis.

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

13.0 Method Performance

13.1 Experimental conditions used for single laboratory testing of the method are summarized in Table 1.

13.2 Table 2 contains precision and recovery data obtained from a single laboratory analysis of a fortified and a non-fortified sample of NASS-3. The samples were prepared using the procedure described in Section 11.1. Four replicates of the non-fortified samples were analyzed and the average of the replicates was used for determining the sample analyte concentration. The fortified samples of NASS-3 were also analyzed and the average percent recovery and the percent relative standard deviation is reported. The reference material certified values are also listed for comparison.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation (e.g., Section 7.8). When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

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

15.0 Waste Management

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

16.0 References

1. A. Siraraks, H.M. Kingston and J.M. Riviello, *Anal Chem.* 62 1185 (1990).

2. E.M. Heithmar, T.A. Hinners, J.T. Rowan and J.M. Riviello, *Anal Chem.* 62 857 (1990).

3. OSHA Safety and Health Standards, General Industry, (29 CFR 1910), Occupational Safety and Health Administration, OSHA 2206, (Revised, January 1976).

4. Carcinogens - Working With Carcinogens, Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.

5. Proposed OSHA Safety and Health Standards,Laboratories, Occupational Safety and Health Administration, *Federal Register,* July 24, 1986.

6. Safety in Academic Chemistry Laboratories, American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition, 1979.

7. Rohrbough, W.G. et al. *Reagent Chemicals,* American Chemical Society Specifications, 7th edition. American Chemical Society, Washington, DC, 1986.

8. *Code of Federal Regulations 40,* Ch. 1, Pt. 136 Appendix B.

9. Winefordner, J.D., Trace Analysis: Spectroscopic Methods for Elements, *Chemical Analysis,* Vol. 46, pp. 41-42,1976.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

Table 1. Method Detection Limits for Total Recoverable Analytes in Reagent Water[1]

Element	Slit, nm	Recommended analytical Wavelengths, nm	Char Temp, •C	Atomization Temp, •C	MDL[2], µg/L
Cadmium	0.7	228.8	800	1600	0.016
Cobalt	0.2	242.5	1400	2500	-
Copper	0.7	324.8	1300	2600	0.36
Lead	0.7	283.3	1250	2000	0.28
Nickel	0.2	232.4	1400	2500	*

[1] MDLs were calculated using NASS-3 as the matrix.
[2] MDLs were calculated based on a 10-mL sample loop.
* MDL was not calculated because the concentration in the matrix exceeds the MDL spike level.
- Not Determined.

Table 2. Precision and Recovery Data for NASS-3 Using System Illustrated in Figure 1[1,2]

Analyte	Certified Value, µg/L[3]	Sample Conc., µg/L[3]	Fortified Conc., µg/L	Avg. Recovery, %	% RSD
Cd	0.029 ± 0.004	0.026 ± 0.012	0.25	93	3.3
Co	0.004 ± 0.001	-	-	-	-
Cu	0.109 ± 0.011	<0.36	5.0	87	1.4
Pb	0.039 ± 0.006	<0.28	5.0	90	3.7
Ni	0.257 ± 0.027	0.260 ± 0.04	5.0	117	8.3

[1] Data collected using 10-mL sample loop.
[2] Matrix modifier is $Pd/Mg(NO_3)_2/H_2$.
[3] Uncertainties based on 95% confidence limits.
- Not determined.

	Valves		Buffer	Carrier	Sample
	1	2	Pump	Pump	Pump
Sample Loop Loading	Off	On	Off	On	On
Column Loading	On	On	On	Off	Off
Elution of Matrix	On	On	Off	Off	Off
Elution of Analytes	Off	Off	Off	Off	Off
Column Recondition	Off	On	Off	Off	Off

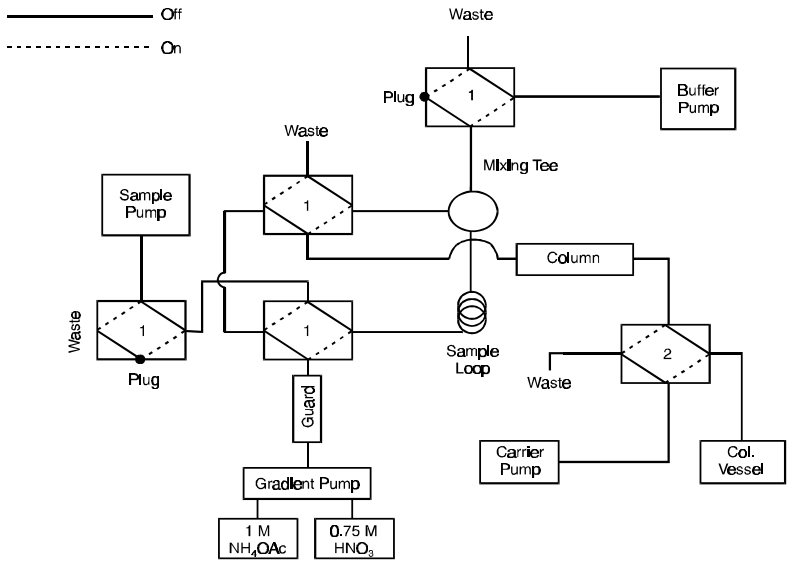


Figure 1. Sample Loop Configuration.

Event	Valves 1	Valves 2	Carrier Pump	Sample Pump
Sample Loading	On	On	On	On
Elution of Matrix	Off	On	Off	Off
Elution of Analytes	Off	Off	Off	Off
Column Recondition	Off	On	Off	On

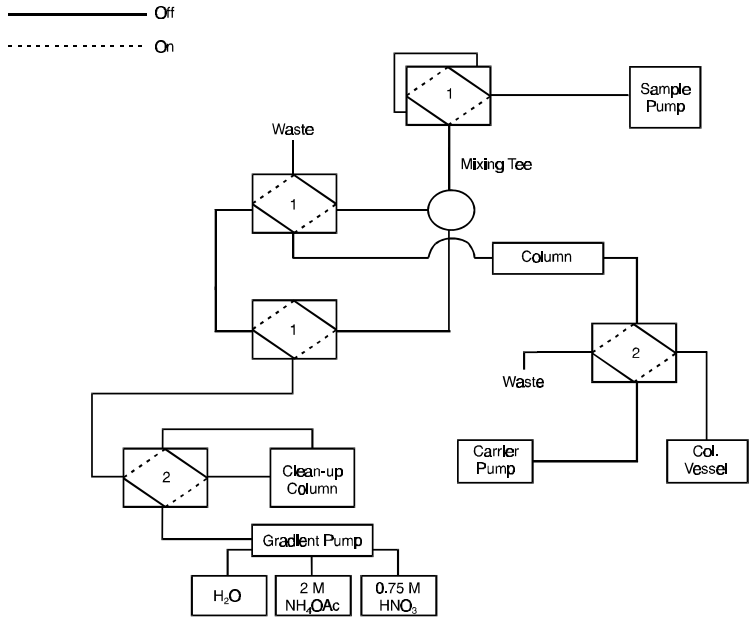


Figure 2. System Diagram without Sample Loop.

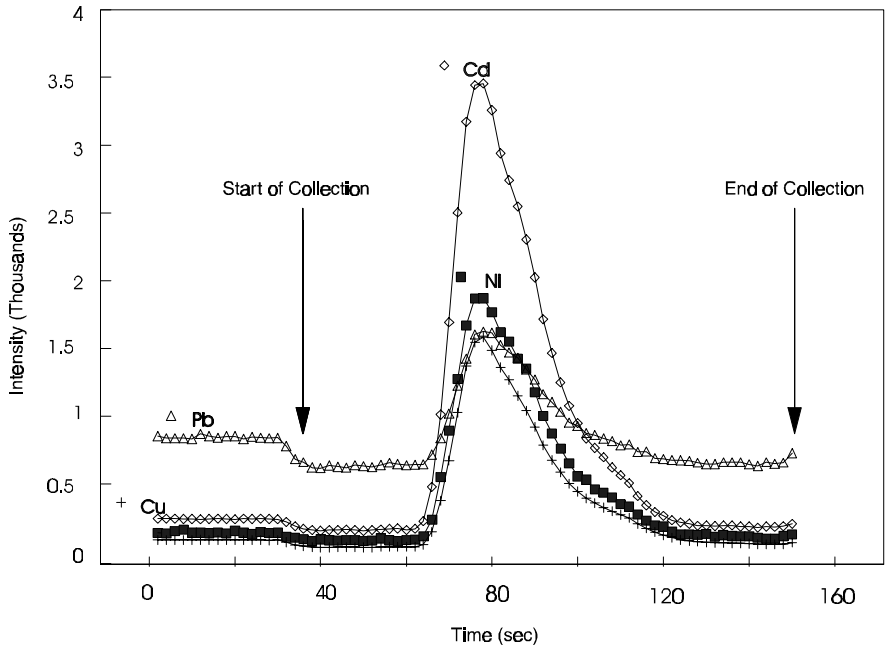


Figure 3. Peak Collection Window from ICP-AES.

Appendix 3

USEPA Method 1631

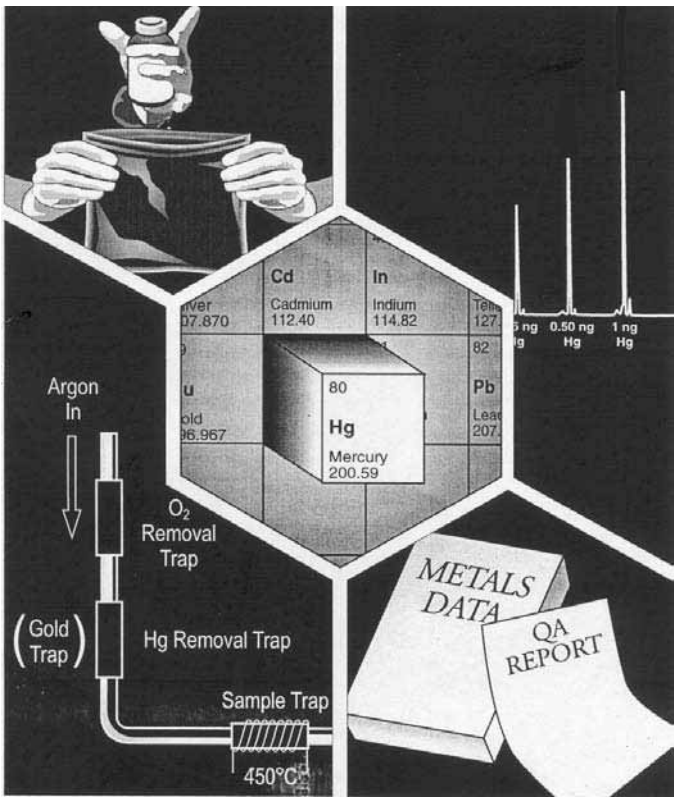
Mercury in Water by Oxidation, Purge and Trap, and Cold Vapour Atomic Fluorescence Spectrometry

Revision E
August 2002

United States
Environmental Protection
Agency

Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry

August 2002



Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry

Acknowledgments

This Method was developed under the direction of William A. Telliard and Maria Gomez-Taylor of the Engineering and Analysis Division (EAD) within the U.S. Environmental Protection Agency's (EPA's) Office of Science and Technology (OST). EPA acknowledges contributions to this method by Frontier Geosciences, Inc., Albion Environmental, Battelle Marine Sciences Laboratory, STL-Canton, and Tekran Inc. Additional assistance in preparing the Method was provided by DynCorp Environmental and Interface, Inc.

Disclaimer

This Method has been reviewed and approved for publication by the Statistics and Analytical Support Branch within EPA's Engineering and Analysis Division. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Questions concerning this Method or its application should be addressed to:

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Statistics and Analytical Support Branch (4303T)
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Table of Contents

Introduction

Method 1631 (the "Method") supports technology-based and water quality-based monitoring programs authorized under the Clean Water Act (CWA; the "Act").

CWA Sections 301 and 306 require EPA to publish effluent standards that restrict the direct discharge of pollutants to the nations waters, and CWA Sections 307(b) and (c) require EPA to promulgate nationally applicable pretreatement standards which restrict pollutant discharges into sewers flowing to publicly owned treatment works (POTWs). The effluent limitations guidelines are published at CFR parts 401-503.

CWA Section 303 requires each State to set a water quality standard for each body of water within its boundaries. A State water quality standard consists of a designated use or uses of a water body or a segment of a water body, the water quality criteria that are necessary to protect the designated use or uses, and an antidegradation policy. CWA Section 304(a) requires EPA to publish water quality criteria that reflect the latest scientific knowledge concerning the physical fate of pollutants, the effects of pollutants on ecological and human health, and the effect of pollutants on biological community diversity, productivity, and stability. These water quality standards serve two purposes: (1) they establish the water quality goals for a specific water body, and (2) they are the basis for establishing water quality-based treatment controls and strategies beyond the technology-based controls required by CWA Sections 301(b) and 306.

In 1987, amendments to the CWA required States to adopt numeric criteria for toxic pollutants (designated in Section 307(a) of the Act) based on EPA Section 304(a) criteria or other scientific data, when the discharge or presence of those toxic pollutants could reasonably be expected to interfere with designated uses. Method 1631 was specifically developed to provide reliable measurements of mercury at EPA WQC levels.

In developing methods for determination of trace metals, EPA found that one of the greatest difficulties was precluding sample contamination during collection, transport, and analysis. The degree of difficulty, however, is highly dependent on the metal and site-specific conditions. Method 1631 is designed to preclude contamination in nearly all situations. It also contains procedures necessary to produce reliable results at the lowest WQC levels published by EPA. In recognition of the variety of situations to which this Method may be applied, and in recognition of continuing technological advances, Method 1631 is performance based. Alternative procedures may be used so long as those procedures are demonstrated to yield reliable results.

Requests for additional copies of this draft Method should be directed to:

U.S. EPA Sample Control Center
6101 Stevenson Avenue
Alexandria, VA 22304-3540
703/461–2100

Note: This Method is performance based. The laboratory is permitted to omit steps or modify procedures provided that all performance requirements in this Method are met. The laboratory must not omit or modify any procedure defined by the term “shall” or “must” and must perform all quality control tests.

Method 1631, Revision E

Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry

1.0 Scope and Application

1.1 Method 1631, Revision E (the "Method") is for determination of mercury (Hg) in filtered and unfiltered water by oxidation, purge and trap, desorption, and cold-vapor atomic fluorescence spectrometry (CVAFS). This Method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act. The Method is based on a contractor-developed procedure (Reference 16.1) and on peer-reviewed, published procedures for the determination of mercury in aqueous samples, ranging from sea water to sewage effluent (References 16.2–16.5).

1.2 This Method is accompanied by Method 1669: *Sampling Ambient Water for Determination of Trace Metals at EPA Water Quality Criteria Levels* (Sampling Method). The Sampling Method guidance document is recommended to preclude contamination during the sampling process.

1.3 This Method is for determination of Hg in the range of 0.5–100 ng/L. Application may be extended to higher levels by selection of a smaller sample size or by calibration of the analytical system across a higher range. For measurement of blank samples, the Method may be extended to a lower level by calibration to a lower calibration point. Section 10.4 gives requirements for extension of the calibration range.

1.4 The ease of contaminating ambient water samples with mercury and interfering substances cannot be overemphasized. This Method includes suggestions for improvements in facilities and analytical techniques that should minimize contamination and maximize the ability of the laboratory to make reliable trace metals determinations. Certain sections of this Method contain suggestions and other sections contain requirements to minimize contamination.

1.5 The detection limit and minimum level of quantitation in this Method usually are dependent on the level of interferences rather than instrument limitations. The method detection limit (MDL; 40 CFR 136, Appendix B) for Hg has been determined to be 0.2 ng/L when no interferences are present. The minimum level of quantitation (ML) has been established as 0.5 ng/L. An MDL as low as 0.05 ng/L can be achieved for low Hg samples by using a larger sample volume, a lower BrCl level (0.2%), and extra caution in sample handling.

1.6 Clean and ultraclean—The terms "clean" and "ultraclean" have been applied to the techniques needed to reduce or eliminate contamination in trace metals determinations. These terms are not used in this Method because they lack an exact definition. However, the information provided in this Method is consistent with the summary guidance on clean and ultraclean techniques (References 16.6-16.7).

1.7 This Method follows the EPA Environmental Methods Management Council's "Guidelines and Format for Methods to Be Proposed at 40 CFR, part 136 or part 141."

1.8 This Method is "performance based." The laboratory is permitted to modify the Method to overcome interferences or lower the cost of measurements if all performance criteria are met. Section 9.1.2.1 gives the requirements for establishing method equivalency.

1.9 Any modification of this Method, beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 CFR 136.4 and 136.5.

1.10 This Method should be used only by analysts experienced in the use of CVAFS techniques and who are trained thoroughly in the sample handling and instrument techniques described in this Method. Each laboratory that uses this Method must demonstrate the ability to generate acceptable results using the procedures in Section 9.2.

1.11 This Method is accompanied by a data verification and validation guidance document, *Guidance on the Documentation and Evaluation of Trace Metals Data Collected for CWA Compliance Monitoring* (Reference 16.8), that can be used for verification and validation of the data obtained.

1.12 This Method uses either a bubbler or flow-injection system for determination of mercury in water. Separate calibration, analysis, and calculation procedures are provided for a bubbler system (Sections 10.2, 11.2.1, and 12.2) and for a flow-injection system (Sections 10.3, 11.2.2, and 12.3).

2.0 Summary of Method

2.1 A 100- to 2000-mL sample is collected directly into a cleaned, pretested, fluoropolymer or glass bottle using sample handling techniques designed for collection of mercury at trace levels (Reference 16.9).

2.2 For dissolved Hg, the sample is filtered through a 0.45-µm capsule filter prior to preservation.

2.3 The sample is preserved by adding either pretested 12N hydrochloric acid (HCl) or bromine monochloride (BrCl) solution. If a sample will also be used for the determination of methyl mercury, it should be preserved according to procedures in the method that will be used for determination of methylmercury.

2.4 Prior to analysis, all Hg in a 100-mL sample aliquot is oxidized to Hg(II) with BrCl.

2.5 After oxidation, the sample is sequentially reduced with $NH_2OH{\cdot}HCl$ to destroy the free halogens, then reduced with stannous chloride ($SnCl_2$) to convert Hg(II) to volatile Hg(0).

2.6 The Hg(0) is separated from solution either by purging with nitrogen, helium, or argon, or by vapor/liquid separation. The Hg(0) is collected onto a gold trap (Figures 1, 2, and 3).

2.7 The Hg is thermally desorbed from the gold trap into an inert gas stream that carries the released Hg(0) to a second gold (analytical) trap. The Hg is desorbed from the analytical trap into a gas stream that carries the Hg into the cell of a cold-vapor atomic fluorescence spectrometer (CVAFS) for detection (Figures 2 and 3).

2.8 Quality is assured through calibration and testing of the oxidation, purging, and detection systems.

3.0 Definitions

3.1 Total mercury—all BrCl-oxidizable mercury forms and species found in an unfiltered aqueous solution. This includes, but is not limited to, Hg(II), Hg(0), strongly organo-complexed Hg(II) compounds, adsorbed particulate Hg, and several tested covalently bound organo-mercurials (e.g., CH_3HgCl, $(CH_3)_2Hg$, and $C_6H_5HgOOCCH_3$). The recovery of Hg bound within microbial cells may require the additional step of UV photo-oxidation. In this Method, total mercury and total recoverable mercury are synonymous.

3.2 Dissolved mercury—all BrCl-oxidizable mercury forms and species found in the filtrate of an aqueous solution that has been filtered through a 0.45-μm filter.

3.3 Apparatus—Throughout this Method, the sample containers, sampling devices, instrumentation, and all other materials and devices used in sample collection, sample processing, and sample analysis that come in contact with the sample and therefore require careful cleaning will be referred to collectively as the Apparatus.

3.4 Definitions of other terms used in this Method are given in the glossary (Section 17.0).

4.0 Contamination and Interferences

4.1 Preventing samples from becoming contaminated during the sampling and analysis process constitutes one of the greatest difficulties encountered in trace metals determinations. Over the last two decades, marine chemists have come to recognize that much of the historical data on the concentrations of dissolved trace metals in seawater are erroneously high because the concentrations reflect contamination from sampling and analysis rather than ambient levels. Therefore, it is imperative that extreme care be taken to avoid contamination when collecting and analyzing samples for trace metals.

4.2 Samples may become contaminated by numerous routes. Potential sources of trace metals contamination during sampling include: metallic or metal-containing labware (e.g., talc gloves that contain high levels of zinc), containers, sampling equipment, reagents, and reagent water; improperly cleaned or stored equipment, labware, and reagents; and atmospheric inputs such as dirt and dust. Even human contact can be a source of trace metals contamination. For example, it has been demonstrated that dental work (e.g., mercury amalgam fillings) in the mouths of laboratory personnel can contaminate samples directly exposed to exhalation (Reference 16.9).

4.3 Contamination Control

4.3.1 Philosophy—The philosophy behind contamination control is to ensure that any object or substance that contacts the sample is metal free and free from any material that may contain mercury.

4.3.1.1 The integrity of the results produced cannot be compromised by contamination of samples. This Method and the Sampling Method give requirements and suggestions for control of sample contamination.

4.3.1.2 Substances in a sample cannot be allowed to contaminate the laboratory work area or instrumentation used for trace metals measurements. This Method gives requirements and suggestions for protecting the laboratory.

4.3.1.3 Although contamination control is essential, personnel health and safety remain the highest priority. The Sampling Method and Section 5 of this Method give suggestions and requirements for personnel safety.

4.3.2 Avoiding contamination—The best way to control contamination is to completely avoid exposure of the sample to contamination in the first place. Avoiding exposure means performing operations in an area known to be free from contamination. Two of the most important factors in avoiding/reducing sample contamination are (1) an awareness of potential sources of contamination and (2) strict attention to work being done. Therefore, it is imperative that the procedures described in this Method be carried out by well-trained, experienced personnel.

4.3.3 Use a clean environment—The ideal environment for processing samples is a class-100 clean room. If a clean room is not available, all sample preparation should be performed in a class-100 clean bench or a nonmetal glove box fed by mercury-and particle-free air or nitrogen. Digestion should be performed in a nonmetal fume hood equipped with HEPA filtration and ideally situated in a clean room.

4.3.4 Minimize exposure—The Apparatus that will contact samples, blanks, or standard solutions should be opened or exposed only in a clean room, clean bench, or glove box so that exposure to an uncontrolled atmosphere is minimized. When not being used, the Apparatus should be covered with clean plastic wrap, stored in the clean bench or in a plastic box or glove box, or bagged in clean zip-type bags. Minimizing the time between cleaning and use will also minimize contamination.

4.3.5 Clean work surfaces—Before a given batch of samples is processed, all work surfaces in the hood, clean bench, or glove box in which the samples will be processed should be cleaned by wiping with a lint-free cloth or wipe soaked with reagent water.

4.3.6 Wear gloves—Sampling personnel must wear clean, non-talc gloves during all operations involving handling of the Apparatus, samples, and blanks. Only clean gloves may touch the Apparatus. If another object or substance is touched, the glove(s) must be changed before again handling the Apparatus. If it is even suspected that gloves have become contaminated, work must be halted, the contaminated gloves removed, and a new pair of clean gloves put on. Wearing multiple layers of clean gloves will allow the old pair to be quickly stripped with minimal disruption to the work activity.

4.3.7 Use metal-free Apparatus—All Apparatus used for determination of mercury at ambient water quality criteria levels must be nonmetallic, free of material that may contain metals, or both.

4.3.7.1 Construction materials—Only fluoropolymer or glass containers must be used for collection of samples that will be analyzed for mercury because mercury vapors can diffuse in or out of other materials, leading to results that are biased low or high. Polyethylene and/or polypropylene labware may be used for digestion and other purposes because the time of sample exposure to these materials is relatively short. All materials, regardless of construction, that will directly or

indirectly contact the sample, must be known to be clean and free of Hg at the levels specified in this Method before proceeding.

4.3.7.2 Serialization—It is recommended that serial numbers be indelibly marked or etched on each piece of reusable Apparatus so that contamination can be traced, and logbooks should be maintained to track the sample from the container through the labware to introduction into the instrument. It may be useful to dedicate separate sets of labware to different sample types; e.g., receiving waters vs. effluents. However, the Apparatus used for processing blanks and standards must be mixed with the Apparatus used to process samples so that contamination of all labware can be detected.

4.3.7.3 The laboratory or cleaning facility is responsible for cleaning the Apparatus used by the sampling team. If there are any indications that the Apparatus is not clean when received by the sampling team (e.g., ripped storage bags), an assessment of the likelihood of contamination must be made. Sampling must not proceed if it is possible that the Apparatus is contaminated. If the Apparatus is contaminated, it must be returned to the laboratory or cleaning facility for proper cleaning before any sampling activity resumes.

4.3.8 Avoid sources of contamination—Avoid contamination by being aware of potential sources and routes of contamination.

4.3.8.1 Contamination by carryover—Contamination may occur when a sample containing a low concentration of mercury is processed immediately after a sample containing a relatively high concentration of mercury. The Hg concentration at which the analytical system (purge, traps, detector) will carry greater than 0.5 ng/L of Hg into a succeeding bubbler or system blank must be determined by analyzing calibration solutions containing successively larger concentrations of Hg. This test must be run prior to first use of the analytical system and whenever a change is made that would increase the amount of carryover. When a sample contains ½ or greater of this determined Hg concentration, a bubbler blank (bubbler system) or system blank (flow injection system) must be analyzed to demonstrate no carryover at the blank criteria level. For the bubbler system, the blank must be run using the same bubbler and sample trap used to run the high concentration sample. Samples analyzed following a sample that has been determined to result in carryover must be reanalyzed. Samples that are known or suspected to contain the lowest concentration of mercury should be analyzed first followed by samples containing higher levels.

4.3.8.2 Contamination by samples—Significant laboratory or instrument contamination may result when untreated effluents, in-process waters, landfill leachates, and other undiluted samples containing concentrations of mercury greater than 100 ng/L are processed and analyzed. Samples known or suspected to contain Hg concentrations greater than 100 ng/L should be diluted prior to bringing them into the clean room or laboratory dedicated for processing trace metals samples.

4.3.8.3 Contamination by indirect contact—Apparatus that may not directly come in contact with the samples may still be a source of contamination. For example, clean tubing placed in a dirty plastic bag may pick up contamination from the bag and subsequently transfer the contamination to the sample. It is imperative that every piece of the Apparatus that is directly or indirectly used in the collection, processing, and analysis of water samples be thoroughly cleaned (Section 6.1.2).

4.3.8.4 Contamination by airborne particulate matter—Less obvious substances capable of contaminating samples include airborne particles. Samples may be contaminated by airborne dust, dirt, particles, or vapors from unfiltered air supplies; nearby corroded or rusted pipes, wires, or other fixtures; or metal-containing paint. Whenever possible, sample processing and analysis should occur as far as possible from sources of airborne contamination.

4.3.8.5 Contamination from reagents— Contamination can be introduced into samples from method reagents used during processing and analysis. Reagent blanks must be analyzed for contamination prior to use (see Section 9.4.3). If reagent blanks are contaminated, a new batch of reagents must be prepared (see Section 9.4.3.2).

4.4 Interferences

4.4.1 At the time of promulgation of this Method, gold and iodide were known interferences. At a mercury concentration of 2.5 ng/L and at increasing iodide concentrations from 30 to 100 mg/L, test data have shown that mercury recovery will be reduced from 100 to 0 percent. At iodide concentrations greater than 3 mg/L, the sample should be pre-reduced with $SnCl_2$ (to remove the brown color) and additional or more concentrated $SnCl_2$ should be added. To preclude loss of Hg, the additional $SnCl_2$ should be added in a closed vessel or analysis should proceed immediately. If samples containing iodide concentrations greater than 30 mg/L are analyzed, it may be necessary to clean the analytical system with 4N HCl after the analysis (Reference 16.10).

4.4.2 The potential exists for destruction of the gold traps if free halogens are purged onto them, or if they are overheated (>500 °C). When the instructions in this Method are followed, neither of these outcomes is likely.

4.4.3 Water vapor may collect in the gold traps and subsequently condense in the fluorescence cell upon desorption, giving a false peak due to scattering of the excitation radiation. Condensation can be avoided by predrying the gold trap. Traps that tend to absorb large quantities of water vapor should not be used.

4.4.4 The fluorescent intensity is strongly dependent upon the presence of molecular species in the carrier gas that can cause "quenching" of the excited atoms. The dual amalgamation technique eliminates quenching due to trace gases, but it remains the laboratory's responsibility to ensure high purity inert carrier gas and a leak-free analytical train.

5.0 Safety

5.1 The toxicity or carcinogenicity of each chemical used in this Method has not been precisely determined; however, each compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

5.1.1 Chronic mercury exposure may cause kidney damage, muscle tremors, spasms, personality changes, depression, irritability and nervousness. Organo-mercurials may cause permanent brain damage. Because of the toxicological and physical properties of Hg, pure standards should be handled only by highly trained personnel thoroughly familiar with handling and cautionary procedures and the associated risks.

5.1.2 It is recommended that the laboratory purchase a dilute standard solution of the Hg in this Method. If primary solutions are prepared, they shall be prepared in a hood, and a NIOSH/MESA-approved toxic gas respirator shall be worn.

5.2 This Method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a current file of OSHA regulations for safe handling of the chemicals specified in this Method. OSHA rules require that a reference file of material safety data sheets (MSDSs) must be made available to all personnel involved in these analyses (29 CFR 1917.28, Appendix E). It also is suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this Method and that the results of this monitoring be made available to the analyst. Personal hygiene monitoring should be performed using OSHA or NIOSH approved personal hygiene monitoring methods. Additional information on laboratory safety can be found in References 16.11-16.14. The references and bibliography included in Reference 16.14 are particularly comprehensive in dealing with the general subject of laboratory safety.

5.3 Samples suspected to contain concentrations of Hg at µg/L or higher levels are handled using essentially the same techniques employed in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. Assistance in evaluating the health hazards of particular laboratory conditions may be obtained from certain consulting laboratories and from State Departments of Health or Labor, many of which have an industrial health service. Each laboratory must develop a safety program for handling Hg.

5.3.1 Facility—When samples known or suspected of containing high concentrations of mercury are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leak-tight or in a fume hood demonstrated to have adequate airflow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in an accident.

5.3.2 Protective equipment—Disposable plastic gloves, apron or lab coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be used. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters.

5.3.3 Training—Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

5.3.4 Personal hygiene—Hands and forearms should be washed thoroughly after each manipulation and before breaks (coffee, lunch, and shift).

5.3.5 Confinement—Isolated work areas posted with signs, segregated glassware and tools, and plastic absorbent paper on bench tops will aid in confining contamination.

5.3.6 Effluent vapors—The effluent from the CVAFS should pass through either a column of activated charcoal or a trap containing gold or sulfur to amalgamate or react mercury vapors.

5.3.7 Waste handling—Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel must be trained in the safe handling of waste.

5.3.8 Decontamination

5.3.8.1 Decontamination of personnel—Use any mild soap with plenty of scrubbing action.

5.3.8.2 Glassware, tools, and surfaces—Sulfur powder will react with Hg to produce mercuric sulfide, thereby eliminating the possible volatilization of Hg. Satisfactory cleaning may be accomplished by dusting a surface lightly with sulfur powder, then washing with any detergent and water.

5.3.9 Laundry—Clothing known to be contaminated should be collected in plastic bags. Persons that convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. If the launderer knows of the potential problem, the clothing may be put into a washer without contact. The washer should be run through a cycle before being used again for other clothing.

5.3.10 Wipe tests—A useful method of determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper. Extraction and analysis by this Method can achieve a limit of detection of less than 1 ng per wipe. Less than 0.1 μg per wipe indicates acceptable cleanliness; anything higher warrants further cleaning. More than 10 μg on a wipe constitutes an acute hazard and requires prompt cleaning before further use of the equipment or work space, and indicates that unacceptable work practices have been employed.

6.0 Apparatus and Materials

Disclaimer: The mention of trade names or commercial products in this Method is for illustrative purposes only and does not constitute endorsement or recommendation for use by the Environmental Protection Agency. Equivalent performance may be achievable using apparatus, materials, or cleaning procedures other than those suggested here. The laboratory is responsible for demonstrating equivalent performance.

6.1 Sampling equipment

6.1.1 Sample collection bottles-fluoropolymer or glass, 125- to 1000-mL, with fluoropolymer or fluoropolymer-lined cap.

6.1.2 Cleaning

6.1.2.1 New bottles are cleaned by heating to 65–75 °C in 4 N HCl or concentrated HNO_3 for at least 48 h. The bottles are cooled, rinsed three times with reagent water, and filled with reagent water containing 1% HCl. These bottles are capped and placed in a clean oven at 60-70°C overnight. After cooling, they are rinsed three more times with reagent water, filled with reagent water containing 0.4% (v/v) HCl, and placed in a mercury-free Class-100 clean bench until the outside surfaces are dry. The bottles are tightly capped (with a wrench), double-bagged in new polyethylene zip-type bags until needed, and stored in wooden or plastic boxes until use. The bottles may be shipped to the sampling site containing dilute HCl solution (e.g., 0.04%), containing reagent water, or empty.

6.1.2.2 Used bottles known not to have contained mercury at high (>100 ng/L) levels are cleaned as above, except for only 6–12 h in hot 4 N HCl.

6.1.2.3 Bottle blanks must be analyzed as described in Section 9.4.7. To verify the effectiveness of the cleaning procedures, bottle blanks must be demonstrated to be free of mercury at the ML of this Method.

6.1.2.4 As an alternative to cleaning by the laboratory, bottles may be purchased from a commercial supplier and each lot certified to be clean. Bottles from the lot must be tested as bottle blanks (Section 9.4.7) and demonstrated to be free of mercury at the ML of this Method. If mercury is present above this level in any bottle, either the lot must be rejected or the bottles must be re-cleaned.

6.1.3 Filtration Apparatus

6.1.3.1 Filter—0.45-µm, 15-mm diameter capsule filter (Gelman Supor 12175, or equivalent)

6.1.3.2 Peristaltic pump—115-V a.c., 12-V d.c., internal battery, variable-speed, single-head (Cole-Parmer, portable, "Masterflex L/S," Catalog No. 07570-10 drive with Quick Load pump head, Catalog No. 07021-24, or equivalent).

6.1.3.3 Tubing—styrene/ethylene/butylene/silicone (SEBS) resin for use with peristaltic pump, approx 3/8-in ID by approximately 3 ft (Cole-Parmer size 18, Catalog No. 06424-18, or approximately 1/4-in OD, Cole-Parmer size 17, Catalog No. 06424-17, or equivalent). Tubing is cleaned by soaking in 5–10% HCl solution for 8–24 h, rinsing with reagent water in a clean bench in a clean room, and drying in the clean bench by purging with metal-free air or nitrogen. After drying, the tubing is double-bagged in clear polyethylene bags, serialized with a unique number, and stored until use.

6.2 Equipment for bottle and glassware cleaning

6.2.1 Vat, 100–200 L, high-density polyethylene (HDPE), half filled with 4 N HCl in reagent water.

6.2.2 Panel immersion heater, 500-W, all-fluoropolymer coated, 120 vac (Cole-Parmer H-03053-04, or equivalent)

WARNING: *Read instructions carefully!! The heater will maintain steady state, without temperature feedback control, of 60–75°C in a vat of the size described. However, the equilibrium temperature will be higher (up to boiling) in a smaller vat. Also, the heater plate MUST be maintained in a vertical position, completely submerged and away from the vat walls to avoid melting the vat or burning out!*

6.2.3 Laboratory sink—in Class-100 clean area, with high-flow reagent water (Section 7.1) for rinsing.

6.2.4 Clean bench—Class-100, for drying rinsed bottles.

6.2.5 Oven—stainless steel, in Class-100 clean area, capable of maintaining ± 5°C in the 60–70°C temperature range.

6.3 Cold vapor atomic fluorescence spectrometer (CVAFS): The CVAFS system used may either be purchased from a supplier, or built in the laboratory from commercially available components.

6.3.1 Commercially available CVAFS—Tekran (Toronto, ON) Series 2600 CVAFS, Brooks-Rand (Seattle, WA) Model III CVAFS, Leeman Labs Hydra AF Gold*plus* CVAFS, or equivalent

6.3.2 Custom-built CVAFS (Reference 16.15). Figure 2 shows the schematic diagram. The system consists of the following:

6.3.2.1 Low-pressure 4-W mercury vapor lamp

6.3.2.2 Far UV quartz flow-through fluorescence cell—12 mm x 12 mm x 45 mm, with a 10-mm path length (NSG Cells, or equivalent).

6.3.2.3 UV-visible photomultiplier (PMT)—sensitive to < 230 nm. This PMT is isolated from outside light with a 253.7-nm interference filter (Oriel Corp., Stamford, CT, or equivalent).

6.3.2.4 Photometer and PMT power supply (Oriel Corp. or equivalent), to convert PMT output (nanoamp) to millivolts

6.3.2.5 Black anodized aluminum optical block—holds fluorescence cell, PMT, and light source at perpendicular angles, and provides collimation of incident and fluorescent beams (Frontier Geosciences Inc., Seattle, WA, or equivalent).

6.3.2.6 Flowmeter—with needle valve capable of reproducibly keeping the carrier gas flow rate at 30 mL/min

6.4 Hg purging system—Figure 2 shows the schematic diagram for the purging system. The system consists of the following:

6.4.1 Flow meter/needle valve—capable of controlling and measuring gas flow rate to the purge vessel at 350 ± 50 mL/min.

6.4.2 Fluoropolymer fittings—connections between components and columns are made using 6.4-mm OD fluoropolymer tubing and fluoropolymer friction-fit or threaded tubing connectors. Connections between components requiring mobility are made with 3.2-mm OD fluoropolymer tubing because of its greater flexibility.

6.4.3 Acid fume pretrap—10-cm long x 0.9-cm ID fluoropolymer tube containing 2–3 g of reagent grade, nonindicating, 8–14 mesh soda lime chunks, packed between wads of silanized glass wool. This trap is cleaned of Hg by placing on the output of a clean cold vapor generator (bubbler) and purging for 1 h with N_2 at 350 mL/min.

6.4.4 Cold vapor generator (bubbler)—200-mL borosilicate glass (15 cm high x 5.0 cm diameter) with standard taper 24/40 neck, fitted with a sparging stopper having a coarse glass frit that extends to within 0.2 cm of the bubbler bottom (Frontier Geosciences, Inc. or equivalent).

6.5 The dual-trap Hg(0) preconcentrating system

6.5.1 Figures 2 and 3 show the dual-trap amalgamation system (Reference 16.5).

6.5.2 Gold-coated sand traps—10-cm long x 6.5-mm OD x 4-mm ID quartz tubing. The tube is filled with 3.4 cm of gold-coated 45/60 mesh quartz sand (Frontier Geosciences Inc., Seattle, WA, or equivalent). The ends are plugged with quartz wool.

6.5.2.1 Traps are fitted with 6.5-mm ID fluoropolymer friction-fit sleeves for making connection to the system. When traps are not in use, fluoropolymer end plugs are inserted in trap ends to eliminate contamination.

6.5.2.2 At least six traps are needed for efficient operation, one as the "analytical" trap, and the others to sequentially collect samples.

6.5.3 Heating of gold-coated sand traps—To desorb Hg collected on a trap, heat for 3.0 min to 450–500 °C (a barely visible red glow when the room is darkened) with a coil consisting of 75 cm of 24-gauge Nichrome wire at a potential of 10-14 vac. Potential is applied and finely adjusted with an autotransformer.

6.5.4 Timers—The heating interval is controlled by a timer-activated 120-V outlet (Gralab, or equivalent), into which the heating coil autotransformer is plugged. Two timers are required, one each for the "sample" trap and the "analytical" trap.

6.5.5 Air blowers—After heating, traps are cooled by blowing air from a small squirrel-cage blower positioned immediately above the trap. Two blowers are required, one each for the "sample" trap and the "analytical" trap.

6.6 Recorder—Any multi-range millivolt chart recorder or integrator with a range compatible with the CVAFS is acceptable. By using a two-pen recorder with pen sensitivity offset by a factor of 10, the dynamic range of the system is extended to 10^3.

6.7 Pipettors—All-plastic pneumatic fixed-volume and variable pipettors in the range of 10 µL to 5.0 mL.

6.8 Analytical balance capable of weighing to the nearest 0.01 g

7.0 Reagents and Standards

Note: The quantities of reagents and the preparation procedures in this section are for illustrative purposes. Equivalent performance may be achievable using quantities of reagents and procedures other than those suggested here. The laboratory is responsible for demonstrating equivalent performance.

7.1 Reagent water—18-MΩ minimum, ultrapure deionized water starting from a prepurified (distilled, reverse osmosis, etc.) source. Water should be monitored for Hg, especially after ion exchange beds are changed.

7.2 Air—It is very important that the laboratory air be low in both particulate and gaseous mercury. Ideally, mercury work should be conducted in a new laboratory with mercury-free paint on the walls. A source of air that is very low in Hg should be brought directly into the Class-100 clean bench air intake. If this is not possible, air coming into the clean bench can be cleaned for mercury by placing a gold-coated cloth prefilter over the intake. Gold-coated cloth filter: Soak 2 m^2 of cotton gauze in 500 mL of 2% gold chloride solution at pH 7. In a hood, add 100 mL of 30% $NH_2OH{\cdot}HCl$ solution, and homogenize into the cloth with gloved hands. The material will turn black as colloidal gold is precipitated. Allow the mixture to set for several hours, then rinse

with copious amounts of deionized water. Squeeze-dry the rinsed cloth, and spread flat on newspapers to air-dry. When dry, fold and place over the intake prefilter of the laminar flow hood.

CAUTION: Great care should be taken to avoid contaminating the laboratory with gold dust. This could cause interferences with the analysis if gold becomes incorporated into the samples or equipment. The gilding procedure should be done in a remote laboratory if at all possible.

7.3 Hydrochloric acid—trace-metal purified reagent-grade HCl containing less than 5 pg/mL Hg. The HCl should be analyzed for Hg before use.

7.4 Hydroxylamine hydrochloride—Dissolve 300 g of $NH_2OH{\cdot}HCl$ in reagent water and bring to 1.0 L. This solution may be purified by the addition of 1.0 mL of $SnCl_2$ solution and purging overnight at 500 mL/min with Hg-free N_2. Flow injection systems may require the use of less $SnCl_2$ for purification of this solution.

7.5 Stannous chloride—Bring 200 g of $SnCl_2{\cdot}2H_2O$ and 100 mL concentrated HCl to 1.0 L with reagent water. Purge overnight with mercury-free N_2 at 500 mL/min to remove all traces of Hg. Store tightly capped.

7.6 Bromine monochloride (BrCl)—In a fume hood, dissolve 27 g of reagent grade KBr in 2.5 L of low-Hg HCl. Place a clean magnetic stir bar in the bottle and stir for approximately 1 h in the fume hood. Slowly add 38 g reagent grade $KBrO_3$ to the acid while stirring. When all of the $KBrO_3$ has been added, the solution color should change from yellow to red to orange. Loosely cap the bottle, and allow to stir another hour before tightening the lid.

WARNING: *This process generates copious quantities of free halogens (Cl_2, Br_2, BrCl), which are released from the bottle. Add the $KBrO_3$ slowly in a fume hood!*

7.7 Stock mercury standard—NIST-certified 10,000-ppm aqueous Hg solution (NIST-3133). This solution is stable at least until the NIST expiration date.

7.8 Secondary Hg standard—Add approx 0.5 L of reagent water and 5 mL of BrCl solution (Section 7.6) to a 1.00-L Class A volumetric flask. Add 0.100 mL of the stock mercury standard (Section 7.7) to the flask and dilute to 1.00 L with reagent water. This solution contains 1.00 µg/mL (1.00 ppm) Hg. Transfer the solution to a fluoropolymer bottle and cap tightly. This solution is considered stable until the NIST expiration date.

7.9 Working Hg Standard A—Dilute 1.00 mL of the secondary Hg standard (Section 7.8) to 100 mL in a Class A volumetric flask with reagent water containing 0.5% by volume BrCl solution (Section 7.6). This solution contains 10.0 ng/mL and should be replaced monthly, or longer if extended stability is demonstrated.

7.10 Working Hg Standard B—Dilute 0.10 mL of the secondary Hg standard (Section 7.8) to 1000 mL in a Class A volumetric flask with reagent water containing 0.5% by volume BrCl solution (Section 7.6). This solution contains 0.10 ng/mL and should be replaced monthly, or longer if extended stability is demonstrated.

7.11 Initial Precision and Recovery (IPR) and Ongoing Precision and Recovery (OPR) solutions—Using the working Hg standard A (Section 7.9), prepare IPR and OPR solutions at a

concentration of 5 ng/L Hg in reagent water. IPR/OPR solutions are prepared using the same amounts of reagents used for preparation of the calibration standards.

7.12 Nitrogen—Grade 4.5 (standard laboratory grade) nitrogen that has been further purified by the removal of Hg using a gold-coated sand trap.

7.13 Argon—Grade 5.0 (ultra high-purity, GC grade) argon that has been further purified by the removal of Hg using a gold-coated sand trap.

8.0 Sample Collection, Preservation, and Storage

8.1 Before samples are collected, consideration should be given to the type of data required (i.e., dissolved or total), so that appropriate preservation and pretreatment steps can be taken. An excess of BrCl should be confirmed either visually (presence of a yellow color) or with starch iodide indicating paper, using a separate sample aliquot, prior to sample processing or direct analysis to ensure the sample has been properly preserved.

8.2 Samples are collected into rigorously cleaned fluoropolymer bottles with fluoropolymer or fluoropolymer-lined caps. Glass bottles may be used if Hg is the only target analyte. It is critical that the bottles have tightly sealing caps to avoid diffusion of atmospheric Hg through the threads (Reference 16.4). Polyethylene sample bottles must not be used (Reference 16.15).

8.3 Collect samples using guidance provided in the Sampling Method (Reference 16.9). Procedures in the Sampling Method are based on rigorous protocols for collection of samples for mercury (References 16.4 and 16.15).

NOTE: *Discrete samplers have been found to contaminate samples with Hg at the ng/L level. Therefore, great care should be exercised if this type of sampler is used. It may be necessary for the sampling team to use other means of sample collection if samples are found to be contaminated using the discrete sampler.*

8.4 Sample filtration—For dissolved Hg, a sample is filtered through a 0.45-µm capsule filter (Section 6.1.3.1) in a mercury-free clean area prior to preservation. If the sample is filtered, it must be accompanied by a blank that has been filtered under the same conditions. The Sampling Method describes sample filtration procedures.

8.5 Preservation—Samples are preserved by adding either 5 mL/L of pretested 12N HCl or 5 mL/L BrCl solution to the sample bottle. If a sample will be used also for the determination of methyl mercury, it should be collected and preserved according to procedures in the method that will be used for determination of methyl mercury (e.g., HCl or H_2SO_4 solution). Preserved samples are stable for up to 90 days of the date of collection.

8.5.1 Samples to be analyzed for total or dissolved Hg only may be shipped to the laboratory unpreserved and unrefrigerated if they are collected in fluoropolymer or glass bottles and capped tightly. Samples must be either preserved or analyzed within 48 hours of collection. If a sample is oxidized in the sample bottle, the time to preservation can be extended to 28 days.

8.5.2 Samples that are acid-preserved may lose Hg to coagulated organic materials in the water or condensed on the walls (Reference 16.16). The best approach is to add BrCl directly to the sample bottle at least 24 hours before analysis. If other Hg species are to be analyzed, these aliquots must be removed prior to the addition of BrCl. If BrCl

cannot be added directly to the sample bottle, the bottle must be shaken vigorously prior to sub-sampling.

8.5.3 Handling of the samples in the laboratory should be undertaken in a mercury-free clean bench, after rinsing the outside of the bottles with reagent water and drying in the clean air hood.

NOTE: *Because of the potential for contamination, it is recommended that filtration and preservation of samples be performed in the clean room in the laboratory. However, if circumstances prevent overnight shipment of samples, samples should be filtered and preserved in a designated clean area in the field in accordance with the procedures given in Method 1669 (Reference 16.9). If filtered in the field, samples ideally should be filtered into the sample bottle.*

8.6 Storage—Sample bottles should be stored in clean (new) polyethylene bags until sample analysis.

8.7 Sample preservation, storage, and holding time requirements also are given at 40 CFR part 136.3(e) Table II.

9.0 Quality Control

9.1 Each laboratory that uses this Method is required to operate a formal quality assurance program (Reference 16.17). The minimum requirements of this program consist of an initial demonstration of laboratory capability, ongoing analysis of standards and blanks as a test of continued performance, and the analysis of matrix spikes (MS) and matrix spike duplicates (MSD) to assess precision and recovery. Laboratory performance is compared to established performance criteria to determine that the results of analyses meet the performance characteristics of the Method.

9.1.1 The laboratory shall make an initial demonstration of the ability to generate acceptable accuracy and precision. This ability is established as described in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, the laboratory is permitted certain options to improve results or lower the cost of measurements. These options include automation of the dual-amalgamation system, single-trap amalgamation (Reference 16.18), direct electronic data acquisition, calibration using gas-phase elemental Hg standards, use of the bubbler or flow-injection systems, or changes in the detector (i.e., CVAAS) when less sensitivity is acceptable or desired. Changes in the determinative technique, such as the use of colorimetry, are not allowed. If an analytical technique other than the CVAFS technique specified in this Method is used, that technique must have a specificity for mercury equal to or better than the specificity of the technique in this Method.

9.1.2.1 Each time this Method is modified, the laboratory is required to repeat the procedure in Section 9.2 to demonstrate that an MDL (40 CFR part 136, Appendix B) less than or equal to one-third the regulatory compliance limit or less than or equal to the MDL of this Method (Table 1), whichever is greater, can be achieved. If the change will affect calibration, the instrument must be recalibrated according to Section 10.

Note: If the compliance limit is greater than the concentration of Hg in the OPR/OPR (5 ng/L), the acceptance criteria for blanks and the concentrations of mercury spiked into quality control samples may be increased to support measurements at the compliance limit. For example, if the compliance limit is 12

ng/L (National Toxics Rule, 40 CFR 131.36), the MDL must be less than or equal to 4 ng/L; concentrations of the calibration standards may be 5, 10, 20, 50 , and 100 ng/L; concentrations of the IPR/OPR samples may be 10 ng/L; spike concentrations and acceptance criteria for MS/MSD samples would remain as specified in Section 9.3; and an appropriate blank acceptance criterion would be 5 ng/L.

9.1.2.2 The laboratory is required to maintain records of modifications made to this Method. These records include the following, at a minimum:

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and the quality control officer who witnessed and will verify the analyses and modification

9.1.2.2.2 A narrative stating the reason(s) for the modification(s)

9.1.2.2.3 Results from all quality control (QC) tests demonstrating the performance of the modified method, including the following:

(a) Calibration (Section 10)
(b) Initial precision and recovery (Section 9.2.2)
(c) Analysis of blanks (Section 9.4)
(d) Matrix spike/matrix spike duplicate analysis (Section 9.3)
(e) Ongoing precision and recovery (Section 9.5)
(f) Quality control sample (Section 9.6)
(g) Method detection limit (Section 9.2.1)

9.1.2.2.4 Data that will allow an independent reviewer to validate each determination by tracking the instrument output to the final result. These data are to include the following:

(a) Sample numbers and other identifiers
(b) Processing dates
(c) Analysis dates
(d) Analysis sequence/run chronology
(e) Sample weight or volume
(f) Copies of logbooks, chart recorder, or other raw data output
(g) Calculations linking raw data to the results reported

9.1.3 Analyses of MS and MSD samples are required to demonstrate the accuracy and precision and to monitor matrix interferences. Section 9.3 describes the procedure and QC criteria for spiking.

9.1.4 Analyses of blanks are required to demonstrate acceptable levels of contamination. Section 9.4 describes the procedures and criteria for analyzing blanks.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through analysis of the ongoing precision and recovery (OPR) sample and the quality control sample (QCS) that the system is in control. Sections 9.5 and 9.6 describe these procedures, respectively.

9.1.6 The laboratory shall maintain records to define the quality of the data that are generated. Sections 9.3.7 and 9.5.3 describe the development of accuracy statements.

9.1.7 Quality of the analyses is controlled by an analytical batch. An analytical batch is a set of samples oxidized with the same batch of reagents, and analyzed during the same 12-hour shift. A batch may be from 1 to as many as 20 samples. Each batch must be accompanied by 3 system blanks (Section 9.4.2 for the flow-injection system), a

minimum of 3 bubbler blanks (Section 9.4.1 for the bubbler system), 1 OPR sample at the beginning and end of the batch (Section 9.5), a QCS (Section 9.6), and at least 3 method blanks (Section 9.4.4). In addition, there must be 1 MS and 1 MSD sample for every 10 samples (a frequency of 10%). A typical analytical sequence would be:

(a) Three system blanks (Section 9.4.2) or a minimum of 3 bubbler blanks (Section 9.4.1)
(b) A minimum of five, non-zero calibration standards (Section 10.2.2.1)
(c) On-going precision and recovery (Section 9.5)
(d) Quality control sample (Section 9.6)
(e) Method blank (Section 9.4.4)
(f) Seven samples
(g) Method blank (Section 9.4.4)
(h) Three samples
(i) Matrix spike (Section 9.3)
(j) Matrix spike duplicate (Section 9.3)
(k) Four samples
(l) Method blank (Section 9.4.4)
(m) Six samples
(n) Matrix spike (Section 9.3)
(o) Matrix spike duplicate (Section 9.3)
(p) Ongoing precision and recovery (Section 9.5)

The above sequence includes calibration. If system performance is verified at the end of the sequence using the OPR, analysis of samples and blanks may proceed without recalibration (i.e., the analytical sequence would be entered at Step (d) above), unless more than 12 hours has elapsed since verification of system performance. If more than 12 hours has elapsed, the sequence would be initiated at Step (c) above.

9.2 Initial demonstration of laboratory capability

9.2.1 Method detection limit—To establish the ability to detect Hg, the laboratory shall achieve an MDL that is less than or equal to the MDL listed in Section 1.5 or one-third the regulatory compliance limit, whichever is greater. The MDL shall be determined according to the procedure at 40 CFR 136, Appendix B using the apparatus, reagents, and standards that will be used in the practice of this Method. This MDL shall be used for determination of laboratory capability only, and should be determined when a new operator begins work or whenever, in the judgment of the laboratory, a change in instrument hardware or operating conditions would dictate reevaluation of capability.

9.2.2 Initial precision and recovery (IPR)—To establish the ability to generate acceptable precision and recovery, the laboratory shall perform the following operations:

9.2.2.1 Analyze four replicates of the IPR solution (5 ng/L, Section 7.11) according to the procedure beginning in Section 11.

9.2.2.2 Using the results of the set of four analyses, compute the average percent recovery (X), and the standard deviation of the percent recovery (s) for Hg.

9.2.2.3 Compare s and X with the corresponding limits for initial precision and recovery in Table 2. If s and X meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If, however, s exceeds the

precision limit or X falls outside the acceptance range, system performance is unacceptable. Correct the problem and repeat the test (Section 9.2.2.1).

9.3 Matrix spike (MS) and matrix spike duplicate (MSD)—To assess the performance of the Method on a given sample matrix, the laboratory must spike, in duplicate, a minimum of 10% (1 sample in 10) from a given sampling site or, if for compliance monitoring, from a given discharge. Therefore, an analytical batch of 20 samples would require two pairs of MS/MSD samples (four spiked samples total).

9.3.1 The concentration of the spike in the sample shall be determined as follows:

9.3.1.1 If, as in compliance monitoring, the concentration of Hg in the sample is being checked against a regulatory compliance limit, the spiking level shall be at that limit or at 1–5 times the background concentration of the sample (as determined in Section 9.3.2), whichever is greater.

9.3.1.2 If the concentration of Hg in a sample is not being checked against a limit, the spike shall be at 1–5 times the background concentration or at 1-5 times the ML in Table 1, whichever is greater.

9.3.2 To determine the background concentration (B), analyze one sample aliquot from each set of 10 samples from each site or discharge according to the procedure in Section 11. If the expected background concentration is known from previous experience or other knowledge, the spiking level may be established *a priori*.

9.3.2.1 If necessary, prepare a standard solution to produce an appropriate level in the sample (Section 9.3.1).

9.3.2.2 Spike two additional sample aliquots with identical amounts of the spiking solution and analyze these aliquots as described in Section 11.1.2 to determine the concentration after spiking (A).

9.3.3 Calculate the percent recovery (R) in each aliquot using the following equation:

$$\%\ R = 100\ \frac{(A-B)}{T}$$

where:

A = Measured concentration of analyte after spiking
B = Measured concentration of analyte before spiking
T = True concentration of the spike

9.3.4 Compare percent recovery (R) with the QC acceptance criteria in Table 2.

9.3.4.1 If results of the MS/MSD are similar and fail the acceptance criteria, and recovery for the OPR standard (Section 9.5) for the analytical batch is within the acceptance criteria in Table 2, an interference is present and the results may not be reported or otherwise used for permitting or regulatory compliance purposes. If the interference can be attributed to sampling, the site or discharge should be resampled. If the interference can be attributed to a method deficiency, the laboratory must modify the method, repeat the test required in Section 9.1.2, and repeat analysis of the sample and MS/MSD. However, during the development

of Method 1631, very few interferences have been noted in the determination of Hg using this Method. (See Section 4.4 for information on interferences.)

9.3.4.2 If the results of both the spike and the OPR test fall outside the acceptance criteria, the analytical system is judged to be not in control, and the results may not be reported or used for permitting or regulatory compliance purposes. The laboratory must identify and correct the problem and reanalyze all samples in the sample batch.

9.3.5 Relative percent difference (RPD)—Compute the RPD between the MS and MSD results according to the following equation using the concentrations found in the MS and MSD. Do not use the recoveries calculated in Section 9.3.3 for this calculation because the RPD is inflated when the background concentration is near the spike concentration.

$$RPD = 200 \times \frac{(|D1-D2|)}{(D1+D2)}$$

Where:
D1 = concentration of Hg in the MS sample
D2 = concentration of Hg in the MSD sample

9.3.6 The RPD for the MS/MSD pair must not exceed the acceptance criterion in Table 2. If the criterion is not met, the system is judged to be out of control. The problem must be identified and corrected, and the MS/MSD and corresponding samples reanalyzed.

9.3.7 As part of the QC program for the laboratory, method precision and recovery for samples should be assessed and records maintained. After analyzing five samples in which the recovery passes the test in Section 9.3.4, compute the average percent recovery (R_a) and the standard deviation of the percent recovery (s_r). Express the accuracy assessment as a percent recovery interval from $R_a - 2s_r$ to $R_a + 2s_r$. For example, if $R_a = 90\%$ and $s_r = 10\%$ for five analyses, the accuracy interval is expressed as 70–110%. Update the accuracy assessment regularly (e.g., after every five to ten new accuracy measurements).

9.4 Blanks—Blanks are critical to the reliable determination of Hg at low levels. The sections below give the minimum requirements for analysis of blanks. Analysis of additional blanks is recommended as necessary to pinpoint sources of contamination in, and external to, the laboratory.

9.4.1 Bubbler blanks—Bubbler blanks are analyzed to demonstrate that bubbler systems are free from contamination at levels that could affect data quality. At least three bubbler blanks must be run during calibration and with each analytical batch.

9.4.1.1 To analyze a bubbler blank, place a clean gold trap on the bubbler. Purge and analyze previously purged water using the procedure in Section 11, and determine the amount of Hg remaining in the system.

9.4.1.2 If the bubbler blank is found to contain more than 50 pg Hg, the system is out of control. The problem must be investigated and remedied, and the samples run on that bubbler must be reanalyzed. If the blanks from other bubblers contain less than 50 pg Hg, the data associated with those bubblers remain valid, provided that all other criteria in Section 9 also are met.

9.4.1.3 The mean result for all bubbler blanks (from bubblers passing the specification in Section 9.4.1.2) must be < 25 pg (0.25 ng/L) Hg with a standard deviation (n-1) of <10 pg (0.10 ng/L). If the mean is < 25 pg, the average peak area or height is subtracted from all raw data before results are calculated (Section 12.2).

9.4.1.4 If Hg in the bubbler blank exceeds the acceptance criteria in Section 9.4.1.3, the system is out of control. The problem must be resolved and the system recalibrated. Usually, the bubbler blank is too high for one of the following reasons:

(a) Bubblers need rigorous cleaning;
(b) Soda-lime is contaminated; or
(c) Carrier gas is contaminated.

9.4.2 System blanks— System blanks are analyzed to demonstrate that flow injection systems are free from contamination at levels that could affect data quality. Three system blanks must be run during calibration and with each analytical batch.

9.4.2.1 To analyze a system blank, analyze reagent water containing the same amount of reagents used to prepare the calibration standards.

9.4.2.2 If a system blank is found to contain ≥ 0.50 ng/L Hg, the system is out of control. The problem must be investigated and remedied, and the system recalibrated. If the blanks contain < 0.50 ng/L Hg, the data associated with the blanks remain valid, provided that all other criteria in Section 9 also are met.

9.4.2.3 The mean result for the three system blanks must be <0.5 ng/L Hg with a standard deviation (n-1) <0.1 ng/L. If the mean exceeds these criteria, the system is out of control, and the problem must be resolved and the system recalibrated. If the mean is <0.5 ng/L, the average peak height or area is subtracted from all raw data before results are calculated (Section 12.3).

9.4.3 Reagent blanks—Reagent blanks are used to demonstrate that the reagents used to prepare samples for Hg analyses are free from contamination. The Hg concentration in reagent blanks is determined by analyzing the reagent solutions using either the bubbler or flow-injection system. For the bubbler system, reagent may be added directly to previously purged water in the bubbler.

9.4.3.1 Reagent blanks are required when the batch of reagents (bromine monochloride plus hydroxylamine hydrochloride) are prepared. The amount of Hg in a reagent blank containing 0.5% (v/v) BrCl solution (Section 7.6) and 0.2% (v/v) hydroxylamine hydrochloride solution (Section 7.4) must be < 20 pg (0.2 ng/L).

9.4.3.2 The presence of more than 20 pg (0.2 ng/L) of Hg indicates a problem with the reagent solution. The purging of certain reagent solutions, such as $SnCl_2$ or NH_2OH, with mercury-free nitrogen or argon can reduce Hg to acceptable levels. Because BrCl cannot be purified, a new batch must be prepared and tested if the BrCl is contaminated.

9.4.4 Method blanks— Method blanks are used to demonstrate that the analytical system is free from contamination that could otherwise compromise sample results. Method blanks are prepared and analyzed using sample containers, labware, reagents, and analytical procedures identical to those used to prepare and analyze the samples.

9.4.4.1 A minimum of three method blanks per analytical batch are required for both the bubbler and flow-injection systems.

9.4.4.2 If the result for any method blank containing the nominal amount of reagent used to prepare a sample (Section 11.1.1) is found to contain ≥ 0.50 ng/L (50 pg) Hg, the system is out of control. Mercury in the analytical system must be reduced until a method blank is free from contamination at the 0.50 ng/L level. Samples associated with a contaminated method blank must be reanalyzed.

9.4.4.3 Because method blanks are analyzed using procedures identical to those used to analyze samples, any sample requiring an increased amount of reagent must be accompanied by at least one method blank that includes an identical amount of reagent.

9.4.5 Field blanks–Field blanks are used to demonstrate that samples have not been contaminated by the sample collection and transport activities.

9.4.5.1 Analyze the field blank(s) shipped with each set of samples (samples collected from the same site at the same time, to a maximum of 10 samples). Analyze the blank immediately before analyzing the samples in the batch.

9.4.5.2 If Hg or any potentially interfering substance is found in the field blank at a concentration equal to or greater than the ML (Table 1), or greater than one-fifth the level in the associated sample, whichever is greater, results for associated samples may be the result of contamination and may not be reported or otherwise used for regulatory compliance purposes.

9.4.5.3 Alternatively, if sufficient multiple field blanks (a minimum of three) are collected, and the average concentration (of the multiple field blanks) plus two standard deviations is equal to or greater than the regulatory compliance limit or equal to or greater than one-half of the level in the associated sample, results for associated samples may be the result of contamination and may not be reported or otherwise used for regulatory compliance purposes.

9.4.5.4 If contamination of the field blanks and associated samples is known or suspected, the laboratory should communicate this to the sampling team so that the source of contamination can be identified and corrective measures taken before the next sampling event.

9.4.6 Equipment blanks—Before any sampling equipment is used at a given site, the laboratory or cleaning facility is required to generate equipment blanks on all sampling equipment that will be used to demonstrate that the sampling equipment is free from contamination.

9.4.6.1 Equipment blanks are generated in the laboratory or at the equipment cleaning facility by processing reagent water through the sampling devices using the same procedures that are used in the field (see Sampling Method). Therefore, the "clean hands/dirty hands" technique used during field sampling should be followed when preparing equipment blanks at the laboratory or cleaning facility for low level mercury measurements. If grab samples are to be collected using any ancillary equipment, e.g., an extension pole or a dipper, an equipment blank

is generated by submersing this equipment into the reagent water and analyzing the resulting reagent water collected.

9.4.6.2 The equipment blank must be analyzed using the procedures in this Method. If mercury or any potentially interfering substance is detected in the blank at or above the level specified for the field blank (Section 9.4.5), the source of contamination or interference must be identified, and the problem corrected. The equipment must be demonstrated to be free from mercury and interferences before the equipment may be used in the field.

9.4.7 Bottle blanks— Bottles must be subjected to conditions of use to verify the effectiveness of the cleaning procedures. A representative set of sample bottles (Section 6.1.2) should be filled with reagent water acidified to pH <2 and allowed to stand for a minimum of 24 h. At least 5% of the bottles from a given lot should be tested, and the time that the bottles are allowed to stand should be as close as possible to the actual time that the sample will be in contact with the bottle. After standing, the water must be analyzed for any signs of contamination. If a bottle shows contamination at or above the level specified for the field blank (Section 9.4.5), the problem must be identified, the cleaning procedures corrected or cleaning solutions changed, and all affected bottles re-cleaned.

9.5 Ongoing precision and recovery (OPR)—To demonstrate that the analytical system is within the performance criteria of this Method and that acceptable precision and recovery is being maintained within each analytical batch, the laboratory shall perform the following operations:

9.5.1 Analyze the OPR solution (5 ng/L, Section 7.11) prior to the analysis of each analytical batch according to the procedure beginning in Section 11. An OPR also must be analyzed at the end of an analytical sequence or at the end of each 12-hour shift.

9.5.2 Compare the recovery with the limits for ongoing precision and recovery in Table 2. If the recovery is in the range specified, the analytical system is in control and analysis of samples and blanks may proceed. If, however, the concentration is not in the specified range, the analytical process is not in control. Correct the problem and repeat the ongoing precision and recovery test. All reported results must be associated with an OPR that meets the Table 2 performance criteria at the beginning and end of each batch.

9.5.3 The laboratory should add results that pass the specification in Section 9.5.2 to IPR and previous OPR data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory also should develop a statement of laboratory data quality by calculating the average percent recovery (R_a) and the standard deviation of the percent recovery (s_r). Express the accuracy as a recovery interval from $R_a - 2s_r$ to $R_a + 2s_r$. For example, if $R_a = 95\%$ and $s_r = 5\%$, the accuracy is 85–105%.

9.6 Quality control sample (QCS) – The laboratory must obtain a QCS from a source different from the Hg used to produce the standards used routinely in this Method (Sections 7.7–7.10). The QCS should be analyzed as an independent check of system performance.

9.7 Depending on specific program requirements, the laboratory may be required to analyze field duplicates and field spikes collected to assess the precision and accuracy of the sampling, sample transportation, and storage techniques. The relative percent difference (RPD) between field duplicates should be less than 20%. If the RPD of the field duplicates exceeds 20%, the laboratory should communicate this to the sampling team so that the source of error can be identified and corrective measures taken before the next sampling event.

10.0 Calibration and Standardization

10.1 Calibration and standardization— Separate calibration procedures are provided for a bubbler system (Section 10.2) and flow-injection system (Section 10.3). Both systems are calibrated using standards traceable to NIST Standard Reference Materials. If system performance is verified at the end of an analytical batch using the OPR, analysis of samples and blanks may proceed without recalibration, unless more than 12 hours has elapsed since verification of system performance.

10.2 Bubbler system calibration

10.2.1 Establish the operating conditions necessary to purge Hg from the bubbler and to desorb Hg from the traps in a sharp peak. Further details for operation of the purge-and-trap, desorption, and analysis systems are given in Sections 11.2.1 and 11.2.2.

10.2.2 The calibration must contain a minimum of five non-zero points and the results of analysis of three bubbler blanks. The lowest calibration point must be at the Minimum Level (ML).

NOTE: *The purge efficiency of the bubbler system is 100% and is independent of volume at the volumes used in this Method. Calibration of this system is typically performed using units of mass. For purposes of working in concentration, the volume is assumed to be 100 mL.*

10.2.2.1 Standards are analyzed by the addition of aliquots of Hg working standard A (Section 7.9) and Hg working standard B (Section 7.10) directly into the bubblers. Add 0.50 mL of working standard B and 0.5 mL $SnCl_2$ to the bubbler. Swirl to produce a standard containing 50 pg of Hg (0.5 ng/L). Purge under the optimum operating conditions (Section 10.2.1). Sequentially follow with the addition of aliquots of 0.05, 0.25, 0.50 and 1.0 mL of working standard A to produce standards of 500, 2500, 5000, and 10,000 pg Hg (5.0, 25.0, 50.0 and 100.0 ng/L).

NOTE: *If calibration to the higher levels results in carryover (Section 4.3.8.1), calibrate the system across a narrower range (Section 10.4)*

10.2.2.2 Analyze the standards beginning with the lowest concentration and proceeding to the highest. Tabulate the height or area for each peak.

10.2.2.3 Prepare and analyze a minimum of 3 bubbler blanks. If multiple bubblers are used, there must be 1 bubbler blank per bubbler (to a maximum of 4 bubblers). Calculate the mean peak area or height for the bubbler blanks.

10.2.2.4 For each calibration point, subtract the mean peak height or area of the bubbler blanks from the peak height or area for each standard. Calculate the calibration factor (CF_x) for Hg in each of the five standards using the mean bubbler-blank-subtracted peak height or area and the following equation:

$$CF_x = \frac{(A_x) - (\bar{A}_{BB})}{(C_x)}$$

Where:

A_x = *peak height or area for Hg in standard*
$\bar{A}_{BB}$ = *mean peak height or area for Hg in bubbler blank*
C_x = *mass in standard analyzed (ng)*

10.2.2.5 Calculate the mean calibration factor (CF_m), the standard deviation of the calibration factor (SD; n-1), and the relative standard deviation (RSD) of the calibration factor, where RSD = 100 x SD/CF_m.

10.2.2.6 If RSD ≤ 15%, calculate the recovery for the lowest standard using CF_m. If the RSD ≤ 15% and the recovery of the lowest standard is in the range of 75-125%, the calibration is acceptable and CF_m may be used to calculate the concentration of Hg in samples. If RSD > 15% or if the recovery of the lowest standard is not in the range of 75-125%, recalibrate the analytical system and repeat the test.

10.2.2.7 Calculate the concentration of Hg in the bubbler blanks (Section 10.2.2.1) using CF_m. The bubbler blanks must meet the criteria in Section 9.4.1; otherwise, mercury in the system must be reduced and the calibration repeated until the bubbler blanks meet the criteria.

10.3 Flow-injection system calibration

10.3.1 Establish the operating conditions necessary to purge Hg from the gas-liquid separator and dryer tube and desorb Hg from the traps in a sharp peak. Further details for operating the analytical system are given in Section 11.2.1.

10.3.2 The calibration must contain a minimum of 5 non-zero points and the results of analysis of 3 system blanks. The lowest calibration point must be at the minimum level (ML).

10.3.2.1 Place 25-30 mL of reagent water and 250 µL of concentrated BrCl solution (Section 7.6) in each of 5 calibrated 50-mL autosampler vials. Prepare the 0.5 ng/L calibration standard by adding 250 µL of working standard B (Section 7.10) to the vial. Dilute to the mark with reagent water. Sequentially follow with the addition of aliquots of 25, 125, 250 and 500 µL of working standard A (Section 7.9) to produce standards of 5.0, 25.0, 50.0 and 100.0 ng/L, respectively. Cap the vials and invert once to mix.

10.3.2.2 Immediately prior to analysis, remove the caps and add 125 µL of NH_2OH solution (Section 7.4). Re-cap, invert once to mix, and allow to stand until the yellow color disappears. Remove all caps and place vials into the analysis rack.

10.3.2.3 Analyze the standards beginning with the lowest concentration and proceeding to the highest. Tabulate the height or area for the Hg peak.

10.3.2.4 Prepare and analyze a minimum of 3 system blanks and tabulate the peak heights or areas. Calculate the mean peak area or height for the system blanks.

10.3.2.5 For each calibration point, subtract the mean peak height or area of the system blanks (Section 9.4.2) from the peak height or area for each standard. Calculate

the calibration factor (CF_x) for Hg in each of the five standards using the mean reagent-blank-subtracted peak height or area and the following equation:

$$CF_x = \frac{(A_x) - (\overline{A}_{SB})}{(C_x)}$$

Where:

A_x = *peak height or area for Hg in standard*
$\overline{A}_{SB}$ = *mean peak height or area for Hg in calibration blanks*
C_x = *concentration of standard analyzed (ng/L)*

10.3.2.6 Calculate the mean calibration factor (CF_m), the standard deviation of the calibration factor (SD; n-1), and the relative standard deviation (RSD) of the calibration factor, where RSD = 100 x SD/CF_m.

10.3.2.7 If RSD ≤ 15%, calculate the recovery for the lowest standard (0.5 ng/L) using CF_m. If the RSD ≤ 15% and the recovery of the lowest standard is in the range of 75-125%, the calibration is acceptable and CF_m may be used to calculate the concentration of Hg in samples, blanks, and OPRs. If RSD > 15% or if the recovery of the lowest standard is not in the range of 75-125%, recalibrate the analytical system and repeat the test.

10.3.2.8 Calculate the concentration of Hg in the system blanks (Section 9.4.2) using CF_m. The system blanks must meet the criteria in Section 9.4.2; otherwise, mercury in the system must be reduced and the calibration repeated until the system blanks meet the criteria.

10.4 Calibration to a range other than 0.5 to 100 ng/L—This Method may be calibrated to a range other than 0.5 to 100 ng/L, provided that the following requirements are met:

(a) There must be a minimum of five non-zero calibration points.
(b) The difference between successive calibration points must be no greater than a factor of 10 and no less than a factor of 2 and should be approximately evenly spaced on a logarithmic scale over the calibration range.
(c) The relative standard deviation (RSD) of the calibration factors for all calibration points must be less than 15%.
(d) The calibration factor for any calibration point at a concentration greater than 100 ng/L must be within ±15% of the average calibration factor for the points at or below 100 ng/L.
(e) The calibration factor for any point <0.5 ng/L must be within 25% of the average calibration factor for all points.
(f) If calibration is to a higher range and this Method is used for regulatory compliance, the ML must be less than one-third the regulatory compliance limit

11.0 Procedure

NOTE: *The following procedures for analysis of samples are provided as guidelines. Laboratories may find it necessary to optimize the procedures, such as drying time or potential applied to the Nichrome wires, for the laboratory's specific instrument set-up.*

11.1 Sample Preparation

11.1.1 Pour a 100-mL aliquot from a thoroughly shaken, acidified sample, into a 125-mL fluoropolymer bottle. If BrCl was not added as a preservative (Section 8.5), add the amount of BrCl solution (Section 7.6) given below, cap the bottle, and digest at room temperature for a 12 h minimum.

11.1.1.1 For clear water and filtered samples, add 0.5 mL of BrCl; for brown water and turbid samples, add 1.0 mL of BrCl. If the yellow color disappears because of consumption by organic matter or sulfides, more BrCl should be added until a permanent (12-h) yellow color is obtained.

11.1.1.2 Some highly organic matrices, such as sewage effluent, will require high levels of BrCl (e.g., 5 mL/100 mL of sample) and longer oxidation times, or elevated temperatures (e.g., place sealed bottles in oven at 50 °C for 6 h). The oxidation must be continued until it is complete. Complete oxidation can be determined by either observation of a permanent yellow color remaining in the sample or the use of starch iodide indicating paper to test for residual free oxidizer. The sample also may be diluted to reduce the amount of BrCl required, provided that the resulting level of mercury is sufficient for reliable determination.

11.1.2 Matrix spikes and matrix spike duplicates—For every 10 or fewer samples, pour 2 additional 100-mL aliquots from a selected sample (see Section 9.3), spike at the level specified in Section 9.3, and process in the same manner as the samples. There must be a minimum of 2 MS/MSD pairs for each analytical batch of 20 samples.

11.2 Hg reduction and purging—Separate procedures are provided for the bubbler system (Section 11.2.1) and flow-injection (Section 11.2.2).

11.2.1 Hg reduction and purging for the bubbler system

11.2.1.1 Add 0.2-0.25 mL of NH_2OH solution to the BrCl-oxidized sample in the 125-mL sample bottle. Cap the bottle and swirl the sample. The yellow color will disappear, indicating the destruction of the BrCl. Allow the sample to react for 5 min with periodic swirling to be sure that no traces of halogens remain.

NOTE: *Purging of free halogens onto the gold trap will result in damage to the trap and low or irreproducible results.*

11.2.1.2 Connect a fresh trap to the bubbler, pour the reduced sample into the bubbler, add 0.5 mL of $SnCl_2$ solution, and purge the sample onto a gold trap with N_2 at 350 ± 50 mL/min for 20 min.

11.2.1.3 When analyzing Hg samples, the recovery is quantitative, and organic interferents are destroyed. Thus, standards, bubbler blanks, and small amounts of high-level samples may be run directly in previously purged water. After very high samples (Section 4.3.8.1), a small degree of carryover (<0.01%) may occur. Bubblers that contain such samples must be demonstrated to be clean prior to proceeding with low level samples. Samples run immediately following a sample that has been determined to result in carryover must be reanalyzed using a bubbler that is demonstrated to be clean as per Section 4.3.8.1.

11.2.2 Hg reduction and purging for the flow-injection system

11.2.2.1 Add 0.2-0.25 mL of NH_2OH solution (Section 7.4) to the BrCl-oxidized sample in the 125-mL sample bottle or in the autosampler tube (the amount of NH_2OH required will be approximately 30 percent of the BrCl volume). Cap the bottle and swirl the sample. The yellow color will disappear, indicating the destruction of the BrCl. Allow the sample to react for 5 minutes with periodic swirling to be sure that no traces of halogens remain.

NOTE: *Purging of free halogens onto the gold trap will result in damage to the trap and low or irreproducible results.*

11.2.2.2 Pour the sample solution into an autosampler vial and place the vial in the rack.

11.2.2.3 Carryover may occur after analysis of a sample containing a high level of mercury. Samples run immediately following a sample that has been determined to result in carryover (Section 4.3.8.1) must be reanalyzed using a system demonstrated to be clean as per Section 4.3.8.1.

11.3 Desorption of Hg from the gold trap

11.3.1 Remove the sample trap from the bubbler, place the Nichrome wire coil around the trap and connect the trap into the analyzer train between the incoming Hg-free argon and the second gold-coated (analytical) sand trap (Figure 2).

11.3.2 Pass argon through the sample and analytical traps at a flow rate of approximately 30 mL/min for approximately 2 min to drive off condensed water vapor.

11.3.3 Apply power to the coil around the sample trap for 3 minutes to thermally desorb the Hg (as Hg(0)) from the sample trap onto the analytical trap.

11.3.4 After the 3-min desorption time, turn off the power to the Nichrome coil, and cool the sample trap using the cooling fan.

11.3.5 Turn on the chart recorder or other data acquisition device to start data collection, and apply power to the Nichrome wire coil around the analytical trap. Heat the analytical trap for 3 min (1 min beyond the point at which the peak returns to baseline).

11.3.6 Stop data collection, turn off the power to the Nichrome coil, and cool the analytical trap to room temperature using the cooling fan.

11.3.7 Place the next sample trap in line and proceed with analysis of the next sample.

NOTE: *Do not heat a sample trap while the analytical trap is still warm; otherwise, the analyte may be lost by passing through the analytical trap.*

11.4 Peaks generated using this technique should be very sharp and almost symmetrical. Mercury elutes at approximately 1 minute and has a width at half-height of about 5 seconds.

11.4.1 Broad or asymmetrical peaks indicate a problem with the desorption train, such as improper gas flow rate, water vapor on the trap(s), or an analytical trap damaged by chemical fumes or overheating.

11.4.2 Damage to an analytical trap is also indicated by a sharp peak, followed by a small, broad peak.

11.4.3 If the analytical trap has been damaged, the trap and the fluoropolymer tubing downstream from it should be discarded because of the possibility of gold migration onto downstream surfaces.

11.4.4 Gold-coated sand traps should be tracked by unique identifiers so that any trap producing poor results can be quickly recognized and discarded.

12.0 Data Analysis and Calculations

12.1 Separate procedures are provided for calculation of sample results using the bubbler system (Section 12.2) and the flow-injection system (Section 12.3), and for method blanks (Section 12.4).

12.2 Calculations for the bubbler system

12.2.1 Calculate the mean peak height or area for Hg in the bubbler blanks measured during system calibration or with the analytical batch (A_{BB}; n = 3 minimum).

12.2.2 Calculate the concentration of Hg in ng/L (parts-per-trillion; ppt) in each sample according to the following equation:

$$[Hg]\ (ng/L) = \frac{A_s - \overline{A}_{BB}}{CF_m \times V}$$

where:
A_s = *peak height (or area) for Hg in sample*
$\overline{A}_{BB}$ = *peak height (or area) for Hg in bubbler blank*
CF_m = *mean calibration factor (Section* 10.2.2.5)
V = *Volume of sample (L)*

12.3 Calculations for the flow-injection system

12.3.1 Calculate the mean peak height or area for Hg in the system blanks measured during system calibration or with each analytical batch (A_{SB}; n = 3)

12.3.2 Calculate the concentration of Hg in ng/L in each sample according to the following equation:

$$[Hg]\ (ng/L) = \frac{(A_s - \overline{A}_{SB})}{CF_m} \times \frac{V_{std}}{V_{sample}}$$

where:
A_s = *peak height (or area) for Hg in sample*
$\overline{A}_{SB}$ = *mean peak height (or area) for Hg in system blanks*
CF_m = *mean calibration factor (Section* 10.3.2.6)
V_{std} = *volume (mL) used for standards – volume (mL) reagent used in standards*
V_{sample} = *volume (mL) of sample – volume (mL) reagent used in sample*

12.4 Calculations for concentration of Hg in method blanks, field blanks, and reagent blanks.

12.4.1 Calculate the concentration of Hg in the method blanks (C_{MB}), field blanks (C_{FB}), or reagent blanks (C_{RB}) in ng/L, using the equation in Section 12.2.2 (if bubbler system is used) or Section 12.3.2 (if flow injection system is used) and substituting the peak height or area resulting from the method blank, field blank, or reagent blank for A_s.

12.4.2 Determine the mean concentration of Hg in the method blanks associated with the analytical batch (a minimum of three). If a sample requires additional reagent(s) (e.g., BrCl), a corresponding method blank containing an identical amount of reagent must be analyzed (Section 9.4.4.3). The concentration of Hg in the corresponding method blank may be subtracted from the concentration of Hg in the sample per Section 12.5.2.

12.5 Reporting

12.5.1 Report results for Hg at or above the ML, in ng/L, to three significant figures. Report results for Hg in samples below the ML as <0.5 ng/L, or as required by the regulatory authority or in the permit. Report results for Hg in reagent blanks and field blanks at or above the ML, in ng/L, to three significant figures. Report results for Hg in reagent blanks, method blanks, or field blanks below the ML but at or above the MDL to two significant figures. Report results for Hg not detected in reagent blanks, method blanks, or field blanks as <0.2 ng/L, or as required by the regulatory authority or in the permit.

12.5.2 Report results for Hg in samples, method blanks and field blanks separately. In addition to reporting results for the samples and blank(s) separately, the concentration of Hg in the method blanks or field blanks associated with the sample may be subtracted from the results for that sample, or must be subtracted if requested or required by a regulatory authority or in a permit.

12.5.3 Results from tests performed with an analytical system that is not in control must not be reported or otherwise used for permitting or regulatory compliance purposes, but do not relieve a discharger or permittee of reporting timely results.

13.0 Method Performance

13.1 This Method was tested in 12 laboratories using reagent water, freshwater, marine water and effluent (Reference 16.19). The quality control acceptance criteria listed in Table 2 were verified by data gathered in the interlaboratory study, and the method detection limit (MDL) given in Section 1.5 was verified in all 12 laboratories. In addition, the techniques in this Method have been compared with other techniques for low-level mercury determination in water in a variety of studies, including ICES-5 (Reference 16.20) and the International Mercury Speciation Intercomparison Exercise (Reference 16.21).

13.2 Precision and recovery data for reagent water, freshwater, marine water, and secondary effluent are given in Table 3.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address waste generation. When it is not feasible to reduce wastes at the source, the Agency recommends recycling as the next best option. The acids used in this Method should be reused as practicable by purifying by electrochemical techniques. The only other chemicals used in this Method are the neat materials used in preparing standards. These standards are used in extremely small amounts and pose little threat to the environment when managed properly. Standards should be prepared in volumes consistent with laboratory use to minimize the disposal of excess volumes of expired standards.

14.2 For information about pollution prevention that may be applied to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Governmental Relations and Science Policy, 1155 16th Street NW, Washington DC 20036, 202/872–4477.

15.0 Waste Management

15.1 The laboratory is responsible for complying with all Federal, State, and local regulations governing waste management, particularly hazardous waste identification rules and land disposal restrictions, and for protecting the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required. An overview of requirements can be found in *Environmental Management Guide for Small Laboratories* (EPA 233-B-98-001).

15.2 Acids, samples at pH <2, and BrCl solutions must be neutralized before being disposed of, or must be handled as hazardous waste.

15.3 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better: Laboratory Chemical Management for Waste Reduction*, both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

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17.0 Glossary

The definitions and purposes below are specific to this Method, but have been conformed to common usage as much as possible.

17.1 **Ambient Water**—Waters in the natural environment (e.g., rivers, lakes, streams, and other receiving waters), as opposed to effluent discharges.

17.2 **Analytical Batch**—A batch of up to 20 samples that are oxidized with the same batch of reagents and analyzed during the same 12-hour shift. Each analytical batch must also include at least three bubbler blanks, an OPR, and a QCS. In addition, MS/MSD samples must be prepared at a frequency of 10% per analytical batch (one MS/MSD for every 10 samples).

17.3 **Bottle Blank**—The bottle blank is used to demonstrate that the bottle is free from contamination prior to use. Reagent water known to be free of mercury at the MDL of this Method is added to a bottle, acidified to pH <2 with BrCl or HCl, and allowed to stand for a minimum of 24 hours. The time that the bottle is allowed to stand should be as close as possible to the actual time that the sample will be in contact with the bottle. After standing, the water is analyzed.

17.4 **Bubbler Blank**—For this Method, the bubbler blank is specific to the bubbler system and is used to determine that the analytical system is free from contamination. After analysis of a standard, blank, or sample, the solution in the bubbler is purged and analyzed. A minimum of three bubbler blanks is required for system calibration.

17.5 **Equipment Blank**—Reagent water that has been processed through the sampling device at a laboratory or other equipment cleaning facility prior to shipment of the sampling equipment to the sampling site. The equipment blank is used to demonstrate that the sampling equipment is free from contamination prior to use. Where appropriate, the "clean hands/dirty hands" technique used during field sampling should be followed when preparing equipment blanks at the laboratory or cleaning facility.

17.6 **Field Blank**—Reagent water that has been transported to the sampling site and exposed to the same equipment and operations as a sample at the sampling site. The field blank is used to demonstrate that the sample has not been contaminated by the sampling and sample transport systems.

17.7 **Intercomparison Study**—An exercise in which samples are prepared and split by a reference laboratory, then analyzed by one or more testing laboratories and the reference laboratory. The intercomparison, with a reputable laboratory as the reference laboratory, serves as the best test of the precision and accuracy of the analyses at natural environmental levels.

17.8 **Matrix Spike (MS) and Matrix Spike Duplicate (MSD)**—Aliquots of an environmental sample to which known quantities of the analyte(s) of interest is added in the laboratory. The MS and MSD are analyzed exactly like a sample. Their purpose is to quantify the bias and precision caused by the sample matrix. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the MS and MSD corrected for these background concentrations.

17.9 **May**—This action, activity, or procedural step is allowed but not required.

17.10 **May not**—This action, activity, or procedural step is prohibited.

17.11 **Method blank**— Method blanks are used to determine the concentration of mercury in the analytical system during sample preparation and analysis, and consist of a volume of reagent water that is carried through the entire sample preparation and analysis. Method blanks are prepared by placing reagent water in a sample bottle and analyzing the water using reagents and procedures identical to those used to prepare and analyze the corresponding samples. A minimum of three method blanks is required with each analytical batch.

17.12 **Minimum Level (ML)**—The lowest level at which the entire analytical system must give a recognizable signal and acceptable calibration point for the analyte. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed. The ML is calculated by multiplying the MDL by 3.18 and rounding the result to the number nearest to (1, 2, or 5) x 10^n, where n is an integer (See Section 1.5).

17.13 **Must**—This action, activity, or procedural step is required.

17.14 **Quality Control Sample (QCS)**—A sample containing Hg at known concentrations. The QCS is obtained from a source external to the laboratory, or is prepared from a source of standards different from the source of calibration standards. It is used as an independent check of instrument calibration.

17.15 **Reagent blank**—Reagent blanks are used to determine the concentration of mercury in the reagents (BrCl, $NH_2OH{\cdot}HCl$, and $SnCl_2$) that are used to prepare and analyze the samples. In this Method, reagent blanks are required when each new batch of reagents is prepared.

17.16 **Reagent Water**—Water demonstrated to be free of mercury at the MDL of this Method. It is prepared from 18 MΩ ultrapure deionized water starting from a prepurified source. Reagent water is used to wash bottles, as trip and field blanks, and in the preparation of standards and reagents.

17.17 **Regulatory Compliance Limit**—A limit on the concentration or amount of a pollutant or contaminant specified in a nationwide standard, in a permit, or otherwise established by a regulatory authority.

17.18 **Shall**—This action, activity, or procedure is required.

17.19 **Should**—This action, activity, or procedure is suggested, but not required.

17.20 **Stock Solution**— A solution containing an analyte that is prepared from a reference material traceable to NIST, or a source that will attest to the purity and authenticity of the reference material.

17.21 **System Blank**— For this Method, the system blank is specific for the flow-injection system and is used to determine contamination in the analytical system and in the reagents used to prepare the calibration standards. A minimum of three system blanks is required during system calibration.

17.22 **Ultraclean Handling**— A series of established procedures designed to ensure that samples are not contaminated during sample collection, storage, or analysis.

18.0 Tables and Figures

Table 1

Lowest Ambient Water Quality Criterion for Mercury and the Method Detection Limit and Minimum Level of Quantitation for EPA Method 1631

Metal	Lowest Ambient Water Quality Criterion[1]	Method Detection Limit (MDL) and Minimum Level (ML)	
		MDL[2]	ML[3]
Mercury (Hg)	1.3 ng/L	0.2 ng/L	0.5 ng/L

1. Lowest water quality criterion for the Great Lakes System (Table 4, 40 CFR 132.6). The lowest Nationwide criterion is 12 ng/L (40 CFR 131.36).
2. Method detection limit (40 CFR 136, Appendix B)
3. Minimum level of quantitation (see Glossary)

Table 2

Quality Control Acceptance Criteria for Performance Tests in EPA Method 1631

Acceptance Criteria	Section	Limit (%)
Initial Precision and Recovery (IPR)	9.2.2	
Precision (RSD)	9.2.2.3	21
Recovery (X)	9.2.2.3	79-121
Ongoing Precision and Recovery (OPR)	9.5.2	77-123
Matrix Spike/Matrix Spike Duplicate (MS/MSD)	9.3	
Recovery	9.3.4	71-125
Relative Percent Difference (RPD)	9.3.5	24

Table 3

Precision and Recovery for Reagent Water, Fresh Water, Marine Water, and Effluent Water Using Method 1631

Matrix	*Mean Recovery (%)	*Precision (% RSD)
Reagent Water	98.0	5.6
Fresh Water (Filtered)	90.4	8.3
Marine Water (Filtered)	92.3	4.7
Marine Water (Unfiltered)	88.9	5.0
Secondary Effluent (Filtered)	90.7	3.0
Secondary Effluent (Unfiltered)	92.8	4.5

*****Mean percent recoveries and RSDs are based on expected Hg concentrations.

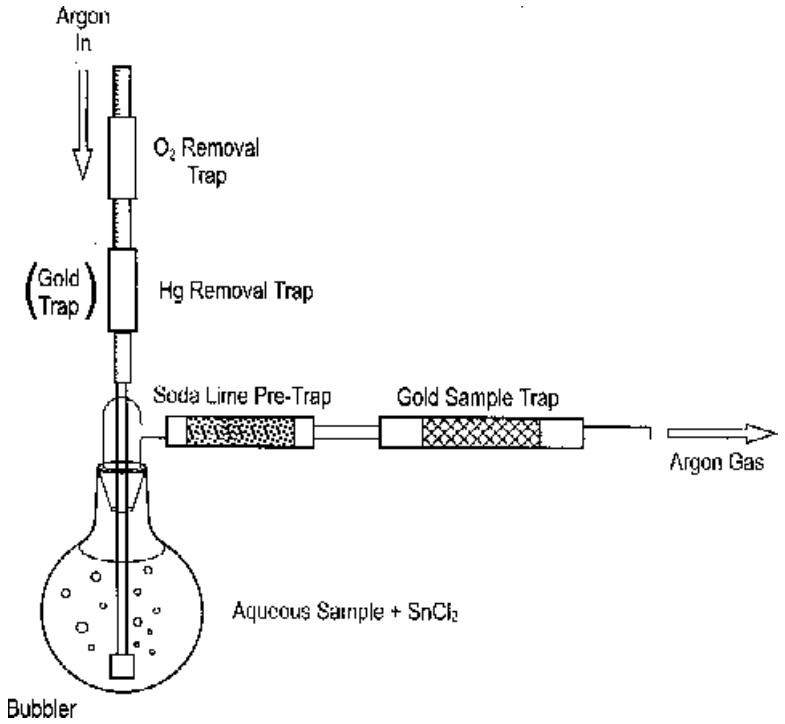

Figure 1. Schematic Diagram of Bubbler Setup

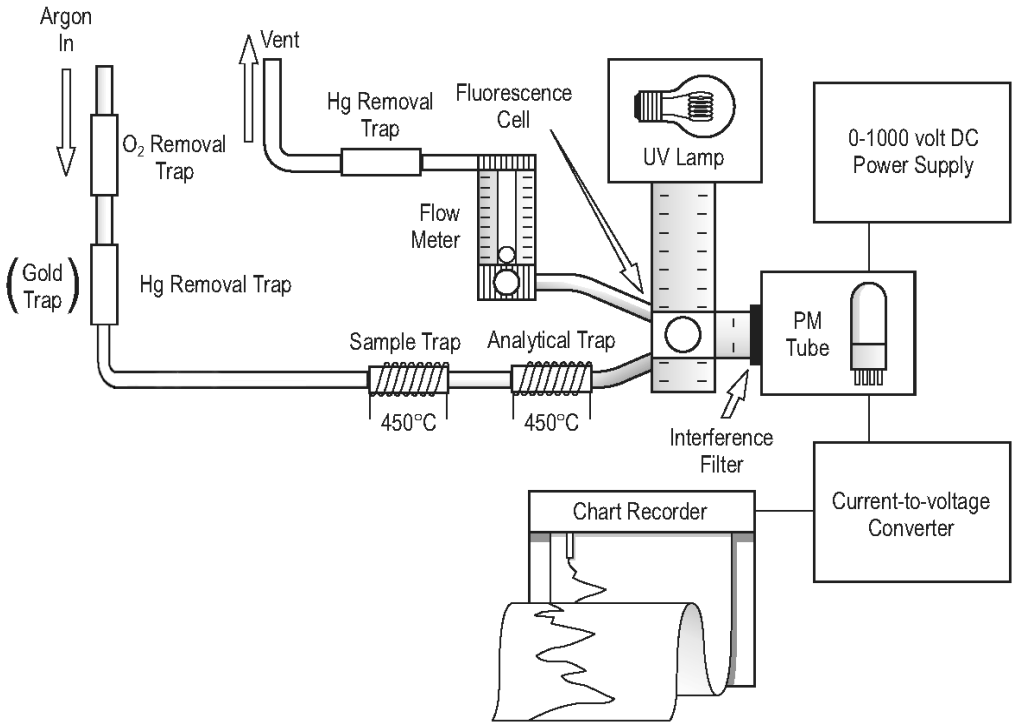

Figure 2. Schematic Diagram of the Bubbler, Purge and Trap, Cold Vapor Atomic Fluorescence Spectrometer (CVAFS) System

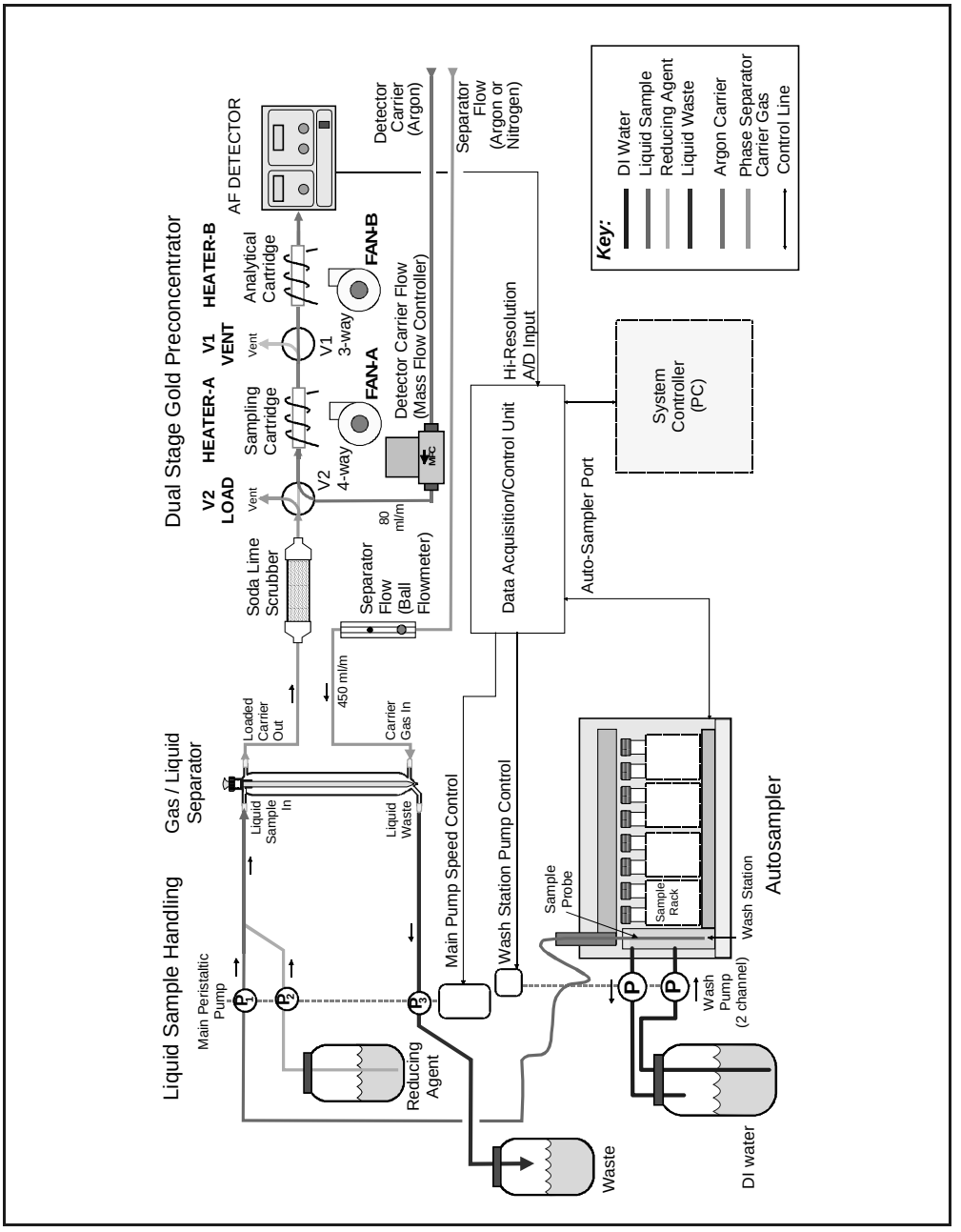

Figure 3. Schematic Diagram of the Flow-Injection, Cold Vapor Atomic Fluorescence Spectrometer (CVAFS) System

Appendix 4

USEPA Method 1632

Chemical Speciation of Arsenic in Water and Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry

Revision A

January 2001

EPA-821-R-01-006
January 2001

Method 1632

Chemical Speciation of Arsenic in Water and Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry

Revision A

January 2001

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Acknowledgments

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Disclaimer

This draft method has been reviewed and approved for publication by the Analytical Methods Staff within the Engineering and Analysis Division of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. This method version contains minor editorial changes to the September 2000 version.

EPA welcomes suggestions for improvement of this method. Suggestions and questions concerning this method or its application should be addressed to:

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Introduction

This analytical method supports water quality monitoring programs authorized under the Clean Water Act (CWA, the "Act"). CWA Section 304(a) requires EPA to publish water quality criteria that reflect the latest scientific knowledge concerning the physical fate (e.g., concentration and dispersal) of pollutants, the effects of pollutants on ecological and human health, and the effect of pollutants on biological community diversity, productivity, and stability.

CWA Section 303 requires each State to set a water quality standard for each body of water within its boundaries. A State water quality standard consists of a designated use or uses of a water body or a segment of a water body, the water quality criteria that are necessary to protect the designated use or uses, and an anti-degradation policy. These water quality standards serve two purposes: (1) they establish the water quality goals for a specific water body, and (2) they are the basis for establishing water quality-based treatment controls and strategies beyond the technology-based controls required by CWA Sections 301(b) and 306.

In defining water quality standards, a State may use narrative criteria, numeric criteria, or both. However, the 1987 amendments to CWA required States to adopt numeric criteria for toxic pollutants (designated in Section 307(a) of the Act) based on EPA Section 304(a) criteria or other scientific data, when the discharge or presence of those toxic pollutants could reasonably be expected to interfere with designated uses.

In some cases, these water quality criteria (WQC) are as much as 280 times lower than levels measurable using approved EPA methods and required to support technology-based permits. EPA developed new sampling and analysis methods to specifically address State needs for measuring toxic metals at WQC levels, when such measurements are necessary to protect designated uses in State water quality standards. The latest criteria published by EPA are those listed in the National Toxics Rule (58 FR 60848) and the Stay of Federal Water Quality Criteria for Metals (60 FR 22228). These rules include WQC for 13 metals, and it is these criteria on which the new sampling and analysis methods are based. Method 1632 was specifically developed to provide reliable measurements of inorganic arsenic at EPA WQC levels using hydride generation quartz furnace atomic absorption techniques. It has since been modified to include determination of arsenic species.

In developing methods for determination of trace metals, EPA found that one of the greatest difficulties was precluding sample contamination during collection, transport, and analysis. The degree of difficulty, however, is highly dependent on the metal and site-specific conditions. This method is designed to preclude contamination in nearly all situations. It also contains procedures necessary to produce reliable results at the lowest WQC levels published by EPA. In recognition of the variety of situations to which this Method may be applied, and in recognition of continuing technological advances, Method 1632 is performance based. Alternative procedures may be used so long as those procedures are demonstrated to yield reliable results.

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Note: This Method is performance based. The laboratory is permitted to omit any step or modify any procedure provided that all performance requirements in this Method are met. The laboratory may not omit any quality control tests. The terms “shall,” “must,” and “may not” define procedures required for producing reliable data at water quality criteria levels. The terms “should” and “may” indicate optional steps that may be modified or omitted if the laboratory can demonstrate that the modified method produces results equivalent or superior to results produced by this Method.

Method 1632

Chemical Speciation of Arsenic in Water and Tissue by Hydride Generation Quartz Furnace Atomic Absorption Spectrometry

1.0 Scope and Application

1.1 This method is for determination of inorganic arsenic (IA), arsenite (As^{+3}), arsenate (As^{+5}), monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) in filtered and unfiltered water and in tissue by hydride generation and quartz furnace atomic absorption detection. The method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act. The method is based on a contractor-developed method (Reference 16.1) and on peer-reviewed, published procedures for the speciation of As in aqueous samples (Reference 16.2).

1.2 This method is accompanied by Method 1669: *Sampling Ambient Water for Trace Metals at EPA Water Quality Criteria Levels* (the Sampling Guidance). The Sampling Guidance may be necessary to preclude contamination during the sampling process.

1.3 This method is designed for measurement of As species in water in the range 0.01-50 • g/L and in tissue in the range 0.10-500 • g/g dry weight. This method may be applicable to determination of arsenic species in industrial discharges after sample dilution. Existing regulations (40 CFR parts 400-500) typically limit concentrations in industrial discharges to the part-per-billion (ppb) range, whereas ambient As concentrations are normally in the low part-per-trillion (ppt) to low part-per-billion range.

1.4 The method detection limits and minimum levels of quantitation in this method are usually dependent on the level of background elements and interferences rather than instrumental limitations. Table 1 lists method detection limits (MDLs) and minimum levels of quantitation (MLs) in water when no background elements or interferences are present as determined by two laboratories. Table 1 also shows MDLs and MLs in a reference tissue matrix (corn oil).

1.5 The ease of contaminating water samples with As and interfering substances cannot be overemphasized. This method includes suggestions for improvements in facilities and analytical techniques that should maximize the ability of the laboratory to make reliable trace metals determinations and minimize contamination (Section 4.0). Additional suggestions for improvement of existing facilities may be found in EPA's *Guidance on Establishing Trace Metals Clean Rooms in Existing Facilities*, which is available from the National Center for Environmental Publications and Information (NCEPI) at the address listed in the introduction to this document.

1.6 Clean and ultra clean—The terms "clean" and "ultra clean" have been applied to the techniques needed to reduce or eliminate contamination in trace metals determinations. These terms are not used in this method because they lack an exact definition. However, the information provided in this method is consistent with EPA's summary guidance on clean and ultra clean techniques.

1.7 This method follows the EPA Environmental Methods Management Council's "Format for Method Documentation."

1.8 This method is "performance based." The laboratory is permitted to modify the method to overcome interferences or lower the cost of measurements if all performance criteria are met. Section 9.1.2 gives the requirements for establishing method equivalency.

1.9 Any modification of this method, beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures at 40 CFR 136.4 and 136.5.

1.10 Each laboratory that uses this method must demonstrate the ability to generate acceptable results (Section 9.2).

1.11 This method is accompanied by a data verification and validation guidance document, *Guidance on the Documentation and Evaluation of Trace Metals Data Collected for CWA Compliance Monitoring*. This guidance document may be useful for reviewing data collected using this method.

2.0 Summary of Method

2.1 Aqueous sample—A 500- to 1000-mL water sample is collected directly into a cleaned fluoropolymer, conventional or linear polyethylene, polycarbonate, or polypropylene sample bottle using sample handling techniques specially designed for collection of metals at trace levels (Reference 16.3). Water samples are preserved in the field by the addition of 3 mL of pretested 6M HCl per liter of sample. The recommended holding time is 28 days.

2.2 Tissue sample—A 10- to 50-g wet weight sample is collected into a glass or fluoropolymer, conventional or linear polyethylene, polycarbonate, or polypropylene sample bottle, also using sample handling techniques specially designed for collection of metals at trace levels. The tissue sample is either freeze-dried and stored at room temperature or stored frozen at less than -18 •C. Prior to analysis, tissue samples are digested in HCl or NaOH at 80 •C for 16 hours. Matrix spike recoveries indicate that As^{+3} is more stable in HCl than NaOH.

2.3 An aliquot of water sample or tissue digestate is placed in a specially designed reaction vessel, and 6M HCl is added.

2.4 Four percent $NaBH_4$ solution is added to convert IA, MMA, and DMA to volatile arsines.

2.5 Arsines are purged from the sample onto a cooled glass trap packed with 15% OV-3 on Chromosorb® W AW-DMCS, or equivalent.

2.6 The trapped arsines are thermally desorbed, in order of increasing boiling points, into an inert gas stream that carries them into the quartz furnace of an atomic absorption spectrophotometer for detection. The first arsine to be desorbed is AsH_3, which represents IA in the sample. MMA and DMA are desorbed and detected several minutes after the first arsine.

2.7 Quality is ensured through calibration and testing of the hydride generation, purging, and detection systems.

2.8 To determine the concentration of As^{+3}, another aliquot of water sample or tissue digestate is placed in the reaction vessel and Tris-buffer is added. The procedure in Sections 2.4 through 2.7 is repeated to quantify only the arsine produced from As^{+3}.

2.9 The concentration of As^{+5} is the concentration of As^{+3} subtracted from the concentration of IA.

3.0 Definitions

3.1 Apparatus—Throughout this method, the sample containers, sampling devices, instrumentation, and all other materials and devices used in sample collection, sample processing, and sample analysis that come in contact with the sample and therefore require careful cleaning will be referred to collectively as the Apparatus.

3.2 Dissolved Inorganic Arsenic—All $NaBH_4$-reducible As^{+3} and As^{+5} found in aqueous solution filtrate after passing the sample through a 0.45 μm capsule filter.

3.3 Total Inorganic Arsenic—All $NaBH_4$-reducible As^{+3} and As^{+5} found in a sample. In this method, total inorganic arsenic and total recoverable inorganic arsenic are synonymous.

3.4 Definitions of other terms used in this method are given in the glossary at the end of the method.

4.0 Contamination and Interferences

4.1 Preventing ambient water samples from becoming contaminated during the sampling and analytical processes constitutes one of the greatest difficulties encountered in trace metal determinations. Over the last two decades, marine chemists have come to recognize that much of the historical data on the concentrations of dissolved trace metals in seawater are erroneously high because the concentrations reflect contamination from sampling and analysis rather than ambient levels. Therefore, it is imperative that extreme care be taken to avoid contamination when collecting and analyzing ambient water samples for As species at trace levels.

4.2 Samples may become contaminated by numerous routes. Potential sources of trace metal contamination during sampling include: metallic or metal-containing labware, containers, sampling equipment, reagents, and reagent water; improperly cleaned and stored equipment, labware, and reagents; and atmospheric inputs such as dirt and dust. Even human contact can be a source of trace metal contamination.

4.3 Contamination Control

4.3.1 Philosophy—The philosophy behind contamination control is to ensure that any object or substance that contacts the sample is arsenic-free and free from any material that may contain As, As species, or material that might interfere with the analysis of samples.

4.3.1.1 The integrity of the results produced must not be compromised by contamination of samples. This method and the Sampling Method give requirements and suggestions for control of sample contamination.

4.3.1.2 Substances in a sample cannot be allowed to contaminate the laboratory work area or instrumentation used for trace metal measurements. This method gives requirements and suggestions for protecting the laboratory.

4.3.1.3 Although contamination control is essential, personnel health and safety remain the highest priority. The Sampling Method and Section 5.0 of this method give requirements and suggestions for personnel safety.

4.3.2 Avoiding contamination—The best way to control contamination is to completely avoid exposure of the sample to contamination in the first place. Avoiding exposure means performing operations in an area known to be free from contamination. Two of the most important factors in avoiding/reducing sample contamination are (1) an awareness of potential sources of contamination and (2) strict attention to the work being done. Therefore, it is imperative that the procedures described in this method be carried out by well-trained, experienced personnel.

4.3.3 Use a clean environment—The ideal environment for processing samples is a class 100 clean room (Section 1.5). If a clean room is not available, all sample preparation should be performed in a class 100 clean bench or a nonmetal glove box fed by arsenic- and particle-free air or nitrogen. Digestions should be performed in a nonmetal fume hood situated, ideally, in the clean room.

4.3.4 Minimize exposure—Any apparatus that will contact samples, blanks, or standard solutions should be opened or exposed only in a clean room, clean bench, or glove box so that exposure to an uncontrolled atmosphere is minimized. When not in use, the apparatus should be covered with clean plastic wrap and stored in the clean bench, in a plastic box, or in a glove box, or bagged in clean zip-type bags. Minimizing the time between cleaning and use will also minimize contamination.

4.3.5 Clean work surfaces—Before a given batch of samples is processed, all work surfaces in the hood, clean bench, or glove box in which the samples will be processed should be cleaned by wiping with a lint-free cloth or wipe soaked with reagent water.

4.3.6 Wear gloves—Sampling personnel must wear clean, non-talc gloves during all operations involving handling of the apparatus, samples, and blanks. Only clean gloves may touch the apparatus. If another object or substance is touched, the glove(s) must be changed before again handling the apparatus. If it is even suspected that gloves have become contaminated, work must be halted, the contaminated gloves removed, and a new pair of clean gloves put on. Wearing multiple layers of clean gloves will allow the old pair to be quickly stripped with minimal disruption to the work activity.

4.3.7 Use metal-free apparatus—All apparatus used for determination of As and/or As species at ambient water quality criteria levels must be nonmetallic and free of material that may contain metals.

4.3.7.1 Construction materials—Only fluoropolymer (FEP, PTFE), conventional or linear polyethylene, polycarbonate, or polypropylene containers should be used for samples that will be analyzed for As. PTFE is less desirable than FEP because the sintered material in PTFE may contain contaminants and is susceptible to serious memory effects (Reference 16.4). All materials, regardless of construction, that will directly or indirectly contact the sample must be cleaned using the procedures given (Section 6.1.2) and must be known to be clean and arsenic-free before proceeding.

Note: Glass containers may be used for tissue sample collection.

4.3.7.2 Serialization—It is recommended that serial numbers be indelibly marked or etched on each piece of apparatus so that contamination can be traced. Logbooks should be

maintained to track the sample from the container through the labware to injection into the instrument. It may be useful to dedicate separate sets of labware to different sample types; e.g., receiving waters and effluents. However, the apparatus used for processing blanks and standards must be mixed with the apparatus used to process samples so that contamination of all labware can be detected.

4.3.7.3 The laboratory or cleaning facility is responsible for cleaning the apparatus used by the sampling team. If there are any indications that the apparatus is not clean when received by the sampling team (e.g., ripped storage bags), an assessment of the likelihood of contamination must be made. Sampling must not proceed if it is possible that the apparatus is contaminated. If the apparatus is contaminated, it must be returned to the laboratory or cleaning facility for proper cleaning before it is used in any sampling activity.

4.3.8 Avoid sources of contamination—Avoid contamination by being aware of potential sources and routes of contamination.

4.3.8.1 Contamination by carryover—Contamination may occur when a sample containing low concentrations of As is processed immediately after a sample containing relatively high concentrations of As. To reduce carryover, the sample introduction system may be rinsed between samples with dilute acid and reagent water. When an unusually concentrated sample is encountered, it should be followed by analysis of a method blank to check for carryover. Samples known or suspected to contain the lowest concentration of As should be analyzed first followed by samples containing higher levels.

4.3.8.2 Contamination by samples—Significant laboratory or instrument contamination may result when untreated effluents, in-process waters, landfill leachates, and other samples containing high concentrations of As are processed and analyzed. This method is not intended for application to these samples, and samples containing high concentrations should not be permitted into the clean room and laboratory dedicated for processing trace metal samples.

4.3.8.3 Contamination by indirect contact—Apparatus that does not directly come in contact with the samples may still be a source of contamination. For example, clean tubing placed in a dirty plastic bag may pick up contamination from the bag and subsequently transfer the contamination to the sample. Therefore, it is imperative that every piece of the apparatus that is directly or indirectly used in the collection, processing, and analysis of water and tissue samples be thoroughly cleaned (see Section 6.1.2).

4.3.8.4 Contamination by airborne particulate matter—Less obvious substances capable of contaminating samples include airborne particles. Samples may be contaminated by airborne dust, dirt, particles, or vapors from unfiltered air supplies; nearby corroded or rusted pipes, wires, or other fixtures; or metal-containing paint. Whenever possible, sample processing and analysis should occur as far as possible from sources of airborne contamination.

4.4 Interferences—Water vapor may condense in the transfer line between the cold trap and the atomizer if it is not well heated. Such condensation can interfere with the determination of DMA.

5.0 Safety

5.1 The toxicity or carcinogenicity of each chemical used in this method has not been precisely determined; however, each compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level. It is recommended that the laboratory purchase a dilute standard solution of the As and/or As species to be used in this method. If solutions are prepared from pure solids, they shall be prepared in a hood, and a NIOSH/MESA-approved toxic gas respirator shall be worn when high concentrations are handled.

5.2 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a current awareness file of OSHA regulations for the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should also be made available to all personnel involved in these analyses. It is also suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this method and that the results of this monitoring be made available to the analyst. Additional information on laboratory safety can be found in References 16.5-16.8.

5.3 Samples suspected to contain high concentrations of As and/or As species are handled using essentially the same techniques used in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. Assistance in evaluating the health hazards of particular laboratory conditions may be obtained from certain consulting laboratories and from State Departments of Health or Labor, many of which have an industrial health service. Each laboratory must develop a strict safety program for handling As and/or As species.

5.3.1 Facility—When samples known or suspected of containing high concentrations (> 50 μg/Lor >500μg/g) of total As are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leak tight or in a fume hood demonstrated to have adequate air flow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in an accident.

5.3.2 Protective equipment—Disposable plastic gloves, apron or laboratory coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be used when handling arsenic powders. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters.

5.3.3 Training—Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

5.3.4 Personal hygiene—Hands and forearms should be washed thoroughly after each manipulation and before breaks (including coffee, lunch, and shift).

5.3.5 Confinement—Isolated work areas posted with signs, with their own segregated glassware and tools, and with plastic absorbent paper on bench tops will aid in confining contamination.

5.3.6 Effluent vapors—The effluent vapors from the atomic absorption spectrophotometer (AAS) should pass through either a column of activated charcoal or a trap designed to remove As and/or As species.

5.3.7 Waste handling—Good waste handling techniques include minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel must be trained in the safe handling of waste.

5.3.8 Decontamination

5.3.8.1 Decontamination of personnel—Use any mild soap with plenty of scrubbing action.

5.3.8.2 Glassware, tools, and surfaces—Satisfactory cleaning may be accomplished by washing with any detergent and water.

5.3.9 Laundry—Clothing known to be contaminated should be collected in plastic bags. Persons who convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. If the launderer knows of the potential problem, the clothing may be put into a washing machine without contact. The washing machine should be run through a full cycle before being used for other clothing.

6.0 Apparatus and Materials

NOTE: *The mention of trade names or commercial products in this method is for illustrative purposes only and does not constitute endorsement or recommendation for use by the Environmental Protection Agency. Equivalent performance may be achievable using apparatus, materials, or cleaning procedures other than those suggested here. The laboratory is responsible for demonstrating equivalent performance.*

6.1 Sampling Equipment

6.1.1 Sample collection bottles—Fluoropolymer, conventional or linear polyethylene, polycarbonate, or polypropylene, 500-1000 mL for aqueous samples. Glass or plastic (fluoropolymer, etc.) jars for tissue samples.

6.1.2 Cleaning—Sample collection bottles, glass jars, and glass vials are cleaned with liquid detergent and thoroughly rinsed with reagent water. The bottles are then immersed in 1N trace metal grade HCl for at least 48 hours. The bottles are thoroughly rinsed with reagent water, air dried in a class 100 area, and double-bagged in new polyethylene zip-type bags until needed.

NOTE: *Plastic sample bottles should not be cleaned with HNO_3 as it oxidizes chemicals that may remain in the plastic.*

6.1.3 Tissue digestion vials— Glass scintillation vials (25-mL) with fluoropolymer-lined lids are used for the digestion of tissue samples.

6.2 Equipment for bottle and glassware cleaning.

6.2.1 Vats—Up to 200-L capacity, constructed of high-density polyethylene (HDPE) or other nonmetallic, non-contaminating material suitable for holding dilute HCl.

6.2.2 Laboratory sink—In Class 100 clean area, with high-flow reagent water for rinsing.

6.2.3 Clean bench—Class 100, for drying rinsed bottles.

6.3 Atomic absorption spectrophotometer (AAS)—Any AAS may serve as a detector. A bracket is required to hold the quartz atomizer in the optical path of the instrument. Table 3 gives typical conditions for the spectrophotometer.

6.3.1 Electrodeless discharge lamp—For measuring As at 193.7 nm.

6.3.2 Quartz cuvette burner tube (Reference 16.2)—70 mm long and 9 mm in diameter with two 6 mm O.D. side tubes, each 25 mm long. Figure 1A shows a schematic diagram of the tube and bracket.

6.4 Reaction vessel—Figure 1B shows the schematic diagram for the vessel used for the reaction of the sample with sodium borohydride. The system consists of the following:

6.4.1 125-mL gas wash bottle—Corning # 1760-125, or equivalent, onto which an 8 mm O.D. sidearm inlet tube 2 cm long has been grafted. A smaller reaction vessel (30-mL size) can be used for up to 5 mL aqueous samples and tissue digestates.

6.4.2 Silicone rubber stopper septum—Ace Glass #9096-32, or equivalent.

6.4.3 Four-way fluoropolymer stopcock valve—Capable of switching the helium from the purge to the analysis mode of operation.

6.4.4 Flow meter/needle valve—Capable of controlling and measuring gas flow rate to the reaction vessel at 150 (±30) mL/minute.

6.4.5 Silicone tubing—All glass-to-glass connections are made with silicone rubber sleeves.

6.5 Cryogenic trap—Figure 1C shows the schematic diagram for the trap. It consists of the following:

6.5.1 Nichrome wire (22-gauge).

6.5.2 Variacs for controlling Nichrome wire.

6.5.3 A 6 mm O.D. borosilicate glass U-tube about 30 cm long with a 2 cm radius of bend (or similar dimensions to fit into a tall wide mouth Dewar flask), which has been silanized and packed halfway with 15% OV-3 on Chromosorb® W AW DMCS (45-60 mesh), or equivalent. The ends of the tube are packed with silanized glass wool.

6.5.3.1 Conditioning the trap—The input side of the trap (the side that is not packed) is connected with silicone rubber tubing to He at a flow rate of 40 mL/min, and the trap is placed in an oven at 175°C for two hours. At the end of this time, two 25 µL aliquots of GC column conditioner (Silyl-8®, Supelco, Inc., or equivalent) are injected through the silicone tubing into the glass trap. The trap is returned to the oven, with the He still flowing, for 24 hours.

6.5.3.2 After conditioning, the trap is wrapped with approximately 1.8 m of 22-gauge Nichrome wire, the ends of which are affixed to crimp-on electrical contacts.

6.5.3.3 The trap is connected by silicone rubber tubing to the output of the reaction vessel.

The output side of the trap is connected by 6 mm O.D. borosilicate tubing that has been wrapped by Nichrome wire to the input of the flame atomizer.

6.5.4 Dewar flask—Capable of containing the trap described in Section 6.5.3.

6.6 Recorder/integrator—Any integrator with a range compatible with the AAS is acceptable.

6.7 Pipettors—All-plastic pneumatic fixed volume and variable pipettors in the range of 10 μL to 5.0 mL.

6.8 Analytical balance—Capable of weighing to the nearest 0.01 g.

7.0 Reagents and Standards

7.1 River/reagent Water—Water demonstrated to be free from As species at the MDL as well as potentially interfering substances. The water can be prepared by distillation or collected from the field and filtered through a 0.2 • m filter. It has been observed that deionized water can have an oxidizing potential that diminishes As^{+3} response (References 16.1,16.2, and 16.9).

7.2 Hydrochloric acid—Trace-metal grade, purified, concentrated, reagent-grade HCl.

7.2.1 6M hydrochloric acid—Equal volumes of trace metal grade concentrated HCl (Section 7.2) and river/reagent water (Section 7.1) are combined to give a solution approximately 6M in HCl.

7.2.2 2M hydrochloric acid—Trace metal grade concentrated HCl (Section 7.2) and river/reagent water (Section 7.1) are combined in a 1:6 ratio to give a solution approximately 2M in HCl.

7.3 Tris buffer—394 g of Tris-HCl (tris(hydroxymethyl)aminomethane hydrochloride) and 2.5 g of reagent grade NaOH (sodium hydroxide) are dissolved in river/reagent water (Section 7.1) to make 1.0 L of a solution that is 2.5 M tris-HCl and 2.475 M HCl.

7.4 Sodium hydroxide — Reagent grade NaOH.

7.4.1 2M NaOH—Add 80 g of reagent grade NaOH to a 1-L flask. Add about 700 mL of river/reagent water. After the solid dissolves, dilute to 1 L to give a 2M NaOH solution.

7.4.2 0.02M NaOH—Add 10.0 mL of 2M NaOH (Section 7.4.1) to a 1-L flask. Dilute to 1 L with river/reagent water to give a 0.02M NaOH solution.

7.5 Sodium borohydride solution ($NaBH_4$)—Four grams of > 98% $NaBH_4$ (previously analyzed and shown to be free of measurable As) are dissolved in 100 mL of 0.02 M NaOH solution. This solution is stable for only 8-10 hours, and must be made daily.

7.6 Liquid nitrogen (LN_2)—For cooling the cryogenic trap.

7.7 Helium—Grade 4.5 (standard laboratory grade) helium.

7.8 Hydrogen—Grade 4.5 (standard laboratory grade) hydrogen.

7.9 Air—Grade 4.5 (standard laboratory grade) air.

7.10 Ascorbic acid

7.10.1 10% Ascorbic acid—Add 10 g reagent ascorbic acid to about 70 mL of river/reagent water (Section 7.1) and swirl to dissolve. After the powder dissolves, dilute to 100 mL, producing a solution which is stable for one year when stored at 4•€.

7.10.2 0.1% Ascorbic acid—Dilute 10 mL of 10% ascorbic acid solution to 1 L with river/reagent water. This solution should be made as needed.

7.11 Arsenic standards—It is recommended that laboratories purchase standard solutions of 1000 mg/L and dilute them to make working standard solutions (Section 7.13.6). Sections 7.13.1 through 7.13.4 give directions for making stock solutions if a source is not readily available.

7.11.1 Arsenite (As^{+3}) standard—A 1000 mg/L stock solution is made up by the dissolution of 1.73 g of reagent grade $NaAsO_2$ in 1.0 L of the 0.1% ascorbic acid solution (Section 7.12.2). This solution is stable for at least one year if kept refrigerated in an amber bottle.

7.11.2 Arsenate (As^{+5}) standard—To prepare a 1000 mg/L stock solution, 4.16 g of reagent grade Na_2HAsO_4 -$7H_2O$ are dissolved in 1.0 L of river/reagent water (Section 7.1). This stock solution has been found to be stable for at least 10 years.

7.11.3 Monomethylarsonate (MMA) standard—To prepare a stock solution of 1000 mg/L, 3.90 g of $CH_3AsO(ONa)_2$ -$6H_2O$ is dissolved in 1.0 L of river/reagent water (Section 7.1). This stock solution has been found to be stable for at least 10 years.

7.11.4 Dimethylarsinate (DMA) standard—To prepare a stock solution of 1000 mg/L, 2.86 g of reagent grade $(CH_3)_2AsO_2Na$-$3H_2O$ (cacodylic acid, sodium salt) is dissolved in 1.0 L river/reagent water (Section 7.1). This stock solution has been found to be stable for at least 10 years.

7.11.5 Working standard solution A—Prepare an intermediate solution containing 10 mg/L of As^{3+}, MMA and DMA combining measured aliquots of the above stock solutions (7.13.1, 7.13.3 and 7.13.4) and diluting to a measured volume with river/reagent water. Prepare a working standard solution containing 500 • g/L of As^{3+}, MMA and DMA by diluting the intermediate solution in river/reagent water.

NOTE: *As^{3+} is used for calibrating the analytical system for inorganic arsenic (As^{3+} + As^{5+}).*

7.11.6 Working standard solution B—Prepare an intermediate solution containing 10 mg/L of As^{3+}, As^{5+}, MMA and DMA combining measured aliquots of the above stock solutions (7.13.1 through 7.13.4) and diluting to a measured volume with river/reagent water. Prepare a working standard solution containing 500 • g/L of As^{3+},As^{5+}, MMA and DMA by diluting the intermediate solution in river/reagent water.

7.12 Corn oil—Reference matrix for tissue samples.

8.0 Sample Collection, Preservation, and Storage

8.1 Sample collection—Aqueous samples are collected as described in the Sampling Method (Reference 16.3). Tissue samples are collected as described in Reference 16.10.

8.2 Sample filtration—This step is not required if total IA and/or As species are the target analyte(s). For dissolved IA and/or As species, samples and field blanks are filtered through a 0.45 μm capsule filter at the field site as described in the Sampling Method. If the dissolved As species are required analytes, the water sample must be field filtered without contact to air. This can be accomplished by using a capsule filter and exercising care during the filtration process. The extra care is necessary because anoxic water may contain high concentrations of soluble iron and manganese that rapidly precipitate when exposed to air. Iron and manganese hydroxy/oxides precipitates remove dissolved As from water. After the sample is filtered, however, the concern is not as great. The samples are preserved through acidification, and when the water is acidified these precipitates will dissolve.

8.3 Water sample preservation—Sample preservation must be performed in the field to reduce changes in As speciation that may occur during transport and storage. Water samples are acidified to pH <2 with hydrochloric acid (3 mL 6M HCl/L sample) and stored at 0-4• •C from the time of collection until analysis. Other preservation techniques for water and a variety of matrices have been explored (References 16.1 and 16.11 through 16.13) but only the procedure described here is to be used. If As species are not target analytes, the samples may be preserved upon receipt by the laboratory.

8.3.1 Wearing clean gloves, remove the cap from the sample bottle, add the volume of reagent grade acid that will bring the pH to < 2 and recap the bottle immediately. If the bottle is full, withdraw the necessary volume using a precleaned plastic pipette and then add the acid.

NOTE: *When testing pH, do not dip pH paper or a pH meter into the sample; remove a small aliquot with a clean pipette and test the pH of the aliquot.*

8.3.2 Store the preserved sample for a minimum of 48 hours at 0-4• € to allow the As adsorbed on the container walls to completely dissolve in the acidified sample.

8.3.3 Sample bottles should be stored in polyethylene bags at 0-4• € until analysis.

8.3.3 The holding time for aqueous samples is 28 days from the time of collection until the time of analysis.

8.4 Tissue sample preservation—The tissue sample must be frozen in the sampling container at less than -18 • € or freeze-dried and stored at room temperature. The holding time for tissue samples is 2 years.

9.0 Quality Control/Quality Assurance

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 16.3). The minimum requirements of this program consist of an initial demonstration of laboratory capability, analysis of samples spiked with As and/or As species to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. To determine if the results of analyses meet the performance characteristics of the method, laboratory performance is compared to established performance criteria.

9.1.1 The laboratory shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, the laboratory is permitted to exercise certain options to eliminate interferences or lower the costs of measurements. These options include alternate digestion, concentration, and cleanup procedures, and changes in instrumentation. Alternate determinative techniques such as the substitution of a colorimetric technique or changes that degrade method performance are not allowed. If an analytical technique other than the techniques specified in this method is used, that technique must have a specificity equal to or better than the specificity of the techniques in the referenced method for the analytes of interest.

9.1.2.1 Each time this method is modified, the laboratory is required to repeat the procedures in Section 9.2. If the change will affect the detection limit of the method, the laboratory is required to demonstrate that the MDL (40 CFR part 136, Appendix B) is less than or equal to the MDL for this method or one-third the regulatory compliance level, whichever is greater. If the change will affect calibration, the laboratory must recalibrate the instrument according to Section 10.0 of this method.

9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the following, at a minimum:

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.

9.1.2.2.2 A listing of metals measured (As and/or As species), by name and CAS Registry number.

9.1.2.2.3 A narrative stating reason(s) for the modification(s).

9.1.2.2.4 Results from all quality control (QC) tests comparing the modified method to this method, including:

(a) Calibration (Section 10.1)
(b) Calibration verification (Section 9.5 and 10.2)
(c) Initial precision and recovery (Section 9.2.2)
(d) Analysis of blanks (Section 9.6)
(e) Matrix spike/matrix spike duplicate analysis (Section 9.3 and 9.4)
(f) Ongoing precision and recovery (Section 9.7)

9.1.2.2.5 Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include, where possible:

(a) Sample numbers and other identifiers
(b) Preparation dates
(c) Analysis dates and times
(d) Analysis sequence/run chronology

(e) Sample volume
(f) Volume before each preparation step
(g) Volume after each preparation step
(h) Final volume before analysis
(i) Dilution data
(j) Instrument and operating conditions (make, model, revision, modifications)
(k) Sample introduction system (ultrasonic nebulizer, hydride generator, flow injection system, etc.)
(l) Operating conditions (ashing temperature, temperature program, flow rates, etc.)
(m) Detector (type, operating conditions, etc.)
(n) Printer tapes and other recordings of raw data
(o) Quantitation reports, data system outputs, and other data to link the raw data to the results reported

9.1.3 Analyses of blanks are required to demonstrate freedom from contamination. Section 9.6 describes the required blank types and the procedures and criteria for analysis of blanks.

9.1.4 The laboratory shall spike at least 10% of the samples with As species to monitor method performance. Section 9.3 describes this test. When results of these spikes indicate atypical method performance, an alternate extraction or cleanup technique must be used to bring method performance within acceptable limits. If method performance for spikes cannot be brought within the limits given in this method, the result may not be reported or used for permitting or regulatory compliance purposes.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through calibration verification (for water and tissue samples) and through analysis of the ongoing precision and recovery aliquot (for tissue samples) that the analytical system is within specified limits. Sections 9.5 and 9.7 describe these required procedures.

9.1.6 The laboratory shall maintain records to define the quality of data that are generated. Section 9.3.4 describes the development of accuracy statements.

9.2 Initial demonstration of laboratory capability.

9.2.1 Method detection limit—To establish the ability to detect each As species, the laboratory must determine the MDL for each analyte per the procedure in 40 CFR 136, Appendix B using the apparatus, reagents, and standards that will be used in the practice of this method. The laboratory must produce an MDL for each analyte that is no more than one-tenth the regulatory compliance level or that is less than or equal to the MDL listed in Table 1, whichever is greater.

9.2.2 Initial precision and recovery (IPR)—To establish the ability to generate acceptable precision and recovery, the laboratory shall perform the following operations.

9.2.2.1 Analyze four aliquots of river/reagent water (Section 7.1) or corn oil (tissue reference matrix; Section 7.14) spiked with the analyte(s) of interest at one to five times the ML (Table 1). All sample preparation steps, and the containers, labware, and reagents that will be used with samples must be used in this test.

9.2.2.2 Using results of the set of four analyses, compute the average percent recovery (X) of

each analyte in each aliquot and the standard deviation (s) of the recovery of the analyte.

9.2.2.3 Compare X and s for each analyte with the corresponding limits for initial precision and recovery in Table 2. If s and X meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, s exceeds the precision limit or X falls outside the range for accuracy, system performance is unacceptable. The laboratory should correct the problem and repeat the test (Section 9.2.2.1).

9.2.3 Quality control sample (QCS)—The QCS must be prepared from a source different from that used to produce the calibration standards. River/reagent water and marine water that contain certified concentrations of total As may be purchased. Certified reference materials for As species are not currently available. When beginning use of this method and on a quarterly basis, or as required to meet data quality needs, the calibration standards and acceptable instrument performance must be verified with the preparation and analyses of a QCS (Section 7.10). To verify the calibration standards, the determined mean concentration from three analyses of the QCS must be within ± 10% of the stated QCS value. If the QCS is not within the required limits, an immediate second analysis of the QCS is recommended to confirm unacceptable performance. If the calibration standards and/or acceptable instrument performance cannot be verified, the source of the problem must be identified and corrected before proceeding with further analyses.

9.3 Method Accuracy—To assess the performance of the method on a given sample matrix, the laboratory must perform matrix spike (MS) and matrix spike duplicate (MSD) sample analyses on 10% of the samples from each site being monitored, or at least one MS sample analysis and one MSD sample analysis must be performed for each sample set (samples collected from the same site at the same time, to a maximum of 10 samples), whichever is more frequent.

9.3.1 The concentration of the MS and MSD is determined as follows:

9.3.1.1 If, as in compliance monitoring, the concentration of analyte(s) in the sample is being checked against a regulatory concentration limit, the spike must contain the analyte(s) at that limit or at one to five times the background concentration, whichever is greater.

9.3.1.2 If the concentration(s) is not being checked against a regulatory limit, the concentration(s) must be at one to five times the background concentration or at one to five times the ML(s) in Table 1, whichever is greater.

9.3.2 Assessing spike recovery

9.3.2.1 Determine the background concentration (B) of As species by analyzing one sample aliquot according to the procedures in Section 11.0.

9.3.2.2 Prepare a matrix spiking solution that will produce the appropriate level (Section 9.3.1) of analyte(s) of interest in the sample when the spiking solution is added.

9.3.2.3 Spike two additional aliquots with the matrix spiking solution and analyze these aliquots to determine the concentration after spiking (A).

9.3.2.4 Calculate each percent recovery of the matrix spike and matrix spike duplicate by using Equation 1.

Equation 1

$$P = 100 * \frac{A - B}{T}$$

Where P = Percent recovery of the spike
A = Concentration of the spiked aliquot
B = Background concentration of the sample
T = Known value of the spike

9.3.3 Compare the percent recovery (P) with the corresponding QC acceptance criteria in Table 2. If P falls outside the designated range for recovery, the result has failed the acceptance criteria.

9.3.3.1 If the system performance is unacceptable, analyze the calibration verification standard (CALVER, Section 9.5.2) for water samples, or the ongoing precision and recovery sample (Section 9.7) for tissue samples. If the CALVER or OPR is within acceptance criteria (Table 2), the analytical system is within specified limits and the problem can be attributed to the sample matrix.

9.3.3.2 For samples that exhibit matrix problems, further isolate As species from the sample matrix using chelation, extraction, concentration, or other means, and repeat the accuracy test (Sections 9.3.2).

NOTE: *The use of these techniques to reduce matrix problems may affect the speciation of the As in solution.*

9.3.3.3 If matrix problems cannot be corrected and the recovery for As species remains outside the acceptance criteria, the analytical result in the unspiked sample is suspect and may not be reported or used for permitting or regulatory compliance purposes.

9.3.4 Recovery for samples should be assessed and records maintained.

9.3.4.1 After the analysis of five samples of a given matrix type (river water, lake water, etc.) for which As species pass the tests in Section 9.3.3, compute the average percent recovery (P) (P = percent recovery in 9.3.2.4) and the standard deviation of the percent recovery (SP). Express the accuracy assessment as a percent recovery interval from P-2SP to P+2SP for each matrix. For example, if P = 90% and SP = 10% for five analyses of river water, the accuracy interval is expressed as 70-110%.

9.3.4.2 Update the accuracy assessment in each matrix regularly (e.g., after each 5-10 new measurements).

9.4 Precision of MS/MSD

9.4.1 Calculate the relative percent difference (RPD) between the MS and MSD using the concentrations found in the MS and MSD (Equation 1). Do not use the recoveries calculated in Section 9.3.2.4 for this calculation because the RPD of recoveries is inflated when the background concentration is near the spike concentration.

Equation 2

$$RPD = 100 * \frac{\left(\left|D_1 - D_2\right|\right)}{\frac{1}{2}\left(D_1 + D_2\right)}$$

Where:

RPD = Relative percent difference

D_1 = Concentration of the analyte in the MS sample

D_2 = Concentration of the analyte in the MSD sample

9.4.2 Compare the RPD with the limits in Table 2. If the criteria are not met, the analytical system performance is judged to be unacceptable. Correct the problem and reanalyze all samples in the sample set associated with the MS/MSD that failed the RPD test.

9.5 Calibration verification (also see Section 10.2)

9.5.1 Calibration verification (CALVER) shall be performed immediately after the analytical system is calibrated or before analyzing any samples in a sample batch. In addition, the CALVER standard shall be analyzed after every 10 samples and after the last analytical sample in a sample batch. Refer to Section 10.2.2 and 10.2.3 for procedures on analyzing the CALVER standard.

9.5.2 Recovery of the CALVER standard must be within the control limits specified in Table 2. If recovery of the CALVER standard is outside the control limits in Table 2, the analysis must be stopped, the problem corrected, the instrument recalibrated, and the calibration verified. Samples processed after the last satisfactory calibration verification must be re-analyzed.

9.6 Blanks—Blanks are analyzed to demonstrate freedom from contamination.

9.6.1 Calibration blanks– A calibration blank consists of river/reagent water placed in the reaction vessel and analyzed like a sample (Section 11.4 and 11.5). At least one calibration blank must be analyzed after calibration. A calibration blank is also analyzed after each analysis of the CALVER standard (Section 9.5). If As species or any potentially interfering substance is found in the blank at a concentration equal to or greater than the MDL (Table 1), sample analysis must be halted, the source of the contamination determined, the problem corrected, and the sample batch and a fresh calibration blank reanalyzed.

9.6.2 Method blanks—The method blank is an aliquot of river/reagent water or corn oil (tissue reference matrix; Section 7.14) that is treated exactly as a sample including exposure to all glassware, equipment and reagents that are used with samples. It is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

9.6.2.1 Prepare a minimum of 1 method blank with each sample batch (samples of the same matrix started through the preparation process on the same 12-hour shift, to a maximum of 20 samples). Three method blanks are preferred.

NOTE: *Method blanks for water samples are identical to the calibration blanks (see Section 9.6.1). Analyze the method blank immediately after analysis of the CALVER (Section 9.5) for water samples, or OPR (Section 9.7) for tissue samples, to demonstrate freedom from contamination.*

9.6.2.2 If As species or any potentially interfering substance is found in the blank at a concentration equal to or greater than the MDL (Table 1), sample analysis must be halted, the source of the contamination determined, the problem corrected, and the sample batch and a fresh method blank reanalyzed.

9.6.2.3 Alternatively, if a sufficient number of method blanks (three minimum) are analyzed to characterize the nature of a blank, the average concentration plus two standard deviations must be less than the regulatory compliance level.

9.6.2.4 If the result for a single method blank remains above the MDL or if the result for the average concentration plus two standard deviations of three or more blanks exceeds the regulatory compliance level, results for samples associated with those blanks may not be reported or used for permitting or regulatory compliance purposes. Stated another way, results for all initial precision and recovery tests (Section 9.2) and all samples must be associated with an uncontaminated method blank before these results may be reported or used for permitting or regulatory compliance purposes.

9.6.3 Field blanks for water samples

9.6.3.1 Analyze the field blank(s) shipped with each set of samples (samples collected from the same site at the same time, to a maximum of 10 samples). If the samples are filtered for the determination of dissolved As and/or As species, the field blank shall be filtered as well. Analyze the blank immediately before analyzing the samples in the batch.

9.6.3.2 If As species or any potentially interfering substance is found in the field blank at a concentration equal to or greater than the ML (Table 1), or greater than one-fifth the level in the associated sample, whichever is greater, results for associated samples may be the result of contamination and may not be reported or used for permitting or regulatory compliance purposes.

9.6.3.3 Alternatively, if a sufficient number of field blanks (three minimum) are analyzed to characterize the nature of the field blank, the average concentration plus two standard deviations must be less than the regulatory compliance level or less than one-half the level in the associated sample, whichever is greater.

9.6.3.4 If contamination of the field blanks and associated samples is known or suspected, the laboratory should communicate this to the sampling team so that the source of contamination can be identified and corrective measures taken before the next sampling event.

9.6.4 Equipment blanks—Before any sampling equipment is used at a given site, the laboratory or cleaning facility is required to generate equipment blanks to demonstrate that the sampling

equipment is free from contamination. Two types of equipment blanks are required: bottle blanks and sampler check blanks.

9.6.4.1 Bottle blanks—After undergoing appropriate cleaning procedures (Section 6.1.2), bottles should be subjected to conditions of use to verify the effectiveness of the cleaning procedures. A representative set of sample bottles should be filled with river/reagent water (Section 7.1) acidified to pH < 2 and allowed to stand for a minimum of 24 hours. Ideally, the time that the bottles are allowed to stand should be as close as possible to the actual time that sample will be in contact with the bottle. After standing, the water should be analyzed for any signs of contamination. If any bottle shows signs of contamination, the problem must be identified, the cleaning procedures corrected or cleaning solutions changed, and all affected bottles cleaned again.

9.6.4.2 Sampler check blanks for water samples—Sampler check blanks are generated in the laboratory or at the equipment cleaning contractor's facility by processing river/reagent water (Section 7.1) through the sampling devices using the same procedures that are used in the field (see Sampling Method). Therefore, the "clean hands/dirty hands" technique used during field sampling should be followed when preparing sampler check blanks at the laboratory or cleaning facility.

9.6.4.2.1 Sampler check blanks are generated by filling a large carboy or other container with river/reagent water (Section 7.1) and processing the river/reagent water (Section 7.1) through the equipment using the same procedures that are used in the field (see Sampling Method). For example, manual grab sampler check blanks are collected by directly submerging a sample bottle into the water, filling the bottle, and capping. Subsurface sampler check blanks are collected by immersing the sampler into the water and pumping water into a sample container. "Clean hands/dirty hands" techniques must be used.

9.6.4.2.2 The sampler check blank must be analyzed using the procedures in this method. If As and/or As species or any potentially interfering substance is detected in the blank, the source of contamination or interference must be identified and the problem corrected. The equipment must be demonstrated to be free from As and/or As species before the equipment may be used in the field.

9.6.4.2.3 Sampler check blanks must be run on all equipment that will be used in the field. If, for example, samples are to be collected using both a grab sampling device and a subsurface sampling device, a sampler check blank must be run on both pieces of equipment.

9.7 Ongoing Precision and Recovery - Because water samples do not require digestion prior to analysis, OPR samples are only required for tissue samples. CALVER analysis in Section 9.5 is equivalent to the analysis of an aqueous OPR.

9.7.1 For each sample batch (i.e., samples of the same matrix started through the extraction process on the same 12-hour shift, to a maximum of 20 samples), prepare an ongoing precision and recovery (OPR) aliquot in the same manner as IPR aliquots (Section 9.2.2).

9.7.2 Analyze the OPR aliquot before analyzing the method blank and samples from the same batch.

9.7.3 Compute the percent recovery of As species in the OPR aliquot.

9.7.4 Compare the recovery in the OPR sample to the limits for ongoing recovery in Table 2. If the acceptance criteria are met, system performance is acceptable and analysis of blanks and samples may proceed. If, however, recovery falls outside of the range given, the analytical processes are not being performed properly. Correct the problem, prepare the sample batch again, and repeat the OPR test.

9.7.5 Add results that pass the specifications to IPR and previous OPR data for As species. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory accuracy by calculating the average percent recovery (P) and the standard deviation of percent recovery (SP). Express the accuracy as a recovery interval from P-2SP to P+2SP. For example, if P = 95% and SP = 5%, the accuracy is 85-105%.

9.8 The specifications in this method can be met if the instrument used is calibrated properly and then maintained in a calibrated state. A given instrument will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of As and/or As species by this method.

9.9 Depending on specific program requirements, field duplicates may be collected to determine the precision of the sampling technique. The relative percent difference (RPD, Equation 2) between field duplicates should be less than 20%.

10.0 Calibration and Standardization

10.1 Calibration—Calibration is required before any samples or method blanks are analyzed.

10.1.1 Standards are analyzed by addition of measured aliquots of the working standard solution A (Section 7.13.5) directly into the reaction vessel that has been pre-filled with river/reagent water (70 mL for the 125-mL reaction vessel; 5 mL for the 30-mL reaction vessel). Proceed with analysis of the standards following procedures in Section 11.4.

10.1.2 The calibration must contain 3 or more non-zero points. For a given As species, the lowest calibration point must be less than or equal to the ML shown in Table 1.

10.1.3 Calculate the calibration factor (CF) for IA, MMA and DMA in each calibration standard using the following equation.

Equation 3

$$CF = \frac{R_x}{m_x}$$

Where,

CF = Calibration factor [peak area or height units / μg]

R_x = Peak height or area for As species in standard [peak area or height units]

m_x = Mass of As species in standard analyzed (μg)

10.1.4 For each analyte of interest, calculate the mean calibration factor (CF_m), the standard deviation of the CF_m (SD), and the relative standard deviation (RSD) of the mean, where RSD = 100 x SD/CF_m.

10.1.5 Appropriateness of CF—If the RSD as calculated in Section 10.1.4 is less than 20%, the CF_m may be used to calculate sample concentrations. Otherwise, use weighted linear regression to calculate a slope and intercept for the calibration line.

10.1.6 When analyzing for As^{3+}, the calibration line for IA can be used.

10.1.7 Following calibration, analyze a calibration blank. The concentrations of As and As species in the calibration blank be less than the MDL.

10.2 Calibration verification—A calibration verification is performed immediately after calibration and after analysis of a maximum of every 10 samples thereafter (Section 10.2.2). Blanks and samples may not be analyzed until these criteria are met.

10.2.1 Verify the specificity of the instrument for As and adjust the wavelength or tuning until the resolving power (Table 3) specified in this method is met.

10.2.2 Calibration verification for IA, MMA and DMA

10.2.2.1 Calibration verification (CALVER)—Prepare the CALVER standard by adding a measured volume of working standard solution B to the reaction vessel (pre-filled with river/reagent water) corresponding to the mid-level standard used to establish the calibration line. The CALVER standard is then purged and analyzed for IA, MMA and DMA following procedures in Section 11.4. Compute the percent recovery of As species using the initial calibration.

10.2.2.2 Compare the recovery with the corresponding limit for calibration verification in Table 2. If acceptance criteria are met, system performance is acceptable and analysis of blanks and samples may continue using the response from the initial calibration. If acceptance criteria are not met, system performance is unacceptable. Locate and correct the problem and/or prepare a new calibration verification standard and repeat the test (Sections 10.2.1 through 10.2.3), or recalibrate the system (Sections 10.1 and 10.2). All samples after the last

acceptable calibration verification must be reanalyzed.

10.2.3 Calibration verification for As^{3+}

10.2.3.1 Before the As^{3+} analysis of samples, the CALVER standard is analyzed at the beginning of an analytical batch, following every 10 samples, and at the end of an analytical batch. The CALVER standard is prepared by adding a measured volume of working standard solution B to the reaction vessel pre-filled with river/reagent water (70 or 5 mLs). The CALVER standard should correspond to the mid-level standard used to establish the calibration line. The CALVER standard is then purged and analyzed for As^{3+} in Section 11.5. Compute the percent recovery of As^{3+} using the initial calibration.

10.2.3.2 Compare the recovery with the corresponding limit for calibration verification in Table 2. If acceptance criteria are met, system performance is acceptable and analysis of blanks and samples may continue using the response from the initial calibration. If acceptance criteria are not met, system performance is unacceptable. Locate and correct the problem and/or prepare a new calibration check standard and repeat the test (Sections 10.2.1 through 10.2.3), or recalibrate the system (Sections 10.1 and 10.2). If the recovery does not meet the acceptance criteria specified in Table 2, analyses must be halted and the problem corrected. All samples after the last acceptable calibration verification for As^{3+} must be reanalyzed for As^{3+}.

10.3 Analyze a calibration blank following every calibration verification to demonstrate that there is no carryover of the analytes of interest and that the analytical system is free from contamination. The concentrations of As and As species in the calibration blank must be less than the MDL. If the concentration of an analyte in the blank result is equal to or exceeds the MDL, correct the problem, verify the calibration (Section 10.1), and repeat the analysis of the calibration blank.

11.0 Sample Preparation and Analysis

11.1 Set up the AAS system according to manufacturer's instructions. The settings in Tables 3 and 4 can be used as a guide. Calibrate the instrument according to Section 10.1.

NOTE: Precision and sensitivity are affected by gas flow rates and these must be individually optimized for each system using the settings in Table 5 as an initial guide.

11.2 To light the flame, turn on the air and H_2, and expose the end of the quartz cuvette to a flame. At this point, a flame will be burning out the ends of the tube. Allow the tube to heat for approximately five minutes, then place a flat metal spatula over each end of the tube in sequence. An invisible air/hydrogen flame should now be burning in the center of the cuvette. To check for the flame, place a mirror near the end of the tube and observe condensation of water vapor or turn-off the room light to observe the flame.

11.3 Tissue samples large enough to sub-sample must be homogenized to a fine paste with a stainless steel mill, or finely chopped with stainless steel tools on an acid-cleaned, plastic cutting board. Clean sample handling techniques must be followed. Digest tissue samples by adding 10 mL of 2M HCl to 0.5 g of either wet or dry tissue in a 25-mL glass scintillation vial. Cap the vial with a fluoropolymer-lined lid and heat overnight (16 hours) in an oven at 75 - 85 •C. Cool and

analyze the overlying liquid. Tissue may also be digested in 2M NaOH overnight at 75 - 85 •€; however, As^{+3} and As^{+5} are more stable in HCl than NaOH. If only IA, MMA, and DMA are required, the advantage of the NaOH digestion is that, if it is available, ICP-MS can be used to quantify total As (Reference 16.14) in the digestate.

11.4 Inorganic As, MMA, and DMA determination.

11.4.1 Purging of Samples

11.4.1.1 To achieve a detection limit < 0.01 μg/L, place a known volume of aqueous sample (up to 70 mL) into the large (125-mL) reaction vessel. If less than 70 mL of sample is used, add sufficient river/reagent water (Section 7.1) to result in a total volume of 70 mL. Add 5.0 mL of 6M HCl. Set the four-way valve on the reaction vessel to pass the flow of He through the sample and onto the trap and begin purging the vessel with He.

11.4.1.2 To analyze tissue digestates or to analyze water samples with a detection limit > 0.01 • g/L, place a known volume of aqueous sample (up to 5 mL) or tissue digestate (up to 2 mL) into the small (30 mL) reaction vessel. Add 1.0 mL of 6M HCl. Set the four-way valve on the reaction vessel to pass the flow of He through the sample and onto the trap and begin purging the vessel with He.

11.4.1.3 Lower the trap into a Dewar flask containing LN_2 and top the flask off with LN_2 to a constant level.

11.4.1.4 For a large reaction vessel, add 10 mL of $NaBH_4$ solution slowly (over a period of approximately two minutes) through the rubber septum with a disposable hypodermic syringe and begin timing the reaction. For the small reaction vessel, add 2.0 mL of $NaBH_4$ slowly over a 1-minute period. After seven minutes, turn the stopcock on the four-way valve to bypass the reaction vessel and pass helium directly to the trap. Arsines are purged from the sample onto the cooled glass trap packed with 15% OV-3 on Chromosorb® W AW DMCS, or equivalent.

11.4.2 Trap desorption and AAS analysis

11.4.2.1 Quickly remove the trap from the LN_2, activate the heating coils to heat the trap, and begin recording output from the AAS system. The transfer line is maintained at 75 - 85 •€. The trapped arsines are thermally desorbed, in order of increasing boiling points, into an inert gas stream that carries them into the quartz furnace of an atomic absorption spectrophotometer for detection. The first arsine to be desorbed is AsH_3, which represents total inorganic As in the sample. The MMA and DMA are desorbed and detected several minutes after the arsine.

11.4.2.2 To ensure that all organic reduction products have been desorbed from the trap, maintain the trap temperature at 65 - 85 •€ and keep He flowing through the trap for at least three minutes between samples.

11.4.3 The trap should be cooled for one minute before re-using for another analysis to reduce the possibility of cracking.

11.5 Arsenite (As^{+3}) Determination

11.5.1 pH Adjustment

11.5.1.1 To analyze water samples with a detection limit < 0.01 • g/L, place a known volume (up to 70 mL) in the large (125-mL) reaction vessel. If less than 70 mL of sample is used, add sufficient river/reagent water (Section 7.1) to result in a total volume of 70 mL. Add 3.0 mL of Tris buffer to bring the sample's pH to 5 to 7. If the sample is strongly acidic or basic, it must be either neutralized or have more buffer added to obtain a pH of 5 to 7.

11.5.1.2 To analyze tissue digestates or to analyze water samples with a detection limit > 0.01 • g/L, place a known volume of aqueous sample (up to 5 mL) or tissue digestate (up to 2 mL) in the small reaction vessel. Add 1.0 mL of Tris buffer. If the sample is strongly acidic or basic, it must be either neutralized or have more buffer added to obtain a pH of 5 to 7.

11.5.2 Purging of samples—For a large reaction vessel, add 3.0 mL of $NaBH_4$ solution quickly (about 10 seconds) through the rubber septum with a disposable hypodermic syringe and begin timing the reaction. For a small reaction vessel, add 1.0 mL of $NaBH_4$ in a short injection (about 10 seconds). The injections are quicker for As^{+3} determinations than for Inorganic As, MMA, DMA determinations (Section 11.4.1.4) because rapid evolution of H_2 does not occur at a neutral pH. After seven minutes, turn the stopcock on the four-way valve to bypass the reaction vessel and pass helium directly to the trap. Arsines are purged from the sample onto the cooled glass trap packed with 15% OV-3 on Chromosorb® W AW DMCS, or equivalent.

11.5.3 Trap desorption and AAS analysis—Desorption of arsines from the trap follows the same procedure as in Sections 11.4.2 through 11.4.3 to complete the determination of As^{+3} concentration. During this procedure, small, irreproducible quantities of organic arsines may be released at this pH and should be ignored. This separation of arsenite is reproducible and essentially 100% complete.

11.6 Arsenate (As^{+5}) determination—The concentration of As^{+5} is calculated by subtracting the As^{+3} determined in Section 11.5 from the total inorganic As determined on an aliquot of the same sample in Section 11.4.

12.0 Data analysis and calculations

12.1 For water samples, compute the concentration of As species in ng/L using the calibration data (Section 10.1):

Equation 4

$$C\left[\frac{\mu g}{L}\right] = \frac{Rx}{CF_m V_s}$$

Where:

Rx	= Peak height or area for As species in the sample [peak height or area units]
CF_m	= Mean calibration factor for As species [peak height or area units /μg]
V_s	= Volume of sample purged and analyzed [L]

For tissue samples, compute the concentration of As species in μg/g as follows:

Equation 5

$$C\left[\frac{\mu g}{g}\right] = \left(\frac{R_x}{CF_m}\right)\left(\frac{V_{digest}}{V_d}\right)\left(\frac{1}{m_s}\right)$$

Where:

R_x	= Peak height or area As species in the digestate [peak height or area units]
CF_m	= Mean calibration factor for As species [peak height or area units /μg]
V_{digest}	= Total volume of tissue digestate [mL]
V_d	= Volume of digestate added to reaction vessel [mL]
m_s	= mass of sample digested [g]

12.2 If the concentration exceeds the calibration range, dilute the sample by successive factors of 10 until the concentration is within the calibration range.

12.3 Reporting

12.3.1 Report results for each As species at or above the ML, in μg/L or μg/g, to three significant figures. Report results for each As species in samples below the ML as less than the value of the ML, or as required by the regulatory authority or in the permit. Report results for each As species in field blanks at or above the ML, in μg/L or μg/g, to three significant figures. Report results for each As species in field blanks below the ML but at or above the MDL to two significant figures. Report results for each As species not detected in field blanks as less than the value of the MDL, or as required by the regulatory authority or in the permit.

12.3.2 Report results for each As species in samples, method blanks, and field blanks separately, unless otherwise requested or required by a regulatory authority or in a permit. If blank correction is requested or required, subtract the concentration of each As species in the method blank, average of multiple method blanks, or field blank from the concentration of

the respective As species in the sample to obtain the net sample As species concentration. Among the preceding blanks, only one may be subtracted.

12.3.3 Results from tests performed with an analytical system that is not in control must not be reported or otherwise used for permitting or regulatory compliance purposes, but does not relieve a discharger or permittee of reporting timely results.

13.0 Method Performance

Tables 1 contains MDLs and MLs for As species in water and tissue matrices. The QC acceptance criteria in Table 2 are based on quality control data generated during As speciation analysis by Method 1632 for the Cook Inlet Study (1998). Details on how the criteria were developed can be found in Reference 16.16.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option. The acids used in this method should be reused as practicable by purifying with electrochemical techniques. The only other chemicals used in this method are the neat materials used in preparing standards. These standards are used in extremely small amounts and pose little threat to the environment when managed properly. Standards should be prepared in volumes consistent with laboratory use to minimize the disposal of excess volumes of expired standards.

14.2 For information about pollution prevention that may be applied to laboratories and research institutions, consult Less is Better: Laboratory Chemical Management for Waste Reduction, available from the American Chemical Society's Government Affairs Publications , 1155 16th Street NW, Washington DC 20036, 202/872–4600, or govtrelations@acs.org.

15.0 Waste Management

15.1 The laboratory is responsible for complying with all federal, state, and local regulations governing waste management, particularly hazardous waste identification rules and land disposal restrictions, and for protecting the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

15.2 Acids and samples at pH < 2 must be either neutralized before being disposed or handled as hazardous waste.

15.3 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better: Laboratory Chemical Management for Waste Reduction*, both available from the American Chemical Society's Government Affairs Publications, 1155 16th Street NW, Washington, DC 20036.

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17.0 Glossary

The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

17.1 Ambient water—Water in the natural environment (e.g., river, lake, stream, and other receiving water), as opposed to an effluent discharge.

17.2 Equipment blank—An aliquot of river/reagent water (Section 7.1) that is subjected in the laboratory to all aspects of sample collection and analysis, including contact with all sampling devices and apparatus. The purpose of the equipment blank is to determine if the sampling devices and apparatus for sample collection have been adequately cleaned before shipment to the field site. An acceptable equipment blank must be achieved before the sampling devices and apparatus are used for sample collection. In addition, equipment blanks should be run on random, representative sets of gloves, storage bags, and plastic wrap for each lot to determine if these materials are free from contamination before use.

17.3 Field blank—An aliquot of river/reagent water (Section 7.1) that is placed in a sample container in the laboratory, shipped to the field, and treated as a sample in all respects, including contact with the sampling devices and exposure to sampling site conditions, storage, preservation, and all analytical procedures, which may include filtration. The purpose of the field blank is to determine if the field or sample transporting procedures and environments have contaminated the sample.

17.4 Field duplicates (FD1 and FD2)—Two separate samples collected in separate sample bottles at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

17.5 Initial precision and recovery (IPR)—Four aliquots of the ongoing precision and recovery standard analyzed to establish the ability to generate acceptable precision and accuracy. IPR tests are performed before a method is used for the first time and any time the method or instrumentation is modified.

17.6 Matrix spike (MS) and matrix spike duplicate (MSD)—Aliquots of an environmental sample to which known quantities of the analytes are added in the laboratory. The MS and MSD are analyzed exactly like samples. Their purpose is to quantify the bias and precision caused by the sample matrix. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the MS and MSD corrected for background concentrations.

17.7 May—This action, activity, or procedural step is optional.

17.8 May not—This action, activity, or procedural step is prohibited.

17.9 Method blank—An aliquot of river/reagent water (Section 7.1) or corn oil (Section 7.14) that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with samples. The method blank is used to determine if analytes or interferences are present in the laboratory environment, the reagents, or the apparatus.

17.10 Minimum level (ML)—The lowest level at which the entire analytical system must give a recognizable signal and acceptable calibration point for the analyte. It is equivalent to the concentration of the lowest calibration standard, assuming that all method-specified sample weights, volumes, and cleanup procedures have been employed. The ML is calculated by multiplying the MDL by 3.18 and rounding the result to the number nearest to (1, 2, or 5) x 10n, where n is an integer.

17.11 Must—This action, activity, or procedural step is required.

17.12 Ongoing precision and recovery (OPR)—A method blank spiked with known quantities of analytes. The OPR is analyzed exactly like a sample. Its purpose is to assure that the results produced by the laboratory remain within the limits specified in the referenced methods for precision and accuracy.

17.13 Quality control sample (QCS)—A sample containing all or a subset of the analytes at known concentrations. The QCS is obtained from a source external to the laboratory or is prepared from a source of standards different from the source of calibration standards. It is used to check laboratory performance with test materials prepared external to the normal preparation process.

17.14 Reagent water—Water demonstrated to be free of As, As species, and potentially interfering substances at the MDLs for As and/or As species.

17.15 River Water—Freshwater containing arsenic species at concentrations below the MDLs.

17.16 Should—This action, activity, or procedural step is suggested but not required.

17.17 Stock solution—A solution containing an analyte that is prepared using a reference material traceable to EPA, the National Institute of Science and Technology (NIST), or a source that will attest to the purity and authenticity of the reference material.

18.0 Tables and Figures

TABLE 1. ARSENIC SPECIATION ANALYSIS USING METHOD 1632: METHOD DETECTION LIMIT (MDL) AND MINIMUM LEVEL (ML)[1]

	Water[2]		Tissue[3]	
Analyte	**MDL**	**ML**	**MDL**	**ML**
Inorganic Arsenic (As^{+3} +As^{+5})	0.003 • g/L	0.01 • g/L	0.03 • g/g	0.10 • g/g
Arsenite (As^{+3})	0.003 • g/L	0.01 • g/L	0.02 • g/g	0.10 • g/g
Monomethylarsonic acid (MMA)	0.004 • g/L	0.01 • g/L	0.01 • g/g	0.05 • g/g
Dimethylarsinic acid (DMA)	0.02 • g/L	0.05 • g/L	0.04 • g/g	0.10 • g/g

[1] MDL determined by the procedure in 40 CFR Part 136, Appendix B.
[2] MDL for inorganic As in water was obtained from a validation study involving two laboratories (Ref. 16.15). MDL for As^{+3}, MMA and DMA in water was obtained from data provided by Frontier Geosciences (Ref. 16.16).
[3] MDL for tissue was determined from spiked corn oil samples by Battelle Marine Sciences Laboratory (Ref. 16.16).

TABLE 2. QUALITY CONTROL ACCEPTANCE CRITERIA FOR EPA METHOD 1632[1]

	IPR (Section 9.2)				MS/MSD (Section 9.3)	
Analyte[2]	**s**	**X**	**OPR (Section 9.7)**	**Calibration Verification (Section 9.5)**	**%R**	**RPD**
IA	< 25%	60-140%	50-150%	80-120%	50-150%	< 35%
As^{+3}	< 25%	40-160%	30-170%	70-130%	30-170%	< 35%
MMA	< 20%	70-130%	60-140%	80-120%	60-140%	< 25%
DMA	< 30%	50-150%	40-160%	70-130%	40-160%	< 40%

[1] Acceptance criteria based on quality control data generated during As speciation analysis for the Cook Inlet Study (1998). Details can be found in Reference 16.16.
2 IA - Inorganic arsenic (As^{+3} + As^{+5}); MMA - monomethylarsonic acid; DMA - dimethylarsinic acid.

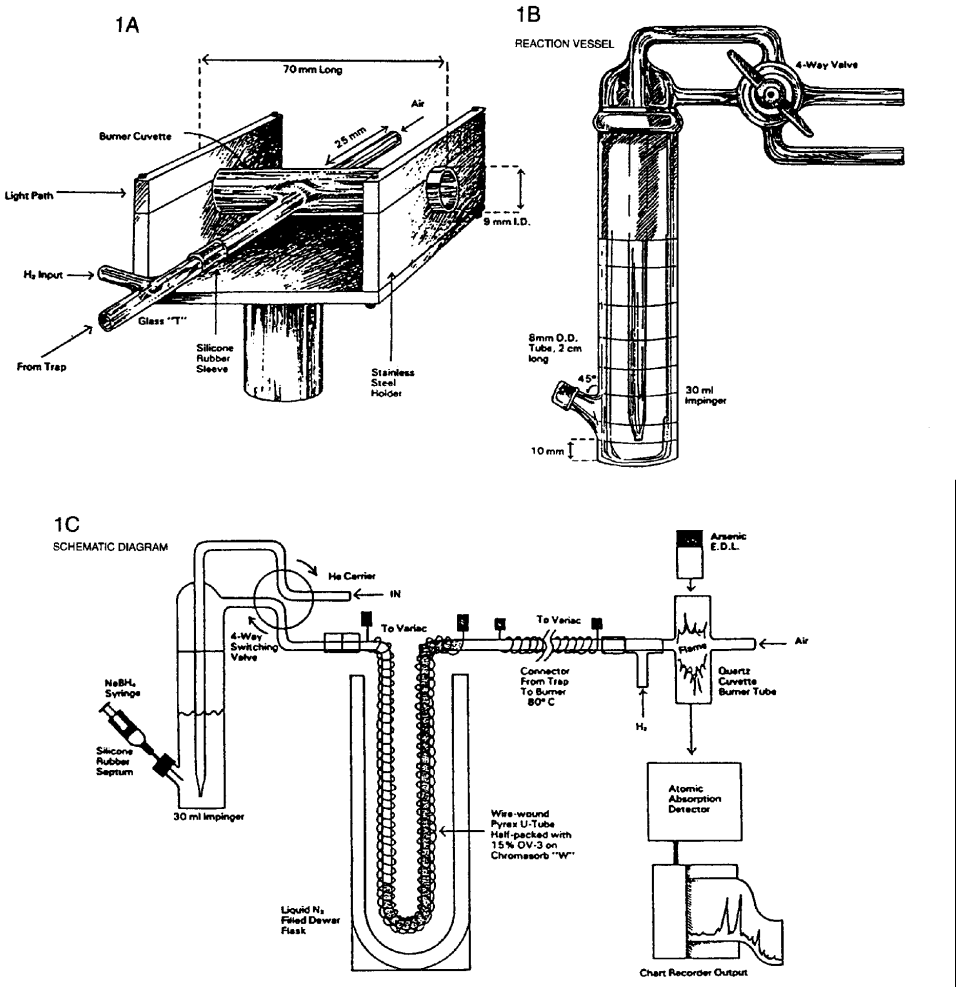
TABLE 3: TYPICAL SPECTROPHOTOMETER SETTINGS

Parameter	Typical Setting
EDL energy	59
EDL power	8 W
Wavelength	193.7 nm
Slit width	0.7 nm

TABLE 4: TYPICAL FLOW RATES AND PRESSURES FOR GASES IN THE HYDRIDE GENERATION SYSTEM

Gas	Flow Rate (mL/min)	Pressure (lb/in^2)
He	150	10
H_2	350	20
Air	180	20

Figure 1. Arsenic Speciation Apparatus: (a) Quartz Cuvette Burner Tube, (b) Reaction Vessel, and (c) Schematic Diagram



Appendix 5

USEPA Method 1636

Determination of Hexavalent Chromium by Ion Chromatography

January 1996

Method 1636

Determination of Hexavalent Chromium by Ion Chromatography

January 1996

U.S. Environmental Protection Agency
Office of Water
Engineering and Analysis Division (4303)
401 M Street S.W.
Washington, D.C. 20460

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Disclaimer

This method has been reviewed and approved for publication by the Engineering and Analysis Division of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Introduction

This analytical method was designed to support water quality monitoring programs authorized under the Clean Water Act. Section 304(a) of the Clean Water Act requires EPA to publish water quality criteria that reflect the latest scientific knowledge about the physical fate (e.g., concentration and dispersal) of pollutants, the effects of pollutants on ecological and human health, and the effect of pollutants on biological community diversity, productivity, and stability.

Section 303 of the Clean Water Act requires states to set a water quality standard for each body of water within its boundaries. A state water quality standard consists of a designated use or uses of a waterbody or a segment of a waterbody, the water quality criteria that are necessary to protect the designated use or uses, and an antidegradation policy. These water quality standards serve two purposes: (1) they establish the water quality goals for a specific waterbody, and (2) they are the basis for establishing water quality-based treatment controls and strategies beyond the technology-based controls required by Sections 301(b) and 306 of the Clean Water Act.

In defining water quality standards, the state may use narrative criteria, numeric criteria, or both. However, the 1987 amendments to the Clean Water Act required states to adopt numeric criteria for toxic pollutants (designated in Section 307(a) of the Act) based on EPA Section 304(a) criteria or other scientific data, when the discharge or presence of those toxic pollutants could reasonably be expected to interfere with designated uses.

In some cases, these water quality criteria are as much as 280 times lower than those achievable using existing EPA methods and required to support technology-based permits. Therefore, EPA developed new sampling and analysis methods to specifically address state needs for measuring toxic metals at water quality criteria levels, when such measurements are necessary to protect designated uses in state water quality standards. The latest criteria published by EPA are those listed in the National Toxics Rule (57 *FR* 60848) and the Stay of Federal Water Quality Criteria for Metals (60 *FR* 22228). These rules include water quality criteria for 13 metals, and it is these criteria on which the new sampling and analysis methods are based. Method 1636 was specifically developed to provide reliable measurements of hexavalent chromium at EPA WQC levels using ion chromatography techniques.

In developing these methods, EPA found that one of the greatest difficulties in measuring pollutants at these levels was precluding sample contamination during collection, transport, and analysis. The degree of difficulty, however, is highly dependent on the metal and site-specific conditions. This analytical method, therefore, is designed to provide the level of protection necessary to preclude contamination in nearly all situations. It is also designed to provide the procedures necessary to produce reliable results at the lowest possible water quality criteria published by EPA. In recognition of the variety of situations to which this method may be applied, and in recognition of continuing technological advances, the method is performance based. Alternative procedures may be used, so long as those procedures are demonstrated to yield reliable results.

Requests for additional copies should be directed to:

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Note: This method is intended to be performance based, and the laboratory is permitted to omit any step or modify any procedure if *all* performance requirements set forth in this method are met. The laboratory is *not* allowed to omit any quality control analyses. The terms "must," "may," and "should" are included throughout this method and are intended to illustrate the importance of the procedures in producing verifiable data at water quality criteria levels. The term "must" is used to indicate that researchers in trace metals analysis have found certain procedures essential in successfully analyzing samples and avoiding contamination; however, these procedures can be modified or omitted if the laboratory can demonstrate that data quality is not affected.

Method 1636
Determination of Hexavalent Chromium by Ion Chromatography

1.0 Scope and Application

1.1 This method is for the determination of dissolved hexavalent chromium (as CrO_4^{2-}) in ambient waters at EPA water quality criteria (WQC) levels using ion chromatography (IC). This method was developed by integrating the analytical procedures in EPA Method 218.6 with the quality control (QC) and sample handling procedures necessary to avoid contamination and ensure the validity of analytical results during sampling and analysis for metals at EPA WQC levels. This method contains QC procedures that will ensure that contamination will be detected when blanks accompanying samples are analyzed. This method is accompanied by Method 1669: *Sampling Ambient Water for Determination of Trace Metals at EPA Water Quality Criteria Levels* (the "Sampling Method"). The Sampling Method is necessary to ensure that contamination will not compromise trace metals determinations during the sampling process.

Analyte	Chemical Abstract Services Registry Number (CASRN)
Hexavalent Chromium (as CrO_4^{2-})	18540-29-9

1.2 Table 1 lists the EPA WQC level, the method detection limit (MDL), and the minimum level (ML) for hexavalent chromium (Cr(VI)). Linear working ranges will be dependent on the sample matrix, instrumentation, and selected operating conditions.

1.3 This method is not intended for determination of metals at concentrations normally found in treated and untreated discharges from industrial facilities. Existing regulations (40 *CFR* Parts 400-500) typically limit concentrations in industrial discharges to the mid to high part-per-billion (ppb) range, whereas ambient metals concentrations are normally in the low part-per-trillion (ppt) to low ppb range.

1.4 The ease of contaminating ambient water samples with the metal(s) of interest and interfering substances cannot be overemphasized. This method includes suggestions for improvements in facilities and analytical techniques that should maximize the ability of the laboratory to make reliable trace metals determinations and minimize contamination. These suggestions are given in Section 4.0 and are based on findings of researchers performing trace metals analyses (References 1-8). Additional suggestions for improvement of existing facilities may be found in EPA's *Guidance for Establishing Trace Metals Clean Rooms in Existing Facilities*, which is available from the National Center for Environmental Publications and Information (NCEPI) at the address listed in the introduction to this document.

1.5 Clean and Ultraclean—The terms "clean" and "ultraclean" have been applied to the techniques needed to reduce or eliminate contamination in trace metals determinations. These terms are not used in this method because they lack an exact definition. However, the information provided in this method is consistent with the summary guidance on clean and ultraclean techniques (Reference 9).

1.6 This method follows the EPA Environmental Methods Management Council's "Format for Method Documentation" (Reference 10).

1.7 This method is "performance based"; i.e., an alternate procedure or technique may be used, as long as the performance requirements in the method are met. Section 9.1.2 gives details of the tests and documentation required to support and document equivalent performance.

1.8 For dissolved Cr(VI) determinations, samples must be filtered through a 0.45 µm capsule filter at the field site. The Sampling Method describes the filtering procedures. The filtered samples should be preserved in the field; otherwise, samples must be analyzed within 24 hours of collection. The Sampling Method details procedures for field preservation.

1.9 Samples containing high levels of anionic species such as sulphate and chloride may cause column overload. Samples containing high levels of organics or sulfides cause rapid reduction of soluble Cr(VI) to Cr(III). Samples must be stored at 4°C and analyzed within 24 hours of collection unless preserved with sodium hydroxide.

1.10 This method should be used by analysts experienced in the use of ion chromatography, and should be used only by personnel thoroughly trained in the handling and analysis of samples for determination of metals at EPA WQC levels. A minimum of six months experience with commercial instrumentation is recommended.

1.11 This method is accompanied by a data verification and validation guidance document titled *Guidance on the Documentation and Evaluation of Trace Metals Data Collected for CWA Compliance Monitoring*. Before using this method, data users should state the data quality objectives (DQOs) required for a project.

2.0 Summary of Method

2.1 An aqueous sample is filtered through a 0.45 µm filter and the filtrate is adjusted to a pH of 9-9.5 with a concentrated buffer solution. A measured volume of the sample (50-250 µL) is introduced into the ion chromatograph. A guard column removes organics from the sample before the Cr(VI), as CrO_4^{2-}, is separated on a high capacity anion exchange separator column. Postcolumn derivatization of the Cr(VI) with diphenylcarbazide is followed by detection of the colored complex at 530 nm.

3.0 Definitions

3.1 Apparatus—Throughout this method, the sample containers, sampling devices, instrumentation, and all other materials and devices used in sample collection, sample processing, and sample analysis activities will be referred to collectively as the Apparatus.

3.2 Other definitions of terms are given in the glossary (Section 18.0) at the end of this method.

4.0 Contamination and Interferences

4.1 Preventing ambient water samples from becoming contaminated during the sampling and analytical process constitutes one of the greatest difficulties encountered in trace metals determinations. Over the last two decades, marine chemists have come to recognize that much of the historical data on the concentrations of dissolved trace metals in seawater are erroneously high because the concentrations reflect contamination from sampling and analysis rather than ambient levels. More recently, historical trace metals data collected from freshwater rivers and streams have been shown to be similarly biased because of contamination during sampling and analysis (Reference 11). Therefore, it is imperative that extreme care be taken to avoid contamination when collecting and analyzing ambient water samples for trace metals.

4.2 Samples may become contaminated by numerous routes. Potential sources of trace metals contamination during sampling include metallic or metal-containing labware (e.g., talc gloves which contain high levels of zinc), containers, sampling equipment, reagents, and reagent water; improperly cleaned and stored equipment, labware, and reagents; and atmospheric inputs such as dirt and dust. Even human contact can be a source of trace metals contamination. For example, it has been demonstrated that dental work (e.g., mercury amalgam fillings) in the mouths of laboratory personnel can contaminate samples that are directly exposed to exhalation (Reference 3).

4.3 Contamination Control

4.3.1 Philosophy—The philosophy behind contamination control is to ensure that any object or substance that contacts the sample is metal free and free from any material that may contain metals.

4.3.1.1 The integrity of the results produced cannot be compromised by contamination of samples. Requirements and suggestions for control of sample contamination are given in this method and the Sampling Method.

4.3.1.2 Substances in a sample cannot be allowed to contaminate the laboratory work area or instrumentation used for trace metals measurements. Requirements and suggestions for protecting the laboratory are given in this method.

4.3.1.3 Although contamination control is essential, personnel health and safety remain the highest priority. Requirements and suggestions for personnel safety are given in the Sampling Method and Section 5 of this method.

4.3.2 Avoiding contamination—The best way to control contamination is to completely avoid exposure of the sample to contamination in the first place. Avoiding exposure means performing operations in an area known to be free from contamination. Two of the most important factors in avoiding/reducing sample contamination are: (1) an awareness of potential sources of contamination and (2) strict attention to work being done. Therefore it is imperative that the procedures described in this method be carried out by well-trained, experienced personnel.

4.3.3 Use a clean environment—The ideal environment for processing samples is a class 100 clean room (Section 6.1.1). If a clean room is not available, all sample preparation should be performed in a class 100 clean bench or a nonmetal glove box fed by particle-free air or nitrogen. Digestion should be performed in a nonmetal fume hood situated, ideally, in the clean room.

4.3.4 Minimize exposure—The Apparatus that will contact samples, blanks, or standard solutions should be opened or exposed only in a clean room, clean bench, or glove box so that exposure to an uncontrolled atmosphere is minimized. When not being used, the Apparatus should be covered with clean plastic wrap, stored in the clean bench or in a plastic box or glove box, or bagged in clean zip-type bags. Minimizing the time between cleaning and use will also minimize contamination.

4.3.5 Clean work surfaces—Before a given batch of samples is processed, all work surfaces in the hood, clean bench, or glove box in which the samples will be processed should be cleaned by wiping with a lint-free cloth or wipe soaked with reagent water.

4.3.6 Wear gloves—Sampling personnel must wear clean, nontalc gloves (Section 6.6.8) during all operations involving handling of the Apparatus, samples, and blanks. Only clean gloves may touch the Apparatus. If another object or substance is touched, the glove(s) must be changed before again handling the Apparatus. If it is even suspected that gloves have become contaminated, work must be halted, the contaminated gloves removed, and a new pair of clean gloves put on. Wearing multiple layers of clean gloves will allow the old pair to be quickly stripped with minimal disruption to the work activity.

4.3.7 Use metal-free Apparatus—All Apparatus used for determination of metals at ambient water quality criteria levels must be nonmetallic, free of material that may contain metals, or both.

4.3.7.1 Construction materials—Only the following materials should come in contact with samples: fluoropolymer (FEP, PTFE), conventional or linear polyethylene, polycarbonate, polypropylene, polysulfone, or ultrapure quartz. PTFE is less desirable than FEP because the sintered material in PTFE may contain contaminates and is susceptible to serious memory contamination (Reference 6). Fluoropolymer or glass containers should be used for samples that will be analyzed for mercury because mercury vapors can diffuse in or out of the other materials, resulting either in contamination or low-biased results (Reference 3). Stainless steel is a major source of chromium contamination. All materials, regardless of construction, that will directly or indirectly contact the sample must be cleaned using the procedures described in Section 11.0 and must be known to be clean and metal free before proceeding.

4.3.7.2 The following materials have been found to contain trace metals and should not contact the sample or be used to hold liquids that contact the sample, *unless* these materials have been shown to be free of the

metals of interest at the desired level: Pyrex, Kimax, methacrylate, polyvinyl chloride, nylon, and Vycor (Reference 6). In addition, highly colored plastics, paper cap liners, pigments used to mark increments on plastics, and rubber all contain trace levels of metals and must be avoided (Reference 12).

4.3.7.3 Serialization—It is recommended that serial numbers be indelibly marked or etched on each piece of Apparatus so that contamination can be traced, and logbooks should be maintained to track the sample from the container through the labware to injection into the instrument. It may be useful to dedicate separate sets of labware to different sample types; e.g., receiving waters vs. effluents. However, the Apparatus used for processing blanks and standards must be mixed with the Apparatus used to process samples so that contamination of all labware can be detected.

4.3.7.4 The laboratory or cleaning facility is responsible for cleaning the Apparatus used by the sampling team. If there are any indications that the Apparatus is not clean when received by the sampling team (e.g., ripped storage bags), an assessment of the likelihood of contamination must be made. Sampling must not proceed if it is possible that the Apparatus is contaminated. If the Apparatus is contaminated, it must be returned to the laboratory or cleaning facility for proper cleaning before any sampling activity resumes.

4.3.8 Avoid sources of contamination—Avoid contamination by being aware of potential sources and routes of contamination.

4.3.8.1 Contamination by carryover—Contamination may occur when a sample containing low concentrations of metals is processed immediately after a sample containing relatively high concentrations of these metals. To reduce carryover, the sample introduction system may be rinsed between samples with dilute acid and reagent water. When an unusually concentrated sample is encountered, it is followed by analysis of a laboratory blank to check for carryover. For samples containing high levels of metals, it may be necessary to acid-clean or replace the connecting tubing or inlet system to ensure that contamination will not affect subsequent measurements. Samples known or suspected to contain the lowest concentration of metals should be analyzed first followed by samples containing higher levels. For instruments containing autosamplers, the laboratory should keep track of which station is used for a given sample. When an unusually high concentration of a metal is detected in a sample, the station used for that sample should be cleaned more thoroughly to prevent contamination of subsequent samples, and the results for subsequent samples should be checked for evidence of the metal(s) that occurred in high concentration.

4.3.8.2 Contamination by samples—Significant laboratory or instrument contamination may result when untreated effluents, in-process waters, landfill leachates, and other samples containing high concentrations of

inorganic substances are processed and analyzed. As stated in Section 1.0, this method is not intended for application to these samples, and samples containing high concentrations should not be permitted into the clean room and laboratory dedicated for processing trace metals samples.

4.3.8.3 Contamination by indirect contact—Apparatus that may not directly come in contact with the samples may still be a source of contamination. For example, clean tubing placed in a dirty plastic bag may pick up contamination from the bag and then subsequently transfer the contamination to the sample. Therefore, it is imperative that every piece of the Apparatus that is directly or indirectly used in the collection, processing, and analysis of ambient water samples be cleaned as specified in Section 11.0.

4.3.8.4 Contamination by airborne particulate matter—Less obvious substances capable of contaminating samples include airborne particles. Samples may be contaminated by airborne dust, dirt, particles, or vapors from unfiltered air supplies; nearby corroded or rusted pipes, wires, or other fixtures; or metal-containing paint. Whenever possible, sample processing and analysis should be done as far as possible from sources of airborne contamination.

4.4 Interferences which affect the accurate determination of Cr(VI) may come from several sources.

4.4.1 Reduction of Cr(VI) to Cr(III) can occur in the presence of reducing species in an acidic medium. At pH 6.5 or greater, however, CrO_4^{2-}, which is less reactive than $HCrO_4^-$, is the predominant species.

4.4.2 Overloading of the analytical column capacity with high concentrations of anionic species, especially chloride and sulphate, will cause a loss of Cr(VI). The column specified in this method can handle samples containing up to 5% sodium sulphate or 2% sodium chloride (Reference 13). Poor recoveries from fortified samples and tailing peaks are typical manifestations of column overload.

5.0 Safety

5.1 Hexavalent chromium is toxic and a suspected carcinogen and should be handled with appropriate precautions. Extreme care should be exercised when weighing the salt for preparation of the stock standard.

5.2 Each laboratory is responsible for maintaining a current awareness file of OSHA regulations for the safe handling of the chemicals specified in this method (References 14-17). A reference file of material safety data sheets (MSDSs) should also be available to all personnel involved in the chemical analysis. It is also suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this method and that the results of this monitoring be made available to the analyst. The references and bibliography at the end of Reference 17 are particularly comprehensive in dealing with the general subject of laboratory safety.

5.3 Concentrated nitric and hydrochloric acids present various hazards and are moderately toxic and extremely irritating to skin and mucus membranes. Use these reagents in a fume hood whenever possible and if eye or skin contact occurs, flush with large volumes of water. Always wear protective clothing and safety glasses or a shield for eye protection, and observe proper mixing when working with these reagents.

6.0 Apparatus, Equipment, and Supplies

DISCLAIMER: *The mention of trade names or commercial products in this method is for illustrative purposes only and does not constitute endorsement or recommendation for use by the Environmental Protection Agency. Equivalent performance may be achievable using apparatus and materials other than those suggested here. The laboratory is responsible for demonstrating equivalent performance.*

6.1 Facility

6.1.1 Clean room—Class 100, 200 ft^2 minimum, with down-flow, positive-pressure ventilation, air-lock entrances, and pass-through doors.

6.1.1.1 Construction materials—Nonmetallic, preferably plastic sheeting attached without metal fasteners. If painted, paints that do not contain the metal(s) of interest should be used.

6.1.1.2 Adhesive mats—for use at entry points to control dust and dirt from shoes.

6.1.2 Fume hoods—nonmetallic, two minimum, with one installed internal to the clean room.

6.1.3 Clean benches—Class 100, one installed in the clean room; the other adjacent to the analytical instrument(s) for preparation of samples and standards.

6.2 Ion Chromatograph

6.2.1 Instrument equipped with a pump capable of withstanding a minimum backpressure of 2000 psi and of delivering a constant flow in the range of 1-5 mL/min and containing no metal parts in the sample, eluent, or reagent flow path.

6.2.2 Helium gas supply—High purity, 99.995%.

6.2.3 Pressurized eluent container—Plastic, 1 L or 2 L size.

6.2.4 Sample loops of various sizes (50-250 µL).

6.2.5 A pressurized reagent delivery module with a mixing tee and beaded mixing coil.

6.2.6 Guard column—A column placed before the separator column and containing a sorbent capable of removing strongly absorbing organics and particles that

would otherwise damage the separator column (Dionex IonPac NG1 or equivalent).

6.2.7 Separator column—A column packed with a high capacity anion exchange resin capable of separating CrO_4^{2-} from other sample constituents (Dionex IonPac AS7 or equivalent).

6.2.8 A low-volume, flow-through cell, visible lamp detector containing no metal parts in contact with the eluent flow path. Detection wavelength is at 530 nm.

6.2.9 Recorder, integrator, or computer for receiving analog or digital signals for recording detector response (peak height or area) as a function of time.

6.3 Alkaline Detergent—Liquinox®, Alconox®, or equivalent.

6.4 pH meter or pH paper.

6.5 Analytical Balance—With capability to measure to 0.1 mg, for use in weighing solids and for preparing standards.

6.6 Labware—For determination of trace levels of elements, contamination and loss are of prime consideration. Potential contamination sources include improperly cleaned laboratory apparatus and general contamination within the laboratory environment from dust, etc. A clean laboratory work area should be designated for trace element sample handling. Sample containers can introduce positive and negative errors in the determination of trace elements by (1) contributing contaminants through surface desorption or leaching, and (2) depleting element concentrations through adsorption processes. All labware must be metal free. Suitable construction materials are fluoropolymer (FEP, PTFE), conventional or linear polyethylene, polycarbonate, and polypropylene. Fluoropolymer should be used when samples are to be analyzed for mercury. All labware should be cleaned according to the procedure in Section 11.4. Gloves, plastic wrap, storage bags, and filters may all be used new without additional cleaning unless results of the equipment blank pinpoint any of these materials as a source of contamination. In this case, either an alternate supplier must be obtained or the materials must be cleaned.

NOTE: *Chromic acid must not be used for cleaning glassware.*

6.6.1 Glassware—Class A volumetric flasks and a graduated cylinder.

6.6.2 Assorted Class A calibrated pipets.

6.6.3 10 mL male luer-lock disposable syringes.

6.6.4 0.45 µm syringe filters.

6.6.5 Storage bottle—High density polypropylene, 1 L capacity.

6.6.6 Wash bottle—One-piece stem fluoropolymer, with screw closure, 125 mL capacity.

6.6.7 Tongs—For removal of Apparatus from acid baths. Coated metal tongs may not be used.

6.6.8 Gloves—Clean, nontalc polyethylene, latex, or vinyl; various lengths. Heavy gloves should be worn when working in acid baths since baths will contain hot, strong acids.

6.6.9 Buckets or basins—5-50 L capacity, for acid soaking of the Apparatus.

6.6.10 Brushes—Nonmetallic, for scrubbing Apparatus.

6.6.11 Storage bags—Clean, zip-type, nonvented, colorless polyethylene (various sizes) to store the Apparatus.

6.6.12 Plastic wrap—Clean, colorless polyethylene to store the Apparatus.

6.7 Sampling Equipment—The sampling team may contract with the laboratory or a cleaning facility that is responsible for cleaning, storing, and shipping all sampling devices, sample bottles, filtration equipment, and all other Apparatus used for the collection of ambient water samples. Before the equipment is shipped to the field site, the laboratory or facility must generate an acceptable equipment blank (Section 9.5.3) to demonstrate that the sampling equipment is free from contamination.

6.7.1 Sampling devices—Before ambient water samples are collected, consideration should be given to the type of sample to be collected and the devices to be used (grab, surface, or subsurface samplers). The laboratory or cleaning facility must clean all devices used for sample collection. The Sampling Method describes various types of samplers. Cleaned sampling devices should be stored in polyethylene bags or wrap.

6.7.2 Sample bottles—Fluoropolymer, conventional or linear polyethylene, polycarbonate, or polypropylene; 500 mL with lids. Cleaned sample bottles should be filled with 0.1% HCl (v/v) until use.

NOTE: *If mercury is a target analyte, fluoropolymer or glass bottles must be used.*

6.7.3 Filtration apparatus

6.7.3.1 Filter—Gelman Supor 0.45 µm, 15 mm diameter capsule filter (Gelman 12175, or equivalent).

6.7.3.2 Peristaltic pump—115 V a.c., 12 V d.c., internal battery, variable-speed, single-head (Cole-Parmer, portable, "Masterflex L/S," Catalog No. H-07570-10 drive with Quick Load pump head, Catalog No. H-07021-24, or equivalent).

6.7.3.3 Tubing for use with peristaltic pump—Styrene/ethylene/butylene/silicone (SEBS) resin, approx 3/8 in i.d. by approximately 3 ft (Cole-Parmer size 18, Catalog No. G-06464-18, or approximately 1/4 in i.d., Cole-Parmer size 17, Catalog No. G-06464-17, or equivalent). Tubing is cleaned by soaking in 5-10% Hcl solution for 8-24 hours, rinsing with reagent water in a clean bench in a clean room, and drying in the clean bench by purging with metal-free air or nitrogen. After drying, the tubing is double-bagged in clear polyethylene bags, serialized with a unique number, and stored until use.

7.0 Reagents and Standards

Reagents may contain elemental impurities that might affect the integrity of analytical data. A trace amount of chromium is sometimes found in reagent grade salts. Since a concentrated buffer solution is used in this method to adjust the pH of samples, each reagent lot should be tested for the metals of interest by diluting and analyzing an aliquot from the lot using the techniques and instrumentation to be used for analysis of samples. The lot will be acceptable if the concentration of the metal of interest is below the MDL listed in this method. All acids used for this method must be of ultra high-purity grade. Suitable acids are available from a number of manufacturers or may be prepared by sub-boiling distillation.

7.1 Reagents for cleaning Apparatus, sample bottle storage, and sample preservation and analysis.

7.1.1 Nitric acid—Concentrated (sp gr 1.41), Seastar or equivalent.

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

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

7.1.4 Hydrochloric acid—Concentrated (sp gr 1.19).

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

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

7.1.7 Hydrochloric acid (HCl)—1 N trace metal grade.

7.1.8 Hydrochloric acid (HCl)—10% wt, trace metal grade.

7.1.9 Hydrochloric acid (HCl)—1% wt, trace metal grade.

7.1.10 Hydrochloric acid (HCl)—0.5% (v/v), trace metal grade.

7.1.11 Hydrochloric acid (HCL)—0.1% (v/v) ultrapure grade.

7.1.12 Ammonium hydroxide, NH_4OH—(sp gr 0.902), (CASRN 1336-21-6).

7.1.13 Ammonium sulphate, $(NH_4)_2SO_4$—CASRN 7783-20-2.

7.1.14 1,5-Diphenylcarbazide—CASRN 140-22-7.

7.1.15 Methanol—HPLC grade.

7.1.16 Sulfuric acid—Concentrated (sp gr 1.84).

7.2 Reagent Water—Water demonstrated to be free from the metal(s) of interest and potentially interfering substances at the MDL for that metal listed in Table 1. Prepared by distillation, deionization, reverse osmosis, anodic/cathodic stripping voltammetry, or other technique that removes the metal(s) and potential interferent(s).

7.3 Cr(VI) Stock Standard Solution—To prepare a 1000 mg/L solution, dissolve 4.501 g of $Na_2CrO_4 \bullet 4H_2O$ in reagent water and dilute to 1 L. Transfer to a polypropylene storage container.

7.3.1 Preparation of calibration standards—Fresh calibration standards should be prepared every two weeks or as needed. Dilute the stock standard solution to levels appropriate to the operating range of the instrument using reagent water. Before final dilution, the standards should be adjusted to pH 90-9.5 with the buffer solution (Section 7.6). Calibration standards should be prepared at a minimum of three concentrations, one of which must be at the ML (Table 1), and another that must be near the upper end of the linear dynamic range. Calibration standards should be verified initially using a quality control sample (Section 7.8).

7.4 Eluent—Dissolve 33 g of ammonium sulphate in 500 mL of reagent water and add 6.5 mL of ammonium hydroxide. Dilute to 1 L with reagent water.

7.5 Postcolumn Reagent—Dissolve 0.5 g of 1,5-diphenylcarbazide in 100 mL of HPLC grade methanol. Add to about 500 mL of reagent water containing 28 mL of 98% sulfuric acid while stirring. Dilute with reagent water to 1 L in a volumetric flask. Reagent is stable for four or five days but should be prepared only as needed.

7.6 Buffer Solution—Dissolve 33 g of ammonium sulphate in 75 mL of reagent water and add 6.5 mL of ammonium hydroxide. Dilute to 100 mL with reagent water.

7.7 Blanks—The laboratory should prepare the following types of blanks. A calibration blank is used to establish the analytical calibration curve; and the laboratory (method) blank is used to assess possible contamination from the sample preparation procedure. In addition to these blanks, the laboratory may be required to analyze field blanks (Section 9.5.2) and equipment blanks (Section 9.5.3).

7.7.1 Calibration blank—Consists of reagent water adjusted to pH 9-9.5 with the buffer solution (Section 7.6).

7.7.2 Laboratory blank—Must contain all the reagents in the same volumes as used in processing the samples. The laboratory blank must be carried through the same entire preparation scheme as the samples.

7.8 Quality Control Sample (QCS)—The QCS should be obtained from a source outside the laboratory. The concentration of the QCS solution analyzed will depend on the sensitivity of the instrument. To prepare the QCS, dilute an appropriate aliquot of analytes to a concentration ≤100 µg/L in reagent water and adjust the pH to 9-9.5 with the buffer solution (Section 7.6). The QCS should be analyzed as needed to meet data quality needs, and a fresh solution should be prepared quarterly or more frequently as needed.

7.9 Ongoing Precision and Recovery (OPR) Sample—To an aliquot of reagent water, add aliquots from the stock standard (Section 7.3) to prepare the OPR. The OPR must be carried through the same entire preparation scheme as the samples.

8.0 Sample Collection, Filtration, Preservation, and Storage

8.1 Before samples are collected, consideration should be given to the type of data required so that appropriate preservation and pretreatment steps can be taken. Filtration and pH adjustment should be performed at the time of sample collection or as soon thereafter as practically possible.

8.2 Sample Collection—Samples are collected as described in the Sampling Method.

8.3 Sample Filtration—For dissolved Cr(VI), samples and field blanks are filtered through a 0.45 µm capsule filter at the field site. The Sampling Method describes filtering procedures.

8.4 Field preservation is advised for hexavalent chromium to provide sample stability for up to 30 days (Reference 18). Samples are preserved with sodium hydroxide as described in the Sampling Method.

8.5 If the samples are not preserved with sodium hydroxide, they must be analyzed within 24 hours of collection.

8.6 Samples should be stored in polyethylene bags at 0-4°C until analysis.

9.0 Quality Assurance/Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 19). The minimum requirements of this program consist of an initial demonstration of laboratory capability, analysis of samples spiked with metals of interest to evaluate and document data quality, and analysis of standards and blanks as tests of continued performance. To determine that results of the analysis meet the performance characteristics of the method, laboratory performance is compared to established performance criteria.

9.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, the analyst is permitted to exercise certain options to eliminate interferences or lower the costs of measurements. These options include alternate digestion, concentration, and cleanup procedures, and changes in instrumentation. Alternate determinative techniques, such as the substitution of a colorimetric technique or changes that degrade method performance, are not allowed. If an analytical technique other than the techniques specified in the method is used, that technique must have a specificity equal to or better than the specificity of the techniques in the method for the analytes of interest.

9.1.2.1 Each time the method is modified, the analyst is required to repeat the procedure in Section 9.2. If the change will affect the detection limit of the method, the laboratory is required to demonstrate that the MDL (40 *CFR* Part 136, Appendix B) is lower than the MDL for that analyte in this method, or one-third the regulatory compliance level, whichever is higher. If the change will affect calibration, the analyst must recalibrates the instrument according to Section 10.0.

9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the following, at a minimum:

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and of the quality control officer who witnessed and will verify the analyses and modification.

9.1.2.2.2 A listing of metals measured, by name and CAS Registry number.

9.1.2.2.3 A narrative stating reason(s) for the modification(s).

9.1.2.2.4 Results from all quality control (QC) tests comparing the modified method to this method, including:

(a) Calibration
(b) Calibration verification
© Initial precision and recovery (Section 9.2)
(d) Analysis of blanks
(e) Accuracy assessment

9.1.2.2.5 Data that will allow an independent reviewer to validate each determination by tracing the instrument output (peak height, area, or other signal) to the final result. These data are to include, where possible:

(a) Sample numbers and other identifiers
(b) Digestion/preparation or extraction dates
© Analysis dates and times
(d) Analysis sequence/run chronology
(e) Sample weight or volume
(f) Volume before each extraction/concentration step

(g) Volume after each extraction/concentration step
(h) Final volume before analysis
(I) Injection volume
(j) Dilution data, differentiating between dilution of a sample or extract
(k) Instrument and operating conditions (make, model, revision, modifications)
(l) Columns (type, resin, etc.)
(m) Operating conditions (background corrections, temperature program, flow rates, etc.)
(n) Detector (type, operating conditions, etc.)
(o) Printer tapes and other recordings of raw data
(p) Quantitation reports, data system outputs, and other data to link raw data to results reported

9.1.3 Analyses of blanks are required to demonstrate freedom from contamination. Section 9.5 describes the required types, procedures, and criteria for analysis of blanks.

9.1.4 The laboratory shall spike at least 10% of the samples with the metal of interest to monitor method performance. This test is described in Section 9.3 of this method. When results of these spikes indicate atypical method performance for samples, an alternative extraction or cleanup technique must be used to bring method performance within acceptable limits. If method performance for spikes cannot be brought within the limits given in this method, the result may not be reported for regulatory compliance purposes.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through calibration verification and through analysis of the ongoing precision and recovery aliquot that the analytical system is in control. Sections 10.4 and 9.6 describe these procedures.

9.1.6 The laboratory shall maintain records to define the quality of data that are generated. Section 9.3.4 describes the development of accuracy statements.

9.2 Initial Demonstration of Laboratory Capability

9.2.1 Method detection limit—To establish the ability to detect hexavalent chromium, the analyst shall determine the MDL for Cr(VI) according to the procedure in 40 *CFR* 136, Appendix B using the apparatus, reagents, and standards that will be used in the practice of this method. The laboratory must produce an MDL that is less than or equal to the MDL listed in Table 1, or one-third the regulatory compliance limit, whichever is greater. MDLs should be determined when a new operator begins work or whenever, in the judgement of the analyst, a change in instrument hardware or operating conditions would dictate that they be redetermined.

9.2.2 Initial precision and recovery (IPR)—To establish the ability to generate acceptable precision and recovery, the analyst shall perform the following operations.

9.2.2.1 Analyze four aliquots of reagent water spiked with Cr(VI) at 2–3 times the ML (Table 1), according to the procedures in Section 12. All digestion, extraction, and concentration steps, and the containers, labware, and reagents that will be used with samples must be used in this test.

9.2.2.2 Using results of the set of four analyses, compute the average percent recovery (X) for the Cr(VI) in each aliquot and the standard deviation of the recovery(s) for each metal.

9.2.2.3 Compare s and X with the corresponding limits for initial precision and recovery in Table 2. If s and X meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may begin. If, however, s exceeds the precision limit or X falls outside the range for accuracy, system performance is unacceptable. Correct the problem and repeat the test (Section 9.2.2.1).

9.2.3 Linear dynamic range (LDR)—The LDR should be determined by analyzing a minimum of seven calibration standards ranging in concentration from 1-5,000 µg/L across all sensitivity settings of the spectrophotometer. To normalize responses, divide the response by the sensitivity setting multiplier. Perform the linear regression of normalized response vs. concentration and obtain the constants m and b, where m is the slope of the line and b is the y-intercept. Incrementally analyze standards of higher concentration until the measured absorbance response, R, of a standard no longer yields a calculated concentration, C_c, that is ± 10% of the known concentration, C, where $C_c = (R\text{-}b)/m$. That concentration defines the upper limit of the LDR for that instrument and analytical operating conditions. Samples having a concentration that is ≥90% of the upper limit of the LDR must be diluted to fall within the bounds of the current calibration curve concentration range and reanalyzed.

9.2.4 Quality control sample (QCS)—When beginning the use of this method, quarterly or as required to meet data quality needs, verify the calibration standards and acceptable instrument performance with the preparation and analyses of a QCS (Section 7.8). To verify the calibration standards the determined mean concentration from three analyses of the QCS must be within ± 10% of the stated QCS value. If the QCS is not within the required limits, an immediate second analysis of the QCS is recommended to confirm unacceptable performance. If the calibration standards, acceptable instrument performance, or both cannot be verified, the source of the problem must be identified and corrected before proceeding with further analyses.

9.3 Method Accuracy—To assess the performance of the method on a given sample matrix, the laboratory must perform matrix spike (MS) and matrix spike duplicate (MSD) sample analyses on 10% of the samples from each site being monitored, or at least one MS sample analysis and one MSD sample analysis must be performed for each sample batch (samples collected from the same site at the same time, to a maximum of 10 samples), whichever is more frequent. Blanks (e.g., field blanks) may not be used for MS/MSD analysis.

9.3.1 The concentration of the MS and MSD is determined as follows:

9.3.1.1 If, as in compliance monitoring, the concentration of Cr(VI) in the sample is being checked against a regulatory concentration limit, the spike must be at that limit or at one to five times the background concentration, whichever is greater.

9.3.1.2 If the concentration is not being checked against a regulatory limit, the concentration must be at one to five times the background concentration or at one to five times the ML in Table 1, whichever is greater.

9.3.2 Assessing spike recovery

9.3.2.1 Determine the background concentration (B) of Cr(VI) by analyzing one sample aliquot according to the procedure in Section 12.0.

9.3.2.2 If necessary, prepare a QC check sample concentrate that will produce the appropriate level (Section 9.3.1) in the sample when the concentrate is added.

9.3.2.3 Spike a second sample aliquot with the QC check sample concentrate and analyze it to determine the concentration after spiking (A) of Cr(VI).

9.3.2.4 Calculate each percent recovery (P) as 100(A-B)/T, where T is the known true value of the spike.

9.3.3 Compare the percent recovery (P) for Cr(VI) with the corresponding QC acceptance criteria found in Table 2. If P falls outside the designated range for recovery, the acceptance criteria have not been met.

9.3.3.1 If the acceptance criteria were not met, analyze the ongoing precision and recovery standard (Section 9.6). If the OPR is within limits for Cr(VI) (Table 2), the analytical system is in control and the problem can be attributed to the sample matrix.

9.3.3.2 For samples that exhibit matrix problems, further isolate the metal(s) from the sample matrix using dilution, chelation, extraction, concentration, hydride generation, or other means and repeat the accuracy test (Section 9.3.2).

9.3.3.3 If the recovery for Cr(VI) remains outside the acceptance criteria, the analytical result for Cr(VI) in the unspiked sample is suspect and may not be reported for regulatory compliance purposes.

9.3.4 Recovery for samples should be assessed and records maintained.

9.3.4.1 After the analysis of five samples of a given matrix type (river water, lake water, etc.) for which Cr(VI) passes the tests in Section 9.3.3, compute the average percent recovery (R) and the standard deviation of

the percent recovery (SR). Express the accuracy assessment as a percent recovery interval from R-2SR to R+2SR for each matrix. For example, if R = 90% and SR = 10% for five analyses of river water, the accuracy interval is expressed as 70-110%.

9.3.4.2 Update the accuracy assessment for Cr(VI) in each matrix on a regular basis (e.g., after each 5-10 new measurements).

9.4 Precision of Matrix Spike and Duplicate

9.4.1 Calculate the relative percent difference (RPD) between the MS and MSD per the equation below using the concentrations found in the MS and MSD. Do not use the recoveries calculated in Section 9.3.2.4 for this calculation because the RPD is inflated when the background concentration is near the spike concentration.

$$RPD = 100 \frac{(|D1-D2|)}{(D1+D2)/2}$$

where,

D1 = Concentration of the analyte in the MS sample.
D2 = Concentration of the analyte in the MSD sample.

9.4.2 The relative percent difference between the matrix spike and the matrix spike duplicate must be less than 20%. If this criterion is not met, the analytical system is be judged to be out of control. Correct the problem and reanalyze all samples in the sample batch associated with the MS/MSD that failed the RPD test.

9.5 Blanks—Blanks are analyzed to demonstrate freedom from contamination.

9.5.1 Laboratory (method) blank

9.5.1.1 Prepare a method blank with each sample batch (samples of the same matrix started through the sample preparation process (Section 12) on the same 12-hour shift, to a maximum of 10 samples). To demonstrate freedom from contamination, analyze the blank immediately after analysis of the OPR (Section 9.6).

9.5.1.2 If Cr(VI) or any potentially interfering substance is found in the blank at a concentration equal to or greater than the MDL (Table 1), sample analysis must be halted, the source of the contamination determined, the samples and a new method blank prepared, and the sample batch and fresh method blank reanalyzed.

9.5.1.3 Alternatively, if a sufficient number of blanks (three minimum) are analyzed to characterize the nature of a blank, the average concentration plus two standard deviations must be less than the regulatory compliance level.

9.5.1.4 If the result for a single blank remains above the MDL or if the result for the average concentration plus two standard deviations of three or more blanks exceeds the regulatory compliance level, results for

samples associated with those blanks may not be reported for regulatory compliance purposes. Stated another way, results for all initial precision and recovery tests (Section 9.2) and all samples must be associated with an uncontaminated method blank before these results may be reported for regulatory compliance purposes.

9.5.2 Field blank

9.5.2.1 Analyze the field blank(s) shipped with each set of samples (samples collected from the same site at the same time, to a maximum of 10 samples). Analyze the blank immediately before analyzing the samples in the batch.

9.5.2.2 If Cr(VI) or any potentially interfering substance is found in the field blank at a concentration equal to or greater than the ML (Table 1), or greater than one-fifth the level in the associated sample, whichever is greater, results for associated samples may be the result of contamination and may not be reported for regulatory compliance purposes.

9.5.2.3 Alternatively, if a sufficient number of field blanks (three minimum) are analyzed to characterize the nature of the field blank, the average concentration plus two standard deviations must be less than the regulatory compliance level or less than one-half the level in the associated sample, whichever is greater.

9.5.2.4 If contamination of the field blanks and associated samples is known or suspected, the laboratory should communicate this to the sampling team so that the source of contamination can be identified and corrective measures taken before the next sampling event.

9.5.3 Equipment Blanks—Before any sampling equipment is used at a given site, the laboratory or cleaning facility is required to generate equipment blanks to demonstrate that the sampling equipment is free from contamination. Two types of equipment blanks are required: bottle blanks and sampler check blanks.

9.5.3.1 Bottle blanks—After undergoing appropriate cleaning procedures (Section 11.4), bottles should be subjected to conditions of use to verify the effectiveness of the cleaning procedures. A representative set of sample bottles should be filled with reagent water adjusted to a pH 9-9.5 with the buffer solution (Section 7.6) and allowed to stand for a minimum of 24 hours. Ideally, the time that the bottles are allowed to stand should be as close as possible to the actual time that sample will be in contact with the bottle. After standing, the water should be analyzed for any signs of contamination. If any bottle shows signs of contamination, the problem must be identified, the cleaning procedures corrected or cleaning solutions changed, and all affected bottles recleaned.

9.5.3.2 Sampler check blanks—Sampler check blanks are generated in the laboratory or at the equipment cleaning contractor's facility by processing reagent water through the sampling devices using the same procedures that are used in the field (see Sampling Method). Therefore, the "clean hands/dirty hands" technique used during field sampling should be followed when preparing sampler check blanks at the laboratory or cleaning facility.

9.5.3.2.1 Sampler check blanks are generated by filling a large carboy or other container with reagent water (Section 7.2) and processing the reagent water through the equipment using the same procedures used in the field (see Sampling Method). For example, manual grab sampler check blanks are collected by directly submerging a sample bottle into the water, filling the bottle, and capping. Subsurface sampler check blanks are collected by immersing the sampler into the water and pumping water into a sample container.

9.5.3.2.2 The sampler check blank must be analyzed using the procedures given in this method. If Cr(VI) or any potentially interfering substance is detected in the blank, the source of contamination or interference must be identified, and the problem corrected. The equipment must be demonstrated to be free from Cr(VI) before the equipment may be used in the field.

9.5.3.2.3 Sampler check blanks must be run on all equipment that will be used in the field. If, for example, samples are to be collected using both a grab sampling device and a subsurface sampling device, a sampler check blank must be run on both pieces of equipment.

9.6 Ongoing Precision and Recovery

9.6.1 Prepare an ongoing precision and recovery sample (laboratory fortified method blank) identical to the initial precision and recovery aliquots (Section 9.2) with each sample batch (samples of the same matrix started through the sample preparation process (Section 12.0) on the same 12-hour shift, to a maximum of 10 samples) by spiking an aliquot of reagent water with the metal(s) of interest.

9.6.2 Analyze the OPR sample before analysis of the method blank and samples from the same batch.

9.6.3 Compute the percent recovery of Cr(VI) in the OPR sample.

9.6.4 Compare the concentration to the limits for ongoing recovery in Table 2. If the acceptance criteria are met, system performance is acceptable and analysis of blanks and samples may proceed. If, however, the recovery falls outside of the range given, the analytical processes are not being performed properly.

Correct the problem, reprepare the sample batch, and repeat the ongoing precision and recovery test (Section 9.6).

9.6.5 Add results that pass the specifications in Section 9.6.4 to initial and previous ongoing data for Cr(VI) in each matrix. Update QC charts to form a graphic representation of continued laboratory performance. Develop a statement of laboratory accuracy for each matrix type by calculating the average percent recovery (R) and the standard deviation of percent recovery (SR). Express the accuracy as a recovery interval from R-2SR to R+2SR. For example, if R = 95% and SR = 5%, the accuracy is 85-105%.

9.7 The specifications contained in this method can be met if the instrument used is calibrated properly and then maintained in a calibrated state. A given instrument will provide the most reproducible results if dedicated to the settings and conditions required for the analyses of metals by this method.

9.8 Depending on specific program requirements, the laboratory may be required to analyze field duplicates collected to determine the precision of the sampling technique. The relative percent difference (RPD) between field duplicates should be less than 20%. If the RPD of the field duplicates exceeds 20%, the laboratory should communicate this to the sampling team so that the source of error can be identified and corrective measures taken before the next sampling event.

10.0 Calibration and Standardization

10.1 Operating Conditions—Because of the diversity of instrument hardware, no detailed instrument operating conditions are provided. The analyst is advised to follow the recommended operating conditions provided by the manufacturer. It is the responsibility of the analyst to verify that the instrument configuration and operating conditions satisfy the quality control requirements in this method. Table 3 lists instrument operating conditions that may be used as a guide for analysts in determining instrument configuration and operating conditions. The flow rate of the eluent pump is set at 1.5 mL/min and the pressure of the reagent delivery module adjusted so that the final flow rate of the postcolumn reagent (Section 7.5) from the detector is 2.0 mL/min. This requires manual adjustment and measurement of the final flow rate using a graduated cylinder and a stop watch. A warm-up period of approximately 30 minutes after the flow rate has been adjusted is recommended, and the flow rate should be checked prior to calibration and sample analysis.

10.2 Injection sample loop size should be chosen based on anticipated sample concentrations and the selected sensitivity setting of the spectrophotometer. The sample volume used to load the sample loop should be at least 10 times the loop size so that all tubing in contact with sample is thoroughly flushed with the new sample to minimize cross-contamination.

10.3 For initial and daily operation, calibrate the instrument according to the instrument manufacturer's recommended procedures using the calibration blank (Section 7.7.1) and calibration standards (Section 7.3.1) prepared at three or more concentrations, one of which must be at the ML (Table 1), and another that must be near the upper end of the linear dynamic range.

10.4 Calibration Verification—Immediately following calibration, an initial calibration verification should be performed. Adjustment of the instrument is performed until verification criteria are met. Only after these criteria are met may blanks and samples be analyzed.

10.4.1 Analyze the mid-point calibration standard (Section 10.3).

10.4.2 Compute the percent recovery of Cr(VI) using the calibration curve obtained in the initial calibration.

10.4.3 Compare the recovery with the corresponding limit for calibration verification in Table 2. If all metals meet the acceptance criteria, system performance is acceptable and analysis of blanks and samples may continue using the response from the initial calibration. If the value falls outside the range given, system performance is unacceptable. Locate and correct the problem and/or prepare a new calibration check standard and repeat the test (Sections 10.4.1 through 10.4.3), or recalibrates the system according to Section 10.3.

10.4.4 Calibration must be verified following every ten samples by analyzing the mid-point calibration standard. If the recovery does not meet the acceptance criteria specified in Table 2, analysis must be halted, the problem corrected, and the instrument recalibrated. All samples after the last acceptable calibration verification must be reanalyzed.

10.5 A calibration blank must be analyzed following every calibration verification to demonstrate that there is no carryover of Cr(VI) and that the analytical system is free from contamination. If the concentration of an analyte in the blank result exceeds the MDL, correct the problem, verify the calibration (Section 10.4), and repeat the analysis of the calibration blank.

11.0 Procedures for Cleaning the Apparatus

11.1 All sampling equipment, sample containers, and labware should be cleaned in a designated cleaning area that has been demonstrated to be free of trace element contaminants. Such areas may include class 100 clean rooms as described by Moody (Reference 20), labware cleaning areas as described by Patterson and Settle (Reference 6), or clean benches.

11.2 Materials such as gloves (Section 6.6.8), storage bags (Section 6.6.11), and plastic wrap (Section 6.6.12) may be used new without additional cleaning unless the results of the equipment blank pinpoint any of these materials as a source of contamination. In this case, either an alternate supplier must be obtained or the materials must be cleaned.

11.3 Cleaning Procedures—Proper cleaning of the Apparatus is extremely important, because the Apparatus may not only contaminate the samples but may also remove the analytes of interest by adsorption onto the container surface.

NOTE: *If laboratory, field, and equipment blanks (Section 9.5) from Apparatus cleaned with fewer cleaning steps than those detailed below show no levels of analytes above the MDL, those cleaning steps that do not eliminate these artifacts may be omitted if all performance criteria outlined in Section 9.0 are met.*

11.3.1 Bottles, labware, and sampling equipment.

11.3.1.1 Fill a precleaned basin (Section 6.6.9) with a sufficient quantity of a 0.5% solution of liquid detergent (Section 6.3), and completely immerse each piece of ware. Allow to soak in the detergent for at least 30 minutes.

11.3.1.2 Using a pair of clean gloves (Section 6.6.8) and clean nonmetallic brushes (Section 6.6.10), thoroughly scrub down all materials with the detergent.

11.3.1.3 Place the scrubbed materials in a precleaned basin. Change gloves.

11.3.1.4 Thoroughly rinse the inside and outside of each piece with reagent water until there is no sign of detergent residue (e.g., until all soap bubbles disappear).

11.3.1.5 Change gloves, immerse the rinsed equipment in a hot (50-60°C) bath of concentrated reagent grade HNO_3 (Section 7.1.1) and allow to soak for at least two hours.

11.3.1.6 After soaking, use clean gloves and tongs to remove the Apparatus and thoroughly rinse with distilled, deionized water (Section 7.2).

11.3.1.7 Change gloves and immerse the Apparatus in a hot (50-60°C) bath of 1N trace metal grade HCL (Section 7.1.7), and allow to soak for at least 48 hours.

11.3.1.8 Thoroughly rinse all equipment and bottles with reagent water. Proceed with Section 11.3.2 for labware and sampling equipment. Proceed with Section 11.3.3 for sample bottles.

11.3.2 Labware and sampling equipment

11.3.2.1 After cleaning, air-dry in a class 100 clean air bench.

11.3.2.2 After drying, wrap each piece of ware or equipment in two layers of polyethylene film.

11.3.3 Fluoropolymer sample bottles—These bottles should be used if mercury is a target analyte.

11.3.3.1 After cleaning, fill sample bottles with 0.1% (v/v) ultrapure HCL (Section 7.1.11) and cap tightly. To ensure a tight seal, it may be necessary to use a strap wrench.

11.3.3.2 After capping, double-bag each bottle in polyethylene zip-type bags. Store at room temperature until sample collection.

11.3.4 Bottles, labware, and sampling equipment—Polyethylene or material other than fluoropolymer.

11.3.4.1 Apply the steps outlined in Sections 11.3.1.1 through 11.3.1.8 to all bottles, labware, and sampling equipment. Proceed with Section 11.3.4.2 for bottles or Section 11.3.4.3 for labware and sampling equipment.

11.3.4.2 After cleaning, fill each bottle with 0.1% (v/v) ultrapure HCL (Section 7.1.11). Double-bag each bottle in a polyethylene bag to prevent contamination of the surfaces with dust and dirt. Store at room temperature until sample collection.

11.3.4.3 After rinsing labware and sampling equipment, air-dry in a class 100 clean air bench. After drying, wrap each piece of ware or equipment in two layers of polyethylene film.

NOTE: *Polyethylene bottles cannot be used to collect samples that will be analyzed for mercury at trace (e.g., 0.012 µg/L) levels because of the potential for vapors to diffuse through the polyethylene.*

11.3.4.4 Polyethylene bags—If polyethylene bags need to be cleaned, clean according to the following procedure:

11.3.4.4.1 Partially fill with cold, (1+1) HNO_3 (Section 7.1.2) and rinse with distilled deionized water (Section 7.2).

11.3.4.4.2 Dry by hanging upside down from a plastic line with a plastic clip.

11.3.5 Silicone tubing, fluoropolymer tubing, and other sampling apparatus—Clean any silicone, fluoropolymer, or other tubing used to collect samples by rinsing with 10% HCL (Section 7.1.8) and flushing with water from the site before sample collection.

11.3.6 Extension pole—Because of its length, it is impractical to submerse the 2 m polyethylene extension pole (used in with the optional grab sampling device) in acid solutions as described above. If such an extension pole is used, a nonmetallic brush (Section 6.6.10) should be used to scrub the pole with reagent water and the pole wiped down with acids described in Section 11.3.4. After cleaning, the pole should be wrapped in polyethylene film.

11.4 Storage—Store each piece or assembly of the apparatus in a clean, single polyethylene zip-type bag. If shipment is required, place the bagged apparatus in a second polyethylene zip-type bag.

11.5 All cleaning solutions and acid baths should be periodically monitored for accumulation of metals that could lead to contamination. When levels of metals in the solutions become too high, the solutions and baths should be changed and the old solutions neutralized and discarded in compliance with state and federal regulations.

12.0 Procedures for Sample Preparation and Analysis

12.1 Filtered, pH-adjusted samples at 4°C should be brought to ambient temperature before analysis.

12.2 Initiate instrument operating configuration and calibrate the instrument as described in Section 10.0.

12.3 Construct a calibration curve of analyte response (peak height or area) vs. analyte concentration over a concentration range of one or two orders of magnitude. The calibration range should bracket the anticipated concentration range of samples. The coefficient of correlation (r) for the curve should be 0.999 or greater.

12.4 Draw into a new, unused syringe (Section 6.6.3) approximately 3 mL of sample. Inject 10 times the volume of the sample loop into the injection valve of the IC. Sample concentrations that exceed the calibration range must be diluted and reanalyzed.

12.5 During analysis of samples, the laboratory must comply with the required quality control described in Sections 9.0 and 10.0.

13.0 Data Analysis and Calculations

13.1 The sample concentration can be calculated from the calibration curve. Report values in µg/L. Report results at or above the ML for metals found in samples and determined in standards. Report all results for metals found in blanks, regardless of level.

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

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

14.0 Method Performance

14.1 The method detection limit (MDL) listed in Table 1 and the quality control acceptance criteria listed in Table 2 were validated in a single laboratory (Reference 21) for dissolved hexavalent chromium.

15.0 Pollution Prevention

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

chemicals used in this method are the neat materials used in preparing standards. These standards are used in extremely small amounts and pose little threat to the environment when managed properly. To minimize the volume of expired standards to be disposed, standards should be prepared in volumes consistent with laboratory use.

15.2 For information about pollution prevention that may be applied to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington DC 20036, 202/872–4477.

16.0 Waste Management

16.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel*, available from the American Chemical Society at the address listed in Section 15.2.

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18.0 Glossary

Many of the terms and definitions listed below are used in the EPA 1600-series methods, but terms have been cross-referenced to terms commonly used in other methods where possible.

18.1 Ambient Water—Waters in the natural environment (e.g., rivers, lakes, streams, and other receiving waters), as opposed to effluent discharges.

18.2 Analyte—A metal tested for by the methods referenced in this method. The analytes are listed in Table 1.

18.3 Apparatus—The sample container and other containers, filters, filter holders, labware, tubing, pipets, and other materials and devices used for sample collection or sample preparation, and that will contact samples, blanks, or analytical standards.

18.4 Calibration Blank—A volume of reagent water acidified with the same acid matrix as in the calibration standards. The calibration blank is a zero standard and is used to calibrate the ICP instrument (Section 7.7.1).

18.5 Calibration Standard (CAL)—A solution prepared from a dilute mixed standard and/or stock solutions and used to calibrate the response of the instrument with respect to analyte concentration.

18.6 Dissolved Analyte—The concentration of analyte in an aqueous sample that will pass through a 0.45 µm membrane filter assembly before sample acidification (Section 8.3).

18.7 Equipment Blank—An aliquot of reagent water that is subjected in the laboratory to all aspects of sample collection and analysis, including contact with all sampling devices and apparatus. The purpose of the equipment blank is to determine if the sampling devices and apparatus for sample collection have been adequately cleaned before shipment to the field site. An acceptable equipment blank must be achieved before the sampling devices and apparatus are used for sample collection. In addition, equipment blanks should be run on random, representative sets of gloves, storage bags, and plastic wrap for each lot to determine if these materials are free from contamination before use.

18.8 Field Blank—An aliquot of reagent water that is placed in a sample container in the laboratory, shipped to the field, and treated as a sample in all respects, including contact with the sampling devices and exposure to sampling site conditions, storage, preservation, and all analytical procedures, which may include filtration. The purpose of the field blank is to determine if the field or sample transporting procedures and environments have contaminated the sample.

18.9 Field Duplicates (FD1 and FD2)—Two separate samples collected in separate sample bottles at the same time and place under identical circumstances and treated exactly the same throughout field and laboratory procedures. Analyses of FD1 and FD2 give a measure of the precision associated with sample collection, preservation, and storage, as well as with laboratory procedures.

18.10 Initial Precision and Recovery (IPR)—Four aliquots of the OPR standard analyzed to establish the ability to generate acceptable precision and accuracy. IPRs are performed before a method is used for the first time and any time the method or instrumentation is modified.

18.11 Instrument Detection Limit (IDL)—The concentration equivalent to the analyte signal which is equal to three times the standard deviation of a series of ten replicate measurements of the calibration blank signal at the selected analytical wavelength.

18.12 Laboratory Blank—An aliquot of reagent water that is treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, internal standards, and surrogates that are used with samples. The laboratory blank is used to determine if method analytes or interferences are present in the laboratory environment, the reagents, or the apparatus (Sections 7.7.2 and 9.5.1).

18.13 Laboratory Control Sample (LCS)—See Ongoing Precision and Recovery (OPR) Standard.

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

18.15 Laboratory Fortified Blank (LFB)—See Ongoing Precision and Recovery (OPR) Standard.

18.16 Laboratory Fortified Sample Matrix (LFM)—See Matrix Spike (MS) and Matrix Spike Duplicate (MSD).

18.17 Laboratory Reagent Blank (LRB)—See Laboratory Blank.

18.18 Linear Dynamic Range (LDR)—The concentration range over which the instrument response to an analyte is linear (Section 9.2.3).

18.19 Matrix Spike (MS) and Matrix Spike Duplicate (MSD)—Aliquots of an environmental sample to which known quantities of the method analytes are added in the laboratory. The MS and MSD are analyzed exactly like a sample. Their purpose is to quantify the bias and precision caused by the sample matrix. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the MS and MSD corrected for background concentrations (Section 9.3).

18.20 May—This action, activity, or procedural step is optional.

18.21 May Not—This action, activity, or procedural step is prohibited.

18.22 Method Blank—See Laboratory Blank.

18.23 Method Detection Limit (MDL)—The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero (Section 9.2.1 and Table 1).

18.24 Minimum Level (ML)—The lowest level at which the entire analytical system gives a recognizable signal and acceptable calibration point (Reference 9).

18.25 Must—This action, activity, or procedural step is required.

18.26 Ongoing Precision and Recovery (OPR) Standard—A laboratory blank spiked with known quantities of the method analytes. The OPR is analyzed exactly like a sample. Its purpose is to determine whether the methodology is in control and to assure that the results produced by the laboratory remain within the method-specified limits for precision and accuracy (Sections 7.9 and 9.6).

18.27 Preparation Blank—See Laboratory Blank.

18.28 Primary Dilution Standard—A solution containing the analytes that is purchased or prepared from stock solutions and diluted as needed to prepare calibration solutions and other solutions.

18.29 Quality Control Sample (QCS)—A sample containing all or a subset of the method analytes at known concentrations. The QCS is obtained from a source external to the laboratory or is prepared from a source of standards different from the source of calibration standards. It is used to check laboratory performance with test materials prepared external to the normal preparation process.

18.30 Reagent Water—Water demonstrated to be free from the method analytes and potentially interfering substances at the MDL for that metal in the method.

18.31 Should—This action, activity, or procedural step is suggested but not required.

18.32 Stock Standard Solution—A solution containing one or more method analytes that is prepared using a reference material traceable to EPA, the National Institute of Science and Technology (NIST), or a source that will attest to the purity and authenticity of the reference material.

TABLE 1. HEXAVALENT CHROMIUM ANALYSIS USING METHOD 1636: LOWEST WATER QUALITY CRITERION, METHOD DETECTION LIMIT, AND MINIMUM LEVEL

Metal	Lowest Ambient Water Quality Criterion (µg/L)[1]	Method Detection Limit (MDL) and Minimum Level (ML); µg/L	
		MDL[2]	ML[3]
Hexavalent Chromium	10	0.23	0.5

[1] Lowest of the freshwater, marine, or human health WQC at 40 *CFR* Part 131 (57 *FR* 60848 for human health criteria and 60 *FR* 22228 for aquatic criteria). Hardness-dependent freshwater aquatic life criteria also calculated to reflect a hardness of 25 mg/L $CaCO_3$, and all aquatic life criteria have been adjusted to reflect dissolved levels in accordance with the equations provided in 60 *FR* 22228.

[2] Method Detection Limits as determined by 40 *CFR* Part 136, Appendix B.

[3] Minimum Level (ML) calculated by multiplying laboratory-determined MDL by 3.18 and rounding result to nearest multiple of 1, 2, 5, 10, etc. in accordance with procedure used by EAD and described in the EPA *Draft National Guidance for the Permitting, Monitoring, and Enforcement of Water Quality-Based Effluent Limitations Set Below Analytical Detection/Quantitation Levels,* March 22, 1994.

TABLE 2. QUALITY CONTROL ACCEPTANCE CRITERIA FOR PERFORMANCE TESTS IN EPA METHOD 1636[1]

Metal	Initial Precision and Recovery (Section 9.2)		Calibration Verification (Section 10.4)	Ongoing Precision and Recovery (Section 9.6)	Spike Recovery (Section 9.3)
	s	X			
Hexavalent Chromium	20	80–120	90–110	79–122	79–122

[1]All specifications expressed as percent.

TABLE 3. RECOMMENDED ION CHROMATOGRAPHIC CONDITIONS

Columns:	Guard Column—Dionex IonPac NG1 Separator Column—Dionex IonPac AS7
Eluent:	250 mM $(NH_4)_2SO_4$ 100 mM NH_4OH Flow rate = 1.5 mL/min
Postcolumn Reagent:	2mM Diphenylcarbohydrazide 10% v/v CH_3OH 1N H_2SO_4 Flow rate = 0.5 mL/min
Detector:	Visible 530 nm
Retention Time:	3.8 minutes

Appendix 6

Determination of Trace Concentrations of Hexavalent Chromium

Michael Gardner and Sean Comber, *Analyst*, **127**, pp. 153-156 (2002).

THE ANALYST
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Determination of trace concentrations of hexavalent chromium

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A simple and sensitive solvent extraction-atomic spectrometric technique has been developed for the determination of hexavalent chromium in fresh and saline waters. The technique is based on the reaction of chromium with diphenylcarbazide. The method has been tested on a variety of water samples over an analytical range of 0–2 μg l^{-1}. A limit of detection of 0.024 μg l^{-1} was achieved. Spiking recoveries in the range 87–115% were achieved in river water, drinking water and marine waters.

Introduction

Metal speciation is usually the key to the fate and behaviour of metals.[1] In the case of chromium, the important issue is the relative proportions of the trivalent (Cr^{III}) and hexavalent Cr^{VI} forms. The biological effects of the metal in the two oxidation states are markedly different. The trivalent form is relatively non-toxic and is regarded as an essential trace element, whilst Cr^{VI} is of relatively high toxicity and has been shown to be a carcinogen in animal studies.

Public concern has been expressed in the US in relation to possible exposure of drinking water consumers to hexavalent chromium. In 1999, the US Office of Environmental Health Hazard Assessment of the Environment Protection Agency established a public health goal of 2.5 μg l^{-1} for total chromium, based on a health protective level of 0.2 μg l^{-1} for hexavalent chromium (derived for a cancer endpoint) and the assumption that the hexavalent chromium is no more than 7% of the total chromium. However, a limited study of drinking water sources conducted in late 1999 indicated that the average percentage of hexavalent chromium may be above 50%.[2] At present, in the UK and other EU states concentrations of total chromium in drinking water are monitored for compliance with a limit concentration of 50 μg l^{-1}. The UK environmental quality standard for total chromium in surface waters is set at 15 μg l^{-1}, though there may be concern about lower concentrations if the metal were present as the hexavalent form. A reduction in the concentration of interest and a focus on Cr^{VI} species generates a requirement for analytical methodology suitable for monitoring purposes in both drinking waters and surface waters. This paper describes a procedure, which was developed and tested with the aim of meeting this requirement.

Methodology

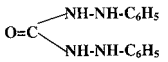
Analytical techniques available for the determination of chromium speciation at trace levels include electrochemical methods (*e.g.* stripping voltammetry) and methods involving separation of species and subsequent determination of the separated fractions using an analytical technique for total metal. Separation may be on to a solid phase,[3,4] or into a solvent.[5,6] There is usually a need to achieve some degree of preconcentration, so that the analytical method is capable of determining suitably low concentrations of chromium. Electrochemical methods are capable of measuring naturally occurring chromium concentrations and species directly.[7] However, the methodology tends to be complex and lacking in robustness. Several separation methods with adequate detection capability for the determination of total chromium and Cr^{III} have been reported[8,9] but none provides a simple means of determining Cr^{VI} directly. Chromium(VI) concentrations may be arrived at by subtraction of the concentration of Cr^{III} from total dissolved Cr. This subtraction may be subject to large uncertainty, particularly if Cr^{III} is the predominant form. Furthermore these methods tend to be complicated and expensive to apply. The method recommended by legislators in the US for the determination of Cr^{VI} is one in which Cr^{VI} is isolated by liquid chromatography and determined colorimetrically. This technique has a reported limit of detection of 0.5 μg l^{-1}.[10] The use of a technique with a limit of detection so close to the proposed water quality standard value may impose a serious limitation on the ability to monitor and regulate levels of chromium^{VI}.

The methodology for the determination of Cr^{VI} by colorimetry using diphenylcarbazide is well established.[11] Direct spectrophotometry can be used to determine Cr^{VI} in clean waters down to a limit of detection of approximately 2–3 μg l^{-1}. This is not adequate to monitor compliance with quality standards or limit values set at the low levels discussed above. This work aimed to develop the diphenylcarbazide methodology for the determination of Cr^{VI}, principally by extending it to sub-microgram per litre levels using preconcentration by solvent extraction.

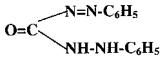
Diphenylcarbazide gives a sensitive and specific colour reaction with Cr^{VI} in mineral acid solution. The pink coloured chromophore is a chelate of Cr^{III} and diphenylcarbazone. The latter is produced and simultaneously combines with chromium when diphenylcarbazide is oxidised by Cr^{VI}. The reaction may be summarised as:

$$2CrO_4^{2-} + 3H_4L + 8H^+ = [Cr^{III}(HL)_2]^+ + Cr^{3+} + H_2L + 8H_2O$$

Where H_4L is diphenylcarbazide:



and H_2L diphenylcarbazone:



Direct reaction of Cr^{III} with diphenylcarbazone does not occur to any appreciable extent on account of the well known inertness of the Cr^{III} aquo-complex. The singly charged chromium–diphenylcarbazone complex can be extracted into

relatively polar solvents as an ion pair with chloride ion. Sandell and Onishi[12] suggest isoamyl alcohol as a suitable solvent.

Target analytical performance

The following performance criteria were defined[13,14,15] as desirable in a method to be used for monitoring of Cr^{VI} in drinking waters and surface waters, assuming that it is necessary to operate in the range 0–2 μg l^{-1} and that the establishment of compliance with a quality standard of 2 μg l^{-1} is of primary interest. The total standard deviation of individual results should be less than 5% of the determinand concentration or 0.01 μg l^{-1}, whichever was the larger; spiking recovery (both saline and fresh water samples) should not be significantly outside the range 90–110%; the limit of detection should be 0.03 μg l^{-1} or better.

Experimental

Reagents

Diphenylcarbazide reagent, 0.25 g of diphenylcarbazide (Sigma Chemicals) was dissolved in 25 ml acetone (prepared freshly each day); sulfuric acid, 5 M: concentrated sulfuric acid (98% w/w) was diluted 3.5 fold with deionised water; iso amyl alcohol (Merck); saturated solution of sodium chloride, 300 g l^{-1}. Cr^{VI} standards were prepared by diluting Merck Spectrosol (1000 mg l^{-1}) standards with deionised water. Cr^{III} standards were prepared from potassium chromium sulfate (Merck).

All water was deionised and all chemicals were of reagent grade. All apparatus was pre-soaked in 5% v/v nitric acid and rinsed with deionised water before use.

Procedure

25 ml of sample was transferred to a graduated 50 ml polypropylene screw capped tube. To this was added 0.25 ml of 5 M sulfuric acid and 0.5 ml of diphenyl carbazide reagent. The sample was swirled to mix and left for10 min to allow colour development. Then 20 ml of saturated sodium chloride solution was added, followed by 2.5 ml of isoamyl alcohol. The tube was capped and shaken for 4 min.

Extractions were performed in batches of 24 samples with the sample tubes held and shaken in a laboratory tube rack. After leaving the samples for at least half an hour for the solvent layer to separate, 0.5 ml of the upper alcohol layer was pipetted off and transferred for analysis by electrothermal atomic absorption spectrophotometry. Standard solutions in deionised water at concentrations of 0, 0.1, 0.5, 1 and 2 μg l^{-1} were extracted along with samples. All determinations were made on a Perkin-Elmer 4000 atomic absorption spectrometer and HGA 400 atomiser at 357.9 nm, with a 0.7 nm bandpass. Background correction was carried out using a deuterium lamp. The furnace programme used is shown in Table 1.

For the purpose of the performance tests reported here, five different water samples were analysed in duplicate, unspiked and spiked with Cr^{VI} at 0.5 and 2 μg l^{-1} over a set of nine analytical runs (see Table 2. All test samples were prepared in bulk by filtration under positive nitrogen pressure through acid-washed cellulose acetate filters (0.45 μm, 47 mm (Sartorius, Watford, UK)).

Results

Fig. 1 shows a typical calibration curve, to which a quadratic fit is appropriate to at least 2 μg l^{-1}, with a consistent reagent blank corresponding to approximately 0.02 μg l^{-1}. The response of the technique to Cr^{III} was tested for a blank sample spiked at Cr^{III} concentrations of 0, 1, 5, 10, 20 and 50 μg l^{-1}. The results, expressed as observed Cr^{VI}, were not statistically significantly different ($p = 0.05$) from zero and ranged between −0.010 and 0.017 μg l^{-1}. The principal performance test characteristics required by UK regulatory agencies for water analysis[13,14] for the six test samples are shown in Table 3. These include within run, between run and total standard deviation, spiking recovery and limit of detection. Performance test results (Table 3), based on 9 batches of analysis, showed that the precision and recovery achieved by the method met the chosen performance criteria.

Spiking the water samples at 0.5 and 2 μg l^{-1} made it possible to assess potential matrix interferences by comparison of the calibration slope for the natural water samples with that obtained for the standard solutions. Fig. 2 shows the ratio of calibration slope in the sample matrix *versus* that in deionised water standard solutions. For the river samples B and C and the saline samples, the ratio is highly consistent and not significantly different from 1.0. For the more highly coloured water (A), there is a marked suppressive interference which is more variable from run to run. This is probably attributable to coextraction of humic material which was clearly visible as both colour and solid precipitate in the alcohol layer. Consequently, data for Sample A have been reported after standard additions

Table 1 ETAAS conditions[a]

Programme	Temperature/°C	Ramp/s	Hold/s
1	90	10	10
2	140	20	10
3	1000	10	10
4	2500	0	3
5	2600	3	1

[a] A pyrolytically coated furnace tube was used. 40 μl of the isoamyl alcohol layer was injected into the graphite furnace. The wavelength was 357.9 nm.

Table 2 Indicative water quality data for natural waters used in performance tests[a]

	Units	River water A	River water B	Estuarine sample C	Seawater sample D	Drinking water E
Calcium	mg l^{-1}	2.5	96	189	360	110
Sodium	mg l^{-1}	nd	nd	3489	10 157	20
Chloride	mg l^{-1}	nd	nd	6268	18 247	13
Magnesium	mg l^{-1}	2	4.4	415	1 200	20
Potassium	mg l^{-1}	nd	nd	122	351	3
DOC	mg l^{-1}	12.1	5.1	4.2	< 1	< 1
pH		4.3	8.1	8	8	7.7
Electrical conductivity	μS cm^{-1}	142	573	na	na	650
Salinity	ppt	na	na	12	35	na

[a] nd = not determined. na = not applicable.

Table 3 Precision and recovery data

Sample (+ spike oncentration μg l^{-1})	Mean value	Within run s^a	Between run s^b	Total s^c	RSD (%)	DF^d	Recovery %)	Recovery confidence limits +/−^{e}
Sample A	0.064	0.020	0.024	0.031	49	12		
Sample A + 0.5 μg l^{-1}	0.50	0.025	0.018	0.031	6	15	87	4
Sample A + 2 μg l^{-1}	2.03	0.061	0.000	0.061	3	17	98	1
Sample B	0.15	0.013	0.012	0.017	11	13		
Sample B + 0.5μg l^{-1}	0.66	0.018	0.030	0.035	5	10	101	5
Sample B + 2 μg l^{-1}	2.10	0.047	0.065	0.080	4	11	97	3
Sample C	0.17	0.013	0.017	0.021	12	11		
Sample C + 0.5 μg l^{-1}	0.75	0.042	0.048	0.064	8	12	115	8
Sample C + 2 μg l^{-1}	2.17	0.038	0.081	0.089	4	10	100	3
Sample D	0.16	0.012	0.025	0.027	17	10		
Sample D + 0.5 μg l^{-1}	0.72	0.029	0.053	0.060	8	10	112	8
Sample E + 2 μg l^{-1}	2.23	0.063	0.135	0.149	7	10	104	5
Sample E	0.086	0.005	0.008	0.010	12	11		
Sample E + 0.5 μg l^{-1}	0.59	0.023	0.019	0.030	5	14	101	4
Sample E + 2 μg l^{-1}	2.06	0.042	0.091	0.100	5	10	99	3
Standard solutions								
0.1 μg l^{-1}	0.10			0.016		12		
0.5 μg l^{-1}	0.51			0.019		12		
1 μg l^{-1}	1.00			0.022		12		
2 μg l^{-1}	2.01			0.010		12		
Limit of detection = 0.024 μg l^{-1}								

[a] Within run s = within run standard deviation estimated as a pooled value with 9 degrees of freedom.[b] Between run s = between run standard deviation estimated with 8 degrees of freedom.[c] Total s = total standard deviation estimated as the combination of (a) and (b) with degrees of freedom indicated (see ref. 14, Cheeseman *et al.*, 1989).[d] Degrees of freedom associated with the estimated total standard deviation (see note c above).[e] 95% confidence limits associated with the observed recovery.

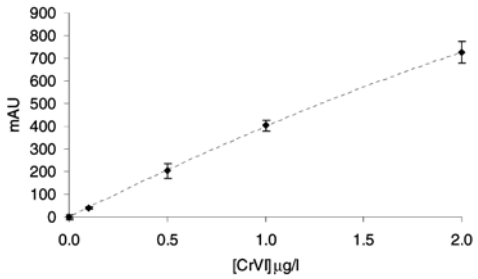


Fig. 1 Typical calibration graph showing response in milli absorbance units (mAU) *versus* concentration of Cr^{VI} in μg l^{-1}. The equation of the quadratic curve fitted to the points is $y = 36.6x^2 + 437x - 1.6$, where y is mAU and x is [Cr^{VI}] in μg l^{-1}. Error bars are 95% confidence limits based on 4 replicate measurements at each concentration.

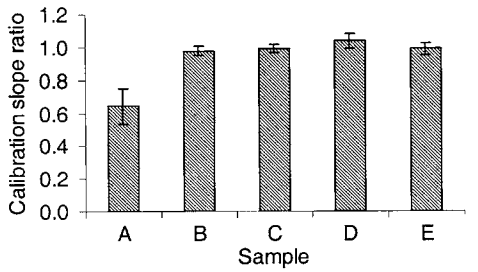


Fig. 2 Ratio of calibration slope in the sample matrix *versus* that in deionised water standard solutions. Error bars are 95% confidence limits on the mean slope for 9 analytical runs.

calibration. Data for the other samples were calculated by direct comparison with aqueous standards.

To assess the likelihood of losses of the determinand by adsorption during filtration, a river water sample was pre-filtered, spiked with Cr^{VI} and analysed with and without filtration. The concentration in the filtered sample was found to be 1.33 ± 0.1 (p = 0.05)for the unfiltered sample and 1.34 ± 0.04 for the filtered sample. This indicates minimal loss of determinand by adsorption during filtration. The stabilty of Cr^{VI} in water samples has been demonstrated as at least one month by Sirinawin and Westerlund,[16] so there does not appear to be a need to extract samples immediately.

Conclusions

This study shows how hexavalent chromium may be determined at sub- microgram per litre concentrations. This complexation/preconcentration procedure is capable of determining dissolved hexavalent chromium in fresh and saline samples in the range 0.03 to 2 μg l^{-1}. The technique is adequately precise to be used for compliance monitoring to a proposed water quality criterion of 2 μg l^{-1}. Recoveries of Cr^{VI} from a range of waters were not significantly outside the range 90–110%, though for samples high in dissolved organic carbon (>6 mg l^{-1} C), standard additions calibration is required. The technique is simple to use and requires instrumentation that is available in most laboratories.

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Appendix 7

Tributyltin Distribution in the Coastal Environment of Peninsular Malaysia

S.L. Tong, F.Y. Pang, S.M. Phang and H.C. Lai,

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TRIBUTYLTIN DISTRIBUTION IN THE COASTAL ENVIRONMENT OF PENINSULAR MALAYSIA

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Abstract

The occurrence of tributyltin (TBT) is reported in the coastal waters of a few selected sites in Peninsular Malaysia. Water, bivalves and sediment samples collected were analysed specifically for TBT using sensitive analytical methods which involved a solvent extraction procedure with appropriate clean-up followed by graphite furnace atomic absorption spectrometric measurements. The levels of TBT in the seawater in unexposed areas were found in the range from <3.4 to 20 ng litre^{-1} as compared to coastal areas with high boat and ship activities where TBT levels in seawater were generally above 30 ng litre^{-1}, with the highest level found at 281.8 ng litre^{-1}. TBT levels in the tissues of random cockle and soft-shell clam samples from local markets were found in the range from <0.5 to 3.7 ng g^{-1} wet weight. The levels of TBT found in green mussel samples both from the market (23.5 ng g^{-1} wet weight) and those from a mussel farm (14.2 ng g^{-1} wet weight) indicate slight accumulation of TBT. In sediments, TBT levels were found ranging from <0.7 ng g^{-1} dry weight in unexposed coastal sites to as high as 216.5 ng g^{-1} dry weight for a site within a port area.

Keywords: Tributyltin, coastal waters, bivalves, sediments, tropical waters.

INTRODUCTION

Organotin compounds have found many important industrial and agricultural applications for more than three decades (Blunden *et al.*, 1985). These include the use of monomethyltins, monobutyltins and dibutyltins as stabilisers for polyvinyl chloride and as catalysts in industrial processes. Progressive introduction of organic groups at the tin atom produces increasing biological activity (Schweinfurth, 1985). Organotin compounds with three alkyl groups attached to the tin atom, such as tributyltin (TBT), triphenyltin and tricyclohexyltin, have found wide applications as antifouling agents in marine paint formulations, wood and stone preservatives, disinfectants, bactericides in cooling water, agricultural fungicides and acaricides. The most important of these is TBT, which is widely used in marine paints as an effective means iof arresting the growth of fouling organisms such as tube worms, barnacles and mussels on seafaring vessels and marine structures.

The ecotoxicological effects of TBT and other triorganotin compounds in the aquatic environment on non-target organisms have caused much concern in recent years leading to the control or banning of the use of these chemicals in a few developed countries. Literature review has shown that TBT is acutely toxic to a variety of freshwater fish species at concentrations down to 10 μg litre^{-1} (Zabel *et al.*, 1988). TBT is particularly toxic to molluscs (oysters) and gastropods (Hall & Pinkney, 1985; Bryan *et al.*, 1986). The decline of dogwhelk populations on various coasts of France and the UK has been attributed to the occurence of TBT in these waters (Bryan *et al.*, 1986; Gibbs & Bryan, 1986). Chronic toxic effects on oysters in the form of shell deformation (Alzieu *et al.*, 1989) and marine gastropods in the form of sterilization of females have been reported occurring at aqueous TBT concentrations of a few nanograms per litre (Bryan *et al.*, 1986).

Many aquatic organisms have been shown to accumulate TBT to high levels in tissues. Tissue TBT concentrations ranging from sub μg g^{-1} to as high as 10 μg g^{-1} have been reported in benthic organisms (Langston *et al.*, 1987), cultivated scallops and Pacific oysters (Davies *et al.*, 1986), Atlantic salmon (Davies & McKie, 1987), mussels in freshwater marinas (Fent & Hunn, 1991) and oysters (Espourteille *et al.*, 1993). Bioconcentration factors from available laboratory and field studies show values ranging from a few thousand to 220000 in molluscs (Waldock & Thain, 1983; Bryan *et al.*, 1993; Kure & Depledge, 1994) while those for a fish species were reported in the order of 1000 in muscle and in the range from 20000 to 52000 in liver (Ward *et al.*, 1981).

Concentrations of TBT of the order of a few hundred ng litre^{-1} have been reported in coastal waters with heavy marine traffic, such as ports, marinas and dockyards, as compared to open water where TBT was found near or below the detection limits (10 ng litre^{-1} or less) for Europe and North America (Maguire, 1987; Waldock *et al.*, 1988; Alzieu *et al.*, 1989; Seligman *et al.*, 1989; Ritsema *et al.*, 1991) and Hong Kong (Lau, 1991). Unlike the methyltin compounds which may be formed naturally in the environment, TBT is exclusively of

anthropogenic origin. As such, its occurrence in the aquatic environment has been directly attributed to its application as an antifouling agent (Dowson *et al.*, 1994). TBT residues in the sediments of harbours, marinas and shipping channels were found to be considerably higher, typically in the range of about 200–1000 $\mu g\ kg^{-1}$ (Maguire, 1987; Matthias *et al.*, 1989; Quevauviller *et al.*, 1994).

Degradation of TBT to DBT and MBT is known to occur in the water column. However, controversies appeared in relation to its half-lives which have been reported in the range between 7–15 days (Seligman *et al.*, 1986) and several months (Maguire & Tkacz, 1985; Quevauviller & Donard, 1990). TBT in sediments is considered to be more persistent and the estimated half-life was in the range of 4–5.5 months (Stang & Seligman, 1986; Maguire & Tkacz, 1985). A recent report indicated that the half-life may be as long as several years (Sarradin *et al.*, 1991).

The first regulatory and legislative control of the use of TBT was adopted in France in 1982 followed by the UK in 1985. Most of the control measures introduced since then involved banning the use of TBT in marine paints for boats of less than 25 m length. For marine water, the UK adopted an Environmental Quality Target of 20 ng $litre^{-1}$ TBT in 1985 and an Environmental Quality Standard of 2 ng $litre^{-1}$ TBT was proposed in 1989 (Cleary, 1990). The US Environmental Protection Agency's proposed limit for TBT in fresh and marine waters were 26 ng $litre^{-1}$ (4-day average) and 10 ng $litre^{-1}$ (4-day average), respectively (Federal Register, 1989). The Canadian Council of the Ministers of Environment (CCME, 1992) derived an Interim Water Quality Guideline of 8 ng $litre^{-1}$ TBT in freshwater and a Water Quality Guideline of 1 ng $litre^{-1}$ in estuarine or seawaters for the protection of aquatic life.

At present, there is no specific legislation controlling the use of TBT in Malaysia. This is primarily due to the lack of supporting data on the occurrence and the impacts of TBT in the Malaysian environment. Very few reports are available on the studies of the impacts of TBT in tropical waters. The present paper reports the results of our study on the occurrence of TBT in the coastal waters, bivalves and sediment samples collected from a few selected sites in Peninsular Malaysia.

MATERIALS AND METHODS

Sampling and sample pretreatment

Coastal waters

Water samples were collected from surface water at a depth of approximately one metre from several coastal sites using a van Dorn water sampler (2-litre capacity). Fig. 1 shows the locations of the sampling sites, which include the coast of Selangor covering Port Klang and the Klang River estuary, the coast of Negeri Sembilan at Port Dickson, and Terengganu at Kemaman and Pulau Redang. A major part of the sampling programme was focused around the coastal waters of Port Klang which is the biggest container cargo port of Peninsular Malaysia. Samplings were carried out in four cruises from the period of 23 April to 30 July 1992. Scattered one-off samples were collected from other sites which covered selected points of interest from both the east and west coasts of the Peninsula during the period from November 1991 until May 1992.

Acid-washed amber borosilicate glass bottles (2-litre capacity) were used for the storage of the water samples. The water samples were acidified with 5 ml of 6 M HCl per litre of sample in the field. Upon return to the laboratory, the water samples were filtered through a 0.45-μm membrane filter and the filtrate stored in the dark 1-litre glass bottles at 4°C prior to analysis.

Biological tissues

Cockle and mussel samples (*Anadara granosa*, *Perna viridis* and *Paphia* sp.) which were harvested mainly from the coasts of Selangor were obtained from local marketplaces in Kuala Lumpur and Petaling Jaya. Field samples of cockles (*A. granosa*) were collected from the mudflats of Sungai Buloh during low tide. Another bivalve (*Perna* sp.) was sampled from the coastal areas of Jeram. The locations of these sampling sites are shown in Fig. 1.

The bivalve tissues were deshelled and homogenised with a blender. The finely homogenised tissues were kept in 100-ml wide-mouth bottles and stored frozen (−20°C) prior to analysis.

Sediments

Sediment samples were collected from different sites, including harbour and unexposed areas. The locations of the sampling sites are shown in Fig. 1. Grab sediment samples were collected during low tide from on-shore, except at sampling site S4 where the sediments were collected from approximately 20 m water depth with a Shipek sediment grab sampler. The sediment samples were stored in 100-ml wide-mouth bottles and stored frozen (−20°C) immediately upon return to the laboratory.

In the preparation of dried sediment samples, a portion of thawed sediment was dried in an oven maintained at 50°C. The dried sediment samples were ground using a porcelain mortar and pestle. The powdered sediment samples were passed through a 50-mesh (300-μm) brass sieve and stored in 100-ml wide mouth bottles in a vacuum desiccator.

Analysis of tributyltin

Seawater samples

The analytical method for the specific determination of TBT in seawater samples basically followed a similar approach to that reported by Apte and Gardner (1988). About 1 litre of filtered water sample was adjusted to pH 6 and then extracted with 50 ml of pentane. The solvent separated was backwashed with 3% w/v NaOH for the removal of mono- and di-alkyltins and inorganic

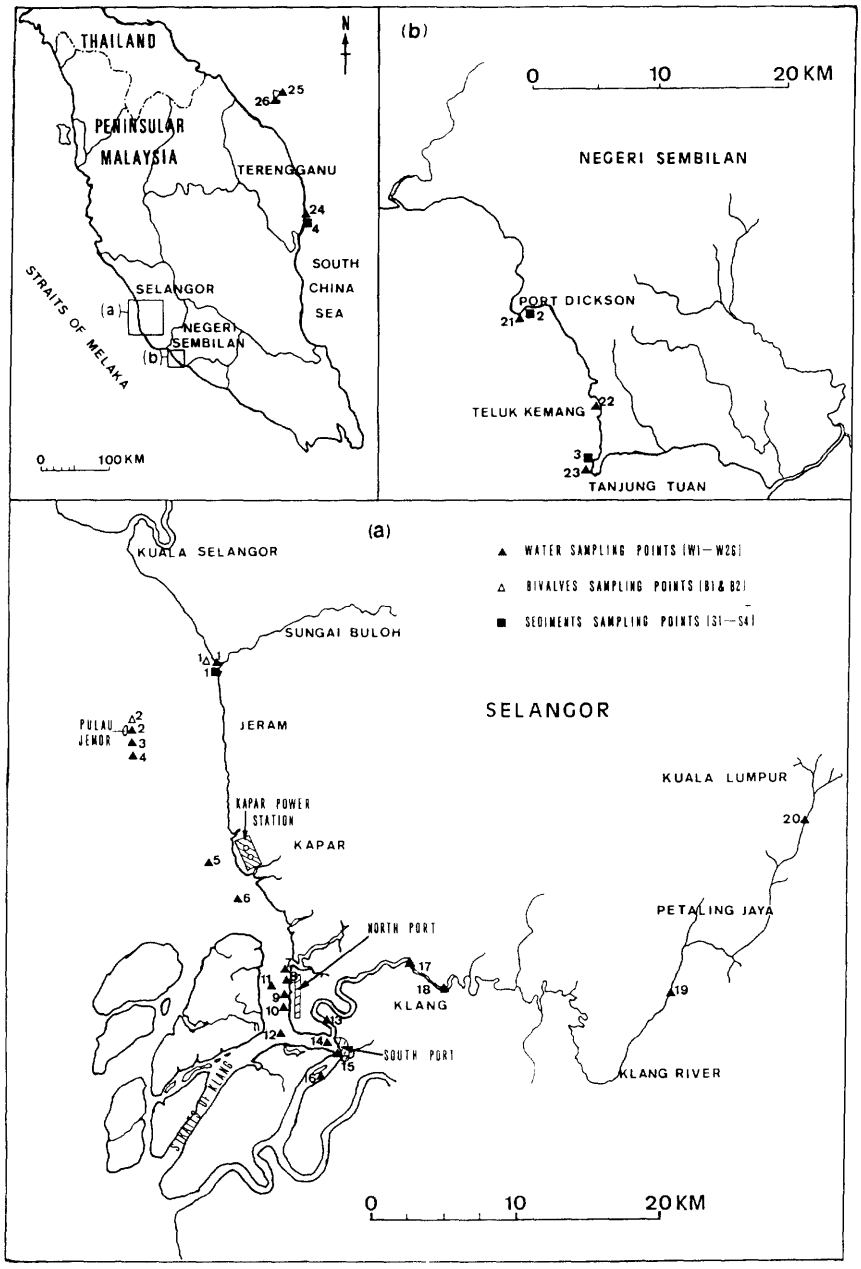

Fig. 1. Sampling sites of water, bivalves and sediment samples of Peninsular Malaysia.

tin. The separated organic layer was then evaporated to dryness at 46°C under a continuous stream of nitrogen. The residue was finally reconstituted with 2 ml of methanol-nitric acid (9:1) and the TBT Sn content was determined by graphite furnace atomic absorption spectrometry (GFAAS) using tungstate-coated graphite tube and pre-optimised operating conditions. The detection limit achieved was estimated to be 3.4 ng

litre^{-1} as TBT (or 1.4 ng litre $^{-1}$ as TBT–Sn), whereas recoveries from synthetic and spiked field samples were found in the range of 93.7 to 100.3% (Pang, 1993).

Triphenyltin (TPT) was found to be co-extracted up to about 80%, if present in the sample, although the backwash step would remove close to half of the extracted TPT. However, since TPT is used in relatively small proportions in antifouling marine paints (Apte & Gardner, 1988), the extraction procedure described has been considered as specific for TBT.

Bivalve tissue samples

Details of the procedure for the specific analysis of TBT in bivalve tissue samples have been published (Pang *et al.*, 1993). The method basically followed a similar approach to those reported by McKie (1987) and Stephenson and Smith (1988). The homogenised tissue was first dissolved in concentrated hydrochloric acid, extracted with pentane followed by an alkaline backwash for the same purpose as described above for water. This was followed by a clean-up step using a small C_{18}-solid phase extraction column for the removal of residual lipids and oils in the extracts. The TBT was recovered in methanol-nitric acid (9:1) mixture and subject to GFAAS measurement. The accuracy of the analytical methods developed have been checked using a certified reference biological tissue material (NIES-11, National Institute of Environmental Study of Japan). The results were well within the 95% confidence limit of the certified values. A detection limit of 0.5 ng g^{-1} for TBT (or 0.2 ng g^{-1} TBT–Sn) has been achieved.

Sediment samples

The procedure for the analysis of TBT in sediment samples has been described previously (Pang, 1993). Basically, the procedure is similar to that for the analysis of TBT in biological tissues. The sediment sample was equilibrated with glacial acetic acid prior to the extraction with pentane. The organic extract was then subject to an alkaline backwash. The organic extract was evaporated to dryness and the residue was taken up with 9:1 methanol–nitric acid and measured by GFAAS. The detection limit obtained was 0.7 ng g^{-1} as TBT (or 0.3 ng g^{-1} as TBT–Sn). Analysis of certified sediment reference PACS-1 (National Research Council of Canada) gave a value of 1.21 ± 0.04 μg g^{-1} TBT–Sn as compared to the certified value of 1.27 ± 0.22 μg g^{-1} TBT–Sn.

RESULTS AND DISCUSSION

Concentrations of TBT in water samples

The concentrations of TBT in the water samples analysed are summarised in Table 1. The concentrations of TBT found appeared to reflect the type of activities in these waters.

For most of the open water sites, TBT concentrations were in the range from below the detection limit (3.4 ng litre^{-1}) to about 14 ng litre^{-1} (W1, W3, W23, W25, W26). Some of these areas are public beaches or coastal fishing villages with only traditional wooden fishing vessels. No aquaculture farms or other apparent TBT sources are known at these sites.

Table 1. Concentration of TBT in water samples from Peninsular Malaysia

Site no.	Location	Type of site	Sampling date	TBT (ng litre^{-1})
W1	Sg. Buloh (river mouth)	Mudflats	5 December 1991	< 3.4
W2	Pulau Jemor	Open water	23 January 1992	100.4 ± 15.2
W3	Pulau Jemor	Open water	01 March 1992	10.8 ± 4.2
W4	Pulau Jemor	Open water	01 March 1992	26.6 ± 4.4
W5	Kapar Power Station	Coastal water	13 March 1992	23.0 ± 6.1
W6	Port Klang (North Port)	Channel entrance	April–July 1992	49.6 ± 23.9
W7	Port Klang (North Port)	Port area	April–July 1992	39.6 ± 22.5
W8	Port Klang (North Port)	Port area	April–July 1992	24.2 ± 12.5
W9	Port Klang (North Port)	Port area	April–July 1992	20.5 ± 5.4
W10	Port Klang (North Port)	Port area	April–July 1992	25.4 ± 8.8
W11	Port Klang (North Port)	Port area	April–July 1992	61.3 ± 27.9
W12	Straits of Klang	Port area	April–July 1992	48.6 ± 27.4
W13	Sg. Klang mouth (Yacht Club)	Estuarine water	April–July 1992	96.0 ± 56.7
W14	Port Klang (Jetty)	Jetty area	April–July 1992	281.8 ± 23.9
W15	Port Klang (South Port)	Port area	April–July 1992	53.0 ± 11.0
W16	Port Klang (West Port) (under construction)	Narrow channel	April–July 1992	57.9 ± 3.2
W17	500 m downstream from Kota Bridge	Estuarine water	23 April 1992	33.5 ± 4.2
W18	Kota Bridge (Klang Town)	Estuarine water	9 and 23 January 1992	23.5 ± 9.3
W19	Off 13th mile Puchong Road	River mouth	16 February 1992	22.2 ± 6.1
W20	Kuala Lumpur	River mouth	16 February 1992	24.2 ± 8.1
W21	Port Dickson	Jetty area	22 April 1992	48.4 ± 3.2
W22	Teluk Kemang	Public beaches	22 April 1992	24.2 ± 3.9
W23	Tanjung Tuan	Resort beaches	December 1991	< 3.4
W24	Kemaman Port	Port area	22 May 1992	62.1 ± 7.1
W25	Pulau Redang (beach)	Open water	15 March 1992	9.5 ± 5.9
W26	Pulau Redang (jetty)	Open water	14 March 1992	13.7 ± 4.6

A few sites which showed TBT concentration of about 24 ng litre^{-1} (W4, W5, W22) are located relatively close to potential TBT sources. A few freshwater samples from Klang River (W18–W20) as it passes through populated and industrialised urban areas also showed levels of about 24 ng litre^{-1}. Site W17 which showed slight elevation of TBT (33.5 ng litre^{-1}) is in the estuarine zone of the river.

A concentration of 100.4 ng litre^{-1} TBT was detected in seawater samples obtained from Pulau Jemor (W2) at the site of a mussel/aquaculture farm. The results indicated the use of TBT in the open water aquaculture farm. Water samples collected later from the surrounding waters of Pulau Jemor (within a radius of approximately 1000 m from the mussel farm; W3 and W4) showed lower TBT contents (10.8–26.6 ng litre^{-1}).

TBT was found at significant levels in seawater samples collected from the port areas, including those with narrow channels. A sample from a jetty site with small docking facilities at Port Dickson (W21) was found to contain 48.4 ng litre^{-1} of TBT. A harbour base at Kemaman, Terengganu (W24) was found with 62.1 ng litre^{-1} TBT in the seawater.

A group of 11 sampling sites (W6–W16) along the coastal waters of Port Klang had been investigated more intensively with four batches of water samples collected from April to July 1992. TBT concentrations measured were plotted in Fig. 2 for the 11 sites. The station mean levels of TBT found ranged from 20.5 to 281.8 ng litre^{-1}. Significant temporal variations were observed for most of these sites for samples taken over the sampling period probably because of the natural distribution resulting from tidal and current movements. However, the trends appear to indicate higher TBT levels around sampling sites W13/W14 and W6/W7. Decreasing TBT concentrations were observed generally for sampling sites away from these two sets of sites which could have been the two main sources of TBT in these waters.

As shown in Fig. 1, site W6 is located at the entrance of a narrow channel to these ports. W7–W12 are located at the wider channel into the North Port. Many commercial container/cargo ships are usually anchored around station W6 while waiting for their turn to berth at the North Port (W7–W12). The North Port handles the bulk of container freights to Malaysia. The South Port is only used for docking of smaller boats and ferries. The docking site of a yacht club where many pleasure crafts were moored is close to the South Port at the Klang River mouth. Sites W13–W16 are located at the narrower channel outside the South Port and the yacht club mooring site at Klang River mouth.

Among the sites shown in Fig. 2, the lowest levels of TBT–Sn were found at stations W8–W10, located at the wider channel to North Port where the station TBT mean levels ranged from 20.5 to 25.4 ng litre^{-1}. Station W13, located at the yacht club at the Klang River mouths showed a mean level of 96.0 ng litre^{-1} of TBT. The highest level of TBT of 281.8 ng litre^{-1} was oberved at station W14 and was probably attributable to the large number of passenger and leisure boats that were moored there. The poor water exchange between this site and the open sea, due to its narrow linking channel, will have probably exacerbated this situation. The station mean levels of TBT found at nearby sites, W15 (53.0 ng litre^{-1}) and W16 (57.9 ng litre^{-1}) were also significantly above background.

The levels of TBT measured above for Port Klang are in the same range as those reported by other workers for port areas. Ochsenkühn-Petropoulou *et al.* (1992) reported TBT levels of 71 and 64 ng litre^{-1}, respectively in seawater from two sites in the Saronikos Gulf in Greece. Harino *et al.* (1992) reported a level of 100 ng litre^{-1} TBT–Sn in seawater from a harbour area in

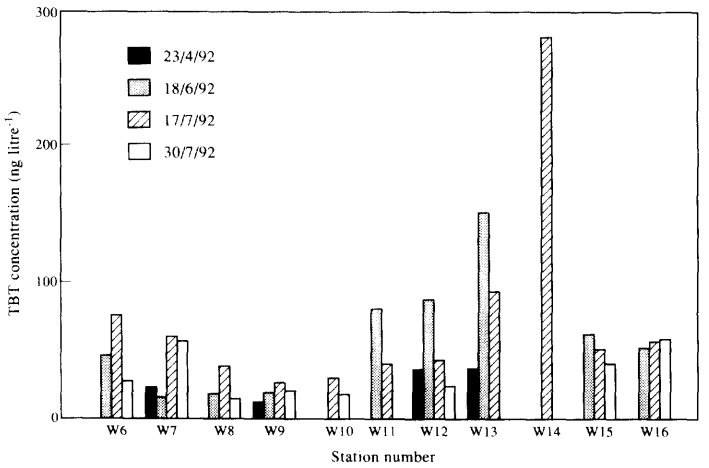

Fig. 2. Temporal variations of TBT concentrations in water samples from Port Klang coastal waters from four sampling cruises.

Table 2. Concentration of TBT in bivalves samples

Site no.	Samples	Common name	Sampling date	TBT (ng g^{-1} wet wt)
Market	*Anadara granosa*	Cockles	22 November 1991	<0.5 ($n = 3$)
Market	*Anadara granosa*	Cockles	29 November 1992	3.7 ± 1.5 ($n = 5$)
Market	*Paphia* sp.	Soft-shelled clams	04 December 1992	3.7 ± 2.0 ($n = 3$)
Market	*Perna viridis*	Green mussels	05 December 1992	23.5 ± 5.6 ($n = 2$)
B1(Sg. Buloh mudflats)	*Anadara granosa*	Cockles	05 December 1992	5.6 ± 1.0 ($n = 2$)
B2(Pulau Jemor)	*Perna* sp.	Green mussels	23 October 1992	14.2 ± 4.9 ($n = 2$)

Table 3. Concentration of TBT in coastal sediment samples

Site no.	Location	Sampling date	TBT (ng g^{-1} wet wt)
1	Sungai Buloh mudflats, Selangor	05 December 1991	29.3 ± 10.3 ($n = 5$)
S2	Jetty area, Port Dickson, Negeri Sembilan	22 April 1992	27.6 ± 5.1 ($n = 3$)
S3	Tanjung Tuan, Port Dickson, Negeri Sembilan	21 October 1991	<0.7 ($n = 2$)
S4	Port area, Kemaman, Terengganu	22 May 1992	216.5 ± 18.3 ($n = 2$)

Osaka, Japan. The southwest of Spain, which has busy shipping activities, shows TBT levels ranging from < 19 to 449 ng $litre^{-1}$ (Gomez-Ariza *et al.*, 1992).

Levels of TBT in bivalves samples

As shown in Table 2, TBT contents in cockles (*A. granosa*) and soft-shell clams (*Paphia* sp.) obtained from local markets were found near the detection limit of 0.5 to 3.7 ng g^{-1} wet weight. The levels of TBT found in green mussel samples from local markets (23.5 ng g^{-1} wet weight) and those from a mussel farm at Pulau Jeram (14.2 ng g^{-1} wet weight) indicate slight accumulation of TBT. The market-purchased green mussel is believed to be also from the coastal waters of Selangor. The levels of TBT found in the present study are significantly lower than those reported for areas with known TBT pollution. For comparison, TBT–Sn contents of 0.18 μg g^{-1} wet weight and 0.77 μg g^{-1} wet weight have been found in the tissues of oysters (*C. gigas*) and salmon (*Salmo salar*), respectively in the UK (Davies *et al.*, 1986; Davies & McKie, 1987). Levels of TBT ranging from 22 to 51 ng g^{-1} wet weight were reported in the tissues of butter clams and oysters (*C. gigas*) in Canada by Cullen *et al.* (1990). Pannier *et al.* (1994) recently reported TBT–Sn concentrations ranging from 112 to 146 ng g^{-1} wet weight in mussels (*Mytilus galloprovincialis*), scallop (*Pecten maximus*) and oyster (*Crassostrea gigas*) from the coasts of France.

Levels of TBT in sediment samples

The levels of TBT–Sn found in sediment samples collected from Tanjung Tuan, Port Dickson (S3) were below the detection limit (< 0.7 ng g^{-1} dry weight) of the analytical method. This site is a recreational beach area which is remote from any significant sources of TBT.

A TBT level of 29.3 ng g^{-1} dry weight was found in sediment samples from the Sungai Buloh mudflats (S1). The site is at the Sungai Buloh river mouth with a small fishing village and some industries located upstream.

Sediment samples collected from a jetty area (S2 at Port Dickson) and a port area in Kemaman, Terengganu Kelantan (S4) showed TBT levels of 27.6 ng g^{-1} and 216.5 ng g^{-1}, respectively. The high level of TBT at site S4 was apparently due to leaching of TBT from the hulls of mooring vessels or barges painted with TBT-based antifouling paints (Table 3).

In comparison, sediment TBT levels ranging from 50 to 1400 ng g^{-1} have been found in commercial marinas in Annapolis, Maryland in USA (Matthias *et al.*, 1989). Cai *et al.* (1994) recently reported a range of TBT–Sn from 1.7 to 460 ng g^{-1} in sediments of the Main River and Hamburg Harbour in Germany. Surface sediments from the Arcachon Harbour in France had TBT–Sn levels ranging from 17 to 596 ng g^{-1} (Quevauviller *et al.*, 1994).

Correlation between TBT in water column, sediments and bivalve tissues

Table 4 summarises the results of TBT found in the water column, sediment and bivalve tissue samples taken from the same sites. Sampling sites W1, S1 and B1 are located at a common site at a river mouth as shown in Fig. 1. TBT was not detectable in the water column probably because of the well mixing of the coastal waters here. Sediment and bivalve samples collected from the mudflats, however, show slight TBT contaminations. Other common sites of water column and sediments sampling, namely, W21 and S2, W23 and S3

Table 4. Relationship between TBT in water, sediments and bivalves

Water Column TBT (ng $litre^{-1}$)	Sediments TBT (ng g^{-1})	Bivlaves TBT (ng g^{-1})
3.4 (W1)	29.3 (S1)	5.6(B1)
100.4 (W2)	—	14.2(B2)
48.4 (W21)	27.6 (S2)	—
< 3.4 (W23)	< 0.7 (S3)	—
62.1 (W24)	216.5 (S4)	—

Parentheses indicate sampling sites.

and W24 and S4 show close correlation in their TBT concentrations. The common sites where water and bivalve samples were taken (W2 and B2) also show significant contaminations in both types of samples.

CONCLUSIONS

The results presented in this paper on the levels of TBT found in coastal waters in Peninsular Malaysia indicate that significant contamination of TBT has occurred in areas with dense shipping-related activities, apparently owing to the continuing use of TBT-based antifouling paints. However, more studies are required to further detail the distribution and fate of TBT in the coastal waters in Malasyia. There is also a need to assess the potential impacts arising from TBT contamination. This should include both field studies of the ecotoxicological effects of TBT in coastal waters and laboratory toxicity testing on local marine organisms.

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Appendix 8

Determination of Organotin Compounds in Environmental Samples by GC-PFPD

Marjorie LE GAC, Gaëtane LESPES, Martine POTIN-GAUTIER

Varian Application Note 02230 GC.

VARIAN

Determination of Organotin compounds in environmental samples by GC-PFPD.

Application Note
02230 GC

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Key Words : organotin compounds, speciation, GC-PFPD

Introduction

In the eighties, the organotins compounds were recognised for the first time as being responsible of very serious environmental contamination and the European Union has listed them as priority pollutants. Organotin compounds are used in a lot of industrial and agricultural applications including poly(vinyl chloride) (PVC) stabilisers, homogeneous catalysts and biocides formulations. So, their presence in the environment is due to anthropogenic uses mainly.

The toxic effects of organotins are widely dependent on the number and nature of alkylated or arylated groups bonded to the tin atom. Recently, they have been considered as possible endocrine disrupters [1]. Negative effects on environment can occur since sub ng*(Sn)* l^{-1} concentrations [2,3]. In these conditions, the development of analytical methods able to speciate and detect these compounds at low concentration levels in the different parts of the environment appears essential.

Recently, the GC-PFPD, newest member of the family of flame-based gas chromatographic detector has been developed for the detection of organotin compounds [4,5]. The PFPD operates in a pulsed-flame rather than continuous-flame mode and offers a number of significant improvements over the conventional flame photometric detector [6].

Experimental

Analytical procedure

The analytical procedure based on one-step ethylation-extraction using sodium tetraethylborate ($NaBEt_4$) followed by GC-PFPD is shown in Figure 1. The derivatisation step involves the ethylation of organotin in order to obtain thermally stable tetrasubstituted species, with preservation of their original speciation, sufficiently volatile for GC separation. Simultaneously, an extraction from the aqueous medium (i.e. water sample or aqueous acidic extract from solid samples) is performed on elliptic stirring table during 30 minutes. The ethylated compounds are extract in 500 µl of organic solvent (iso-octane).

For each sample, the chromatographic responses $K_{i/TPrT}$ of a butyltin (bi) and $K_{i/TDHepT}$ of a phenyltin (pi) relative to the internal standards (tripropyltin as TPrT and diheptyltin as DHepT) were calculated by standard additions.

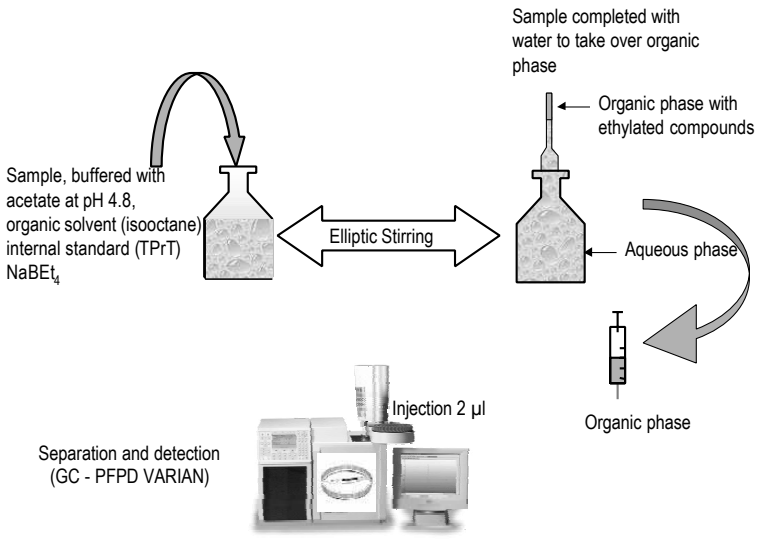

Figure 1: Schematic analytical procedure of the one-step ethylation-extraction followed by GC-PFPD.

Apparatus and conditions

Instruments	Varian 3800 gas chromatograph (GC) equipped with a pulsed flame photometric detector (PFPD) with a 1079 split/splitless injector. The Star Workstation controlled the GC-PFPD and AutoSampler and acquired data.
Column	30 m x 0.25 mm coated with 0.25 μm polydimethylsiloxane. Varian Equivalent VF-1ms, Part No. CP8912
Oven	80°C, 30 seconds, 10 °C/minute to 180 °C, 30°C/min to 270 °C, hold 3 minutes. Nitrogen is used as carrier gas
Injector	1079 at 290°C. Splitless mode : close split at 0.01 minutes, open at 45 seconds. 8200 Autosampler.
Detector	PFPD mode sulphur at 350°C, BG12 filter, Air 1 : 22 ml/min, Air 2 : 30 ml/min, H_2 : 25 ml/min, Gate Delay : 3msec, Gate Width : 2 msec
Conditions of injection	2 μl of iso-octane containing the analytes
Sample	A BCR 646 certified in butyl- and phenyltins A river water spiked with different organotin compounds, Analysis via GC as mono,di and tri-ethyl esters

Figure 2 shows a typical chromatogram obtained, in natural water, under the conditions described above. This method allows the 6 organotins to be analysed in less than 15 minutes.

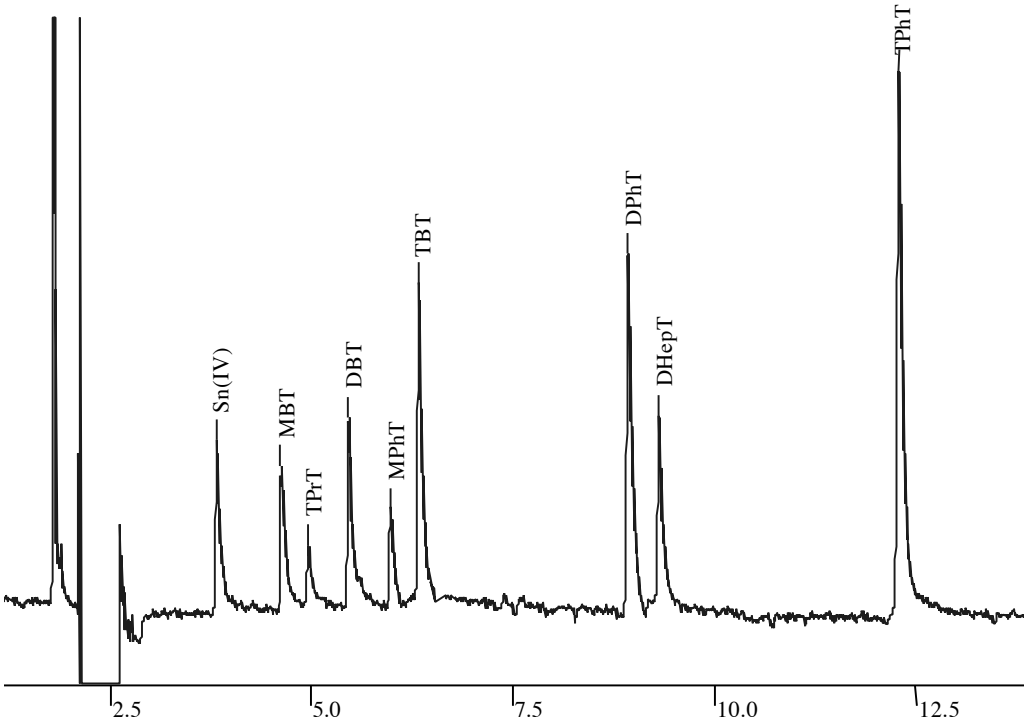

Figure 2: Typical chromatogram of spiked water at 50 ng(Sn)/lobtained by aqueous ethylation/GC-PFPD.

Results and discussion

Application

The method was applied to the analysis of fresh- and marine water, sediments, biological tissues (mussel, fish, oyster, aquatic plants…) and sewage sludge. Some applications are presented here (see Table 1) concerning a spiked river water and a certified reference material (CRM) the BCR 646, which is a river sediment certified for its butyl- and phenyltins contents. The spiked and found values appear to correlate in an interval of confidence of 95% (Student t-test). So, these analysis can be considered as conform and analytical method as accurate. Figure 3 shows calibration curves obtained in these conditions.

Table 1: Determination of organotin compounds in BCR-646 and in river water spiked with butyl- and phenyltins.

Compound	*BRC 646 (concentrations (ng(Sn) g^{-1} dry matter)*		*Spiked river water (ng(Sn) l^{-1})*	
	Certified	*Found values*	*Spiked (±10)*	*Found values*
MBT	411±80	457±57	145	147±21
DBT	392±46	342±18	120	130±3
TBT	196±33	183±3	180	194±30
MPhT	42±11	37±5		
DPhT	16±3	12±7	120	140±18
TPhT	10±4	10±1	180	243±58

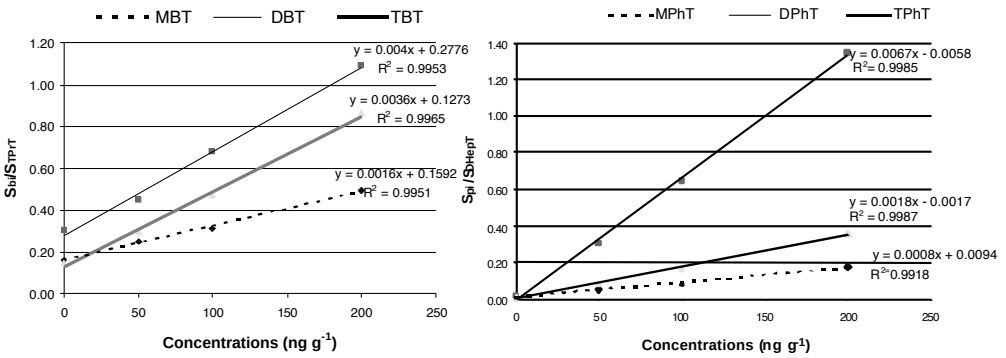

Figure 3: Calibration curve of organotin compounds in BCR-646.

Analytical performances

They were determined, according to the operating conditions described above, by using standard solutions and tripropyltin as internal standard. The limits of detection (LOD) were calculated according to AFNOR XP T 90-210 ($3\sigma/A$, with σ the standard deviation of baseline according to the retention time of the organotins compounds and A the sensitivity (slope) obtained from the corresponding calibration curves). The limits of quantification (LOQ) were calculated as 10 times the σ/A ratio. The LOD, LQ and the repeatability (relative standard deviation, RSD) are presented in Table 2.

Table 2: Detection and quantification limits of the organotin compounds in water and sediment.

Water sample	*MBT*	*DBT*	*TBT*	*MPhT*	*DPhT*	*TPhT*
L0D(ng(Sn) l^{-1})	0.3	0.2	0.3	0.6	0.1	0.1
LQ(ng(Sn) l^{-1})	1.0	0.8	0.8	1.9	0.4	0.4
RSD%*	5	5	5	5	3	7

*n=5, 5 ng(Sn) l^{-1}

Sediment	*MBT*	*DBT*	*TBT*	*MPhT*	*DPhT*	*TPhT*
L0D(ng(Sn) g^{-1})	0.9	0.8	0.8	1.0	0.4	0.4
LQ(ng(Sn) g^{-1})	2.9	2.6	2.7	3.3	1.2	1.4
RSD%*	2	4	9	9	7	8

*n=5, 5 ng(Sn) g^{-1}

The LOD (between 0.1 and 0.6 ng(Sn) l^1 for water and 0.4 and 1 ng(Sn) g^{-1} for sediment) obtained are in agreement with the concentration levels that should be determined in environmental samples, according to the ISO project concerning organotin speciation in water [7]. The repeatability appears also to be satisfactory for a reliable quantitative analysis.

Conclusion

The determination of organotins by ethylation/extraction followed by the GC-PFPD has been demonstrated to be an efficient, rapid and simple technique to analyse organotins. Low limits of detection (sub 1 ng*(Sn)* l^{-1} or g^{-1} for most of the species) can be reached. The application made on CRM have demonstrated the reliability of the analysis.

References

[1] Decision No 2455/2001/EC of the European Parliament and of the Council of 20 November 2001 establishing the list of priority substances in the field of water policy and amending Directive 2000/60/EC (Text with EEA relevance) *Official Journal L 331 , 15/12/2001 P. 0001 - 0005*

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[6] Jacobsen J., Stuer-Lauridsen F., Pritzl G., Organotin speciation in environmental samples by capillary gas chromatography and pulsed flame photometric detection (PFPD), *Applied Organometallic Chemistry, 11 (1997) 737-741.*

[7] ISO/TC 147/SC 2N 0569 -Water quality- Determination of selected organotin compounds-Gas chromatographic method (2002).

Appendix 9

QUASIMEME Laboratory Performance Studies Scheme

(2007-2008)

About QUASIMEME

Background

Between 1993 and 1995 the European Union (EU) supported the Quasimeme project which had the aim to develop a holistic quality assurance program for marine environmental monitoring information in Europe. As a result of this pioneering project a marine network and laboratory performance studies have been established for most of the determinants measured in the marine environmental programs for both monitoring and research purposes. The EU funded Quasimeme project demonstrated that laboratories which followed on a regular basis the learning programs and the laboratory testing schemes improved the quality of their data. The information exchange encouraged by this scheme and the opportunity to verify the laboratory performance was clearly of significant value to those who participated. The advantage of this initial project was that the studies were centrally financed. However, this approach only enabled a limited number of institutes to benefit from this support. After the end of the EU funding in 1995, the Quasimeme scheme continued on subscription basis. Now it is thus possible for any laboratory to participate.
Since 1996 the subscription scheme has included the existing matrix-determinant combinations from the EU QUASIMEME 93-95 programme with additional measurements as needed for national and international marine QA programmes. New determinants are added if there is a demand for them. In addition, workshops and development exercises with the framework of the scheme are organized.

The Quasimeme project has been hosted from its inception in 1993 to April 1, 2005, by the Fisheries Research Institute in Aberdeen, United Kingdom. Wageningen University and Research (WUR) has taken over the responsibility for the Quasimeme project on April 1, 2005. Three groups of WUR cooperate: the Centre for Water and Climate of Alterra, the Netherlands Institute for Fisheries Research (RIVO), and the Wageningen Evaluating Programmes for Analytical Laboratories the Soil Chemistry and Chemical Soil Quality group. Alterra acts as coordinator. In the periode between April 1, 2005 and April 1, 2006, the Fisheries Research Institute in Aberdeen cooperates so as to ensure a smooth transition of the scheme from the Aberdeen, United Kingdom to Wageningen, the Netherlands.

The Name

The acronym QUASIMEME was used during the EU project as the "Quality Assurance of Information for Marine Environmental Monitoring in Europe". The project has kept this acronym, however the studies are available worldwide to all scientists making chemical measurements and not just those which analyse for statutory monitoring programmes.

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The QUASIMEME Laboratory Performance Studies. Year 12 June 2007 to May 2008

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TABLE OF CONTENTS

1. What is QUASIMEME?

QUASIMEME (Quality Assurance of Information in Marine Environmental Monitoring) was founded in 1992. The project was initiated with EU funding (1992-1996) and continued by subscription of the participating institutes. QUASIMEME was co-ordinated by the QUASIMEME Project Office, FRS Marine Laboratory, Aberdeen, United Kingdom until 2005. In 2005 the co-ordination transferred from FRS to Wageningen University and Research Centre, Wageningen, The Netherlands. The QUASIMEME Project Office operates under the guidelines provided in the ISO / IEC guide 43-1: 1996 (E)[1] for the development and operation of proficiency testing schemes and in the Guidelines for the Requirements for the Competence of Providers of Proficiency Testing Schemes: ILAC-G13: 2000[2]. Annex I lists the roles and responsibilities of the QUASIMEME Project Office staff.

QUASIMEME is more than a proficiency-testing scheme. At the heart of the project is a holistic *learn-by-doing* spiral. The routine laboratory performance studies provide the basis of external quality assurance for institutes that make regular chemical measurements in the marine environment. Most studies have two rounds per annum with a minimum of two test materials containing the analytes at different concentrations. The output from these studies is reviewed annually by the QUASIMEME Scientific Assessment Group, which is comprised of experts in each of the main areas of the QUASIMEME Laboratory Performance (LP) studies. Further information relating to the membership and terms of reference for the Scientific Assessment Group is given in Annex I.

As a result of the review it is possible to identify areas of poor performance, which would benefit from a more detailed scrutiny. An improvement programme may be initiated through a workshop run at an institute with sound expertise followed by a series of development exercises to provide detailed tuition and information, with a range of test materials tailored to the specific needs of the problem.

The QUASIMEME LP studies provide external quality assurance (QA) for national and/or international monitoring programmes, individual or collaborative research and for contract studies. The QUASIMEME LP studies support quality management and quality measurement in the participating laboratories.

Participants may use the assessment of the study data to:

- Validate internal laboratory QA
- Support accreditation
- Support QA of environmental monitoring data
- Provide data for national or international programmes

QUASIMEME collaborates with the following organisations:

- Helsinki Commission (HELCOM)
- Oslo and Paris Commission (OSPAR)
- Mediterranean Pollution Monitoring and Research Programme (MEDPOL) - Barcelona Convention
- Arctic Monitoring and Assessment Programme (AMAP)
- International Council for the Exploration of the Seas (ICES)
- European Environment Agency (EEA)
- National Marine Monitoring Programmes of member countries

These organisations are represented on the QUASIMEME Advisory Board. Further information relating to the membership and terms of reference for the Advisory Board is given in Annex I.

The QUASIMEME programme is updated annually and made available to all current and former participants and to third parties that have a close interest in the project and its outcome e.g. OSPAR, HELCOM, MEDPOL and ICES.

[1] ISO / IEC guide 43-1. (1996). (E) Proficiency testing by interlaboratory comparisons - Part 1. Development and operation of proficiency testing schemes. Part 2 Selection and use of proficiency testing schemes by laboratory accreditation bodies.
[2] International Laboratory AccreditationCooperation (ILAC) *Guidelines for the Requirements for the Competence of Proficiency Testing Schemes*. ILAC-G13: 2000.

1.1 Participation

Participation in the QUASIMEME Laboratory Performance studies is open to all institutes and companies world-wide that make chemical measurements in seawater, sediment and biological materials, and require external quality assurance.

The application form to participate in the 2007-2008 programme can be found in section 4.2 of this document as well as on the website www.Quasimeme.org.

A guide to the expected number of participants for each study (round) is taken from the previous year. Normally the minimum number of participants for any study is 10. Where QUASIMEME offer a new type of test material or determinand group, and the number of participants is less than 10, then the study will be cancelled. The project office will determine, on case by case basis, what to do when an existing study has less than 10 participants. Important considerations are costs and the possibilities to establish reliable assigned values and thus meaningful z-scores. Where a study is cancelled, participants will be notified and no costs will be incurred.

1.2 Programme for June 2007 to May 2008

The content of the current scheme is given on pages 10 to 29. Most Laboratory Performance (LP) studies are conducted twice per annum, with a minimum of two test materials per study. The QUASIMEME LP studies routinely includes the following test materials, containing determinands at concentrations similar to those found in estuarine, coastal and open water environments:

Seawater, estuarine and low salinity open water:

Nutrients, trace metals, halogenated organics, volatile organochlorines, pentachlorophenol, organophosphorus pesticides and triazine herbicides, chlorophyll *a,* organotins and PAHs.

Sediment and biological matrices:

Trace metals, PCBs, organochlorine pesticides, PCDDs and PCDFs, PAHs, chlorobornanes (toxaphene), shellfish toxins (Amnesic shellfish poisoning toxins – domoic and epidomoic acid), Brominated flame-retardants and organotins in biota and sediment.

Biological effects measurements:

Imposex and intersex in marine snails.

Development exercises:

Shellfish toxins (Diarrhetic shellfish poisoning toxins – okadaic acid group), Alkylphenols in either seawater and sediment and passive sampling.

Further information on test material preparation, concentration ranges, analytical testing and test material distribution is given on pages 10 to 29.

1.3 QUASIMEME Collaborators

QUASIMEME has a number of collaborators who prepare and provide test materials for the Laboratory Performance (LP) studies, and who analyse these test materials for homogeneity and, where appropriate, stability. All collaborators are experts in their particular field and operate to a traceable standard, which can be audited. This may include:

- Accreditation to a standard acceptable to ILAC e.g. ISO 17025, G13: 2000, ISO 9000 series.
- National reference laboratory.
- Documented evidence of the quality of the test materials provided.

A list of all QUASIMEME collaborators and their role in the provision and testing of materials for the LP studies is given in Annex I.

1.4 Subscription

QUASIMEME is non-profit making and is funded by the participating laboratories. All materials and services are provided at cost. Details of the costs are given on page 31.

The subscription includes:

- Two (or more) test materials for each group delivered to your laboratory mostly twice per year.
- A protocol for each study, which includes information on the analyses required, the timescale for analysis and reporting of the data. This will be provided electronically.
- QUEST Electronic Storage & Transfer system for QA data, to be used with Windows 9x, 2000, NT or XP.
- Assessment and confidential report of performance (data and z-scores) provided as hard copy.
- Electronic data return of each assessment for inclusion in participants' individual QUEST database of performance.
- LP study summary report, provided electronically on the sharepointsite.
- Electronic QUASIMEME study report covers to enable participants to prepare their own paper copies of reports when required.
- Provision of a help desk.
- Access to Quasimeme website and sharepointsite.
- QUASIMEME publications and newsletters.
- Development exercises operated in conjunction with expert laboratories, usually involving one round per year, often accompanied by a workshop.
- Invitation to QUASIMEME workshops, and preferential reduced registration fee.
- Use of excess test materials as a laboratory reference material[3].

QUASIMEME organises specialised workshops in support of the routine and development exercises, in addition to more general conferences. Participants pay for their own travel and accommodation, and for most of the workshops there is a registration fee to cover organisational expenses.

1.5 Timetable

The timetable for the 2007 – 2008 programme is given on page 10. The frequency which the test materials are distributed for each group of determinands is given in section 2.2.2.

1.6 Methods and Procedures

Participants should use their normal validated methods and procedures to analyse the test materials.

Method codes are provided, in the form of a tick list, which cover sample preparation through to sample detection. Participants are requested to complete the method code tick list. The method codes are collated and included in the LP study reports. This allows participants to review the range and similarity of the methodologies used. As part of the new database to be used in 2007-2008, QUASIMEME plan to update and integrate these method codes more interactively, and therefore provide a more in depth assessment relating to the different methodologies used.

1.7 Assessment

Each study is fully assessed using the Cofino Model[4]. All data provided at the time of the assessment, including extreme values and left censored values (LCVs)[5] are used to establish the consensus value.

[3] QUASIMEME supply sufficient quantities of the test materials for each study. Excess test materials can subsequently be used as LRMs with a known assigned value and uncertainty obtained from the QUASIMEME reports.

[4] Cofino, W.P., Wells, D.E., Ariese, F., van Stokkum, I, Wengener, J. W. and Peerboom, R., *J. Chemometrics and Intelligent Laboratory Systems,* **53**, (2000) 37-55; Cofino, W. P., van Stokkum, I.H.M., van Steenwijk, J., and Wells, D E. *Anal. Chim. Acta* (2004) (in press); Wells, D.E., Cofino, W.P. and Scurfield, J. A. *FRS Marine Laboratory, Aberdeen, Collaborative Report* (2004)

[5] Left Censored Values is the correct nomenclature for *ìless thanî* values

At the end of the assessment the consensus value is known as the assigned value. In the assessment a z-score (bias)[6, 7] is used to normalise the data and provide an assessment for each participating institute and a comparison of performance between institutes and studies.

Details of the formulae used to calculate the z-scores are given in Annex II. The constant and proportional errors used to calculate the z-scores, have been established by the QUASIMEME Scientific Assessment Group and are given for each determinand on pages 13 to 28.

Information on the use of the Cofino Model and the assessment rules used for the evaluation of the QUASIMEME Laboratory Performance studies data can be downloaded from the Quasimeme website www.Quasimeme.org.

1.8 Confidentiality and Data Submission to Third Parties

QUASIMEME operates a fully confidential service to all participants. The results remain the property of each participant and full confidentiality is maintained. No information on individual participants' performance is disclosed to any third party.

QUASIMEME will provide each participant with a unique code for each round of the Laboratory Performance (LP) studies. These codes will be used only once, and will randomly change with each round.

QUASIMEME will publish the evaluation and overview of the LP studies in peer review journals, maintaining confidentiality. All data, however presented, will be non-attributable. The codes described above will be the only codes used in publications.

The data generated by participants is valuable to the national and / or international organisations that collate and assess environmental data for the chemical determinands analysed in the QUASIMEME LP studies. QUASIMEME encourages all participants to submit their QA data, including their LP studies results, in the submission of environmental information to the national and / or international marine monitoring programmes. QA data submission to any third party, including submission of LP studies data, is the responsibility of the individual institutes. The assessment files, in text, ASCII, html and QUEST formats, will be provided electronically after the completion of each LP study.

[6] International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories. M Thompson, R Wood, Journal of AOAC International Vol. 76, No. 4, 1993
[7] The formulae used in calculation of the z-scores are given in Annex II

2. Programme for June 2007 to May 2008

QUASIMEME follows an annual timetable as given below, from June to May of the following year, with four rounds (distributions) each year. The time between each round is approximately three months with four months to report the data. This timetable allows all participants to incorporate the test materials into their ongoing analytical programme. This is particularly important for those participants who need to undertake their QA analysis alongside their environmental samples in the laboratory or at sea. The timetable is given in this scheme and a reminder, in the form of a newsletter, is sent to participants prior to the start of each round.

The deadlines for submission of data are fixed. Any data received **after** the deadline may not be included in the assessment. A confidential individual laboratory report, the full study report and the electronic summary files will be provided within two months of the deadline for the submission of data. These reports and summary files will also be provided for data received after the report is issued, but the individual laboratory report will include the statement, "Data received after the report was issued."

2.1 Timetable

Round	Start date [8]	Deadline	Report available
50	July 1, 2007	October 30, 2007	December 30, 2007
51	October 1, 2007	January 30, 2008	March 30, 2008
52	January 1, 2008	April 30, 2008	June 30, 2008
53	April 1, 2008	July 30, 2008	September 30, 2008

2.2 Content of the Studies

2.2.1 New for 2007 ñ 2008

More compounds from Water Framework Directive (WFD) list in the Quasimeme scheme.
Following the Water Framework Directive of the European Parliament and of the council on environmental quality standards in the field of water policy, the Quasimeme project office decided to add more determinands into the Laboratory Performance Study Program. Pentabromodiphenylether, pentachlorobenzene, aldrin, endrin, and isodrin are added to the organochlorine exercise (AQ-5) and as a result the name of this determinand group is changed into halogenated organics. Dichloromethane and trichloromethane are both added to the volatile organochlorine group (AQ-6). The exercise AQ-7 (pentachlorophenol) will be offered again in the new program. Alachlor, chlorpyrifos and isoproturon will be added to the AQ-8 exercise. Organotins in seawater was already changed from a development exercise into a regular exercise (AQ-12), on request of the participants of the Roskilde workshop (March 2006). For PAHs a new exercise will be started (AQ-13) . For alkylphenols in water and sediment development exercises will be started (DE-11 and DE-12, respectively). Finally, a development exercise will be started on passive sampling (DE-13).

Sediment and biota
The development exercise for organotins in sediment (DE-7) and in biota (DE-3) are both changed into regular exercises (MS-6 and BT-8 respectively).

Low salinity water
In both rounds for AQ-2 (round 50 & 52) low salinity water collected from the Baltic sea will be used in stead of simulating low salinity water by diluting seawater from e.g. the North Sea with demineralised water.

[8] The start date is an indication of the beginning of the round. Test materials will be dispatched within a three-week window of this date. The QUASIMEME Project Office will notify all participants of the **exact** date of dispatch in the quarterly newsletter, which will be issued at least three weeks prior to the start date.

2.2.2 Laboratory Performance Study Programme

Round	Group Number	Number of test materials per study	Determinand Group	Matrix
AQUEOUS				
50 & 52	AQ-1	3	Nutrients	Seawater
50 & 52	AQ-2	4	Nutrients (low salinity, 2 high & 2 low concentration)	Estuarine and low salinity open water
51 & 53	AQ-3	3	Metals, other than Mercury (included 1 low salinity test material)	Seawater
51 & 53	AQ-4	3	Mercury	Seawater
53	AQ-5	3	Halogenated organics (included 1 low salinity test material)	Seawater
53	AQ-6	2	Volatile organochlorines	Seawater
53	AQ-7	3	Pentachlorophenol	Seawater
53	AQ-8	3	Triazines & organophosphorus pesticides (included 1 low salinity test material)	Seawater
51 & 53	AQ-11	2	Chlorophyll *a*	Filtered seawater
53	AQ-12	3	Organotins	Seawater
52	AQ-13	3	PAHs	Solution & seawater
SEDIMENTS				
50 & 52	MS-1	2	Trace metals	Sediment
50 & 52	MS-2	2	Chlorinated organics	Sediment
50 & 52	MS-3	2	PAHs	Sediment
50 & 52	MS-6	2	Organotins	Sediment
BIOTA - Stabilised wet biological tissue				
50 & 52	BT-1	2	Trace metals	Fish & shellfish
50 & 52	BT-2	2	Chlorinated organics	Fish & shellfish
50	BT-3	2	Non *ortho* CBs, PCDDs and PCDFs	Fish & shellfish
50 & 52	BT-4	2	PAHs	Shellfish
50	BT-5	2	Chlorobornanes (toxaphene)	Wet tissues & fish oil
51 & 53	BT-7	2	Shellfish toxins (ASP)	Solution & biota
50 & 52	BT-8	2	Organotins	biota
53 (june)	BE-1	2 species	Imposex and intersex in marine snails	Marine snails
BIOTA - Stabilised wet biological tissue and sediment				
50 & 52	BS-1	2	Brominated flame retardants	Fish or shellfish and sediment
DEVELOPMENT EXERCISES				
51 & 53	DE-10	4	Shellfish toxins (DSP)	Solution & biota
53	DE-11	3	Alkylphenols in seawater	Solution & seawater
53	DE-12	3	Alkylphenols in sediment	Solution & sediment
52	DE-13	3	Passive sampling	Solution & passive sampling strips

3 Test Materials.

3.1 Introduction

The minimum and maximum concentrations given in the tables below are indicative of the typical ranges, and reflect the values in test materials used over the past two years. However, there are test materials where the concentration of a determinand may be outside these values. These would be atypical of the normal range of test materials. The constant and proportional errors have been agreed by the Scientific Assessment Group, and are used by QUASIMEME in the calculation of the z-scores used in the data assessment (Annex II).

QUASIMEME has set clear guidelines on the boundaries of the uncertainty of the assigned value. When the allowable target error exceeds 50% of the assigned value, then the assigned value is set to be indicative. However, there have been occasions where the assigned value has been indicative, primarily as a function of the magnitude of the constant error, rather than the performance of the laboratories. Where there is a history of this occurring for a particular determinand, the constant error will be reduced by the Scientific Assessment Group to provide an assigned value.

The constant errors given for the determinand groups on pages 13 to 28 are the numbers as they are on date of issue of this scheme. If there are any changes throughout the year this will be announced on the Quasimeme website.

3.2 Aqueous Test Materials

3.2.1 Nutrients

Two groups are offered for the determination of nutrients: AQ-1, nutrients in seawater and AQ-2, nutrients in estuarine water and low salinity open water. The test materials are prepared in bulk, following the well-defined methods of A. Aminot and R. Kerouel (1991, 1995)[9].

Low nutrient seawater (LNSW), collected from the Eastern Atlantic Ocean during the late spring and summer months after the main plankton bloom, is used to prepare the test materials. LNSW is filtered to remove bacteria and other particles. The estuarine water test materials are prepared by diluting the filtered LNSW with ultrapure demineralised water to the required salinity. The LNSW and diluted LNSW is then adjusted to pH ~ 7.2 using 0.1M hydrochloric acid. The low salinity water is sampled in the Baltic sea. Standard solutions of known concentrations are prepared and these are used to spike the LNSW, diluted LNSW and Baltic seawater. The bulk material is mixed thoroughly and dispensed into clean bottles. The test materials for ammonia, total oxidised nitrogen (TOxN), nitrite and total nitrogen (Total N) are dispensed into 250 ml glass bottles. The test materials for phosphate, silicate and total phosphorus (Total P) are dispensed into 250 ml plastic bottles. The dispensed material is sterilised by autoclaving.

Homogeneity testing is performed on each batch of test materials produced. The nutrient test materials are stable for the period of the test, and have also been shown to be stable for a period of some months, even after opening, if used under the correct conditions i.e. storage in a refrigerator when not being used.

[9] Aminot, A. & Kerouel, R. 1995. Reference material for nutrients in seawater: stability of nitrate, nitrite, ammonia and phosphate in autoclaved samples. *Marine Chemistry* **49**, pp.221-232, Aminot, A. & Kerouel, R. 1991. Autoclaved seawater as a reference material for the determination of nitrate and phosphate in seawater. *Analytical Chimica Acta* **248**, pp.277-283.

AQ-1 Nutrients in seawater

(Salinity > 30 psu)				
Determinands	***Units***	**Indication of Concentration Range**	**Constant Error**	**Proportional Error %**
Ammonia	µM	0.2 - 5	0.1	6
TOxN	µM	0.05 - 15	0.05	6
Nitrite	µM	0.01 - 2	0.01	6
Phosphate	µM	0.05 - 5	0.05	6
Silicate	µM	0.5 - 10	0.1	6
Total N	µM	5 - 25	0.5	6
Total P	µM	0.1 - 5	0.05	6

Data-assessment for unspiked samples will be carried out by calculating with a proportional error of 12.5%

AQ-2 Nutrients in estuarine water and low salinity open water

The AQ-2 studies include both estuarine water test materials and low salinity open water test materials. The same constant errors and proportional errors are used for both types of test material. The different concentration ranges for the estuarine water and low salinity open water test materials reflect naturally occurring concentrations. Salinity is an indicative measurement in support of the methodology.

Estuarine water (Salinity 8 - 10 psu)				
Determinands	**Units**	**Indication of Concentration Range**	**Constant Error**	**Proportional Error %**
Ammonia	µM	2 – 50	0.1	6
TOxN	µM	10 - 100	0.05	6
Nitrite	µM	0.5 - 25	0.01	6
Phosphate	µM	1 – 15	0.05	6
Silicate	µM	5 – 100	0.1	6
Total N	µM	10 - 200	0.5	6
Total P	µM	1 – 20	0.05	6
Salinity	psu		0.001	0.1

Low salinity open water (Salinity 10 – 15 psu)				
Determinands	**Units**	**Indication of Concentration Range**	**Constant Error**	**Proportional Error %**
Ammonia	µM	0.2 – 5	0.1	6
ToxN	µM	0.05 – 15	0.05	6
Nitrite	µM	0.01 – 2	0.01	6
Phosphate	µM	0.02 – 5	0.05	6
Silicate	µM	0.5 – 20	0.1	6
Total N	µM	2 – 20	0.5	6
Total P	µM	0.02 – 2	0.05	6
Salinity	psu		0.001	0.1

Data-assessment for unspiked samples will be carried out by calculating with a proportional error of 12.5%

3.2.2 Trace Metals

Two groups are offered for the determination of trace metals: AQ-3, trace metals in seawater and AQ-4 mercury in seawater. Mercury in seawater is offered as a separate determinand as it is necessary to prepare and ship this element separately. A minimum of two full salinity seawater test materials is provided for both AQ-3 and AQ-4: one of which is an unspiked material. A lower salinity, higher trace metal concentration seawater is also provided for AQ-3.

The seawater used to prepare these test materials is collected from the Eastern Atlantic Ocean and is filtered to remove bacteria and other particles. The low salinity test materials are prepared by diluting the filtered seawater with ultrapure demineralised water to the required salinity. 2 ml trace metal grade nitric acid per 1 litre seawater is added to preserve the test materials. Standard solutions of known concentrations are prepared and these are used to spike the seawater and diluted seawater. The bulk material is mixed thoroughly and dispensed into clean bottles. The bottles are sealed in plastic bags to prevent contamination from dust.

The homogeneity of the aqueous trace metals test materials is assumed, as the materials are prepared in bulk and thoroughly mixed.

AQ-3 Trace metals in seawater

The AQ-3 studies include both full salinity seawater test materials and low salinity test materials. The same constant errors and proportional errors are used for both types of test material. The concentration ranges differ for the full salinity and low salinity test materials.

Full salinity test materials (Salinity > 30 psu)				
Determinands	**Units**	**Indication of Concentration Range**	**Constant Error**	**Proportional Error %**
Arsenic	µg/l	0.5 – 10	0.5	12.5
Boron	µg/l	1000 – 5000	0.4	12.5
Cadmium	µg/l	0.01 – 1	0.005	12.5
Chromium	µg/l	0.1 – 5	0.1	12.5
Cobalt	µg/l	0.01 – 1	0.2	12.5
Copper	µg/l	0.5 – 10	0.2	12.5
Iron	µg/l	0.5 – 10	0.4	12.5
Lead	µg/l	0.02 – 2	0.01	12.5
Manganese	µg/l	0.2 – 5	0.4	12.5
Nickel	µg/l	0.2 – 5	0.2	12.5
Silver	µg/l	0.2 – 2	0.2	12.5
Tin	µg/l	0.2 – 2	0.2	12.5
Vanadium	µg/l	1 – 5	0.2	12.5
Zinc	µg/l	0.5 – 10	0.4	12.5

Low salinity test materials (Salinity ca. 15 psu)				
Determinands	**Units**	**Indication of Concentration Range**	**Constant Error**	**Proportional Error %**
Arsenic	µg/l	2 – 20	0.5	12.5
Boron	µg/l	200 – 5000	0.4	12.5
Cadmium	µg/l	0.5 – 5	0.005	12.5
Chromium	µg/l	5 – 20	0.1	12.5
Cobalt	µg/l	0.1 – 10	0.2	12.5
Copper	µg/l	2 – 10	0.2	12.5
Iron	µg/l	2 – 10	0.4	12.5
Lead	µg/l	1 – 5	0.01	12.5
Manganese	µg/l	1 – 5	0.4	12.5
Nickel	µg/l	1 – 5	0.2	12.5
Silver	µg/l	1 – 5	0.2	12.5
Tin	µg/l	1 – 10	0.2	12.5
Vanadium	µg/l	2 – 10	0.2	12.5
Zinc	µg/l	2 – 20	0.4	12.5

AQ-4 Mercury in seawater

(Salinity > 30 psu)				
Determinands	**Units**	**Indication of Concentration Range**	**Constant Error**	**Proportional Error %**
Mercury	ng/l	0.5 – 30	0.2	12.5

3.2.3 Organic Compounds

The aqueous organic test materials are divided into four groups: AQ-5 halogenated organic compounds, AQ-6 chlorinated volatile organic compounds and AQ-8 triazines and organophosphorus compounds.

AQ-5 Halogenated Organics in seawater

The AQ-5 studies include both full salinity seawater test materials and low salinity test materials. The same constant errors and proportional errors are used for both types of test material. The concentration ranges differ for the full salinity and low salinity test materials.

The seawater is collected from the Eastern Atlantic Ocean and filtered to remove bacteria and other particles. The low salinity test materials are prepared by diluting the filtered seawater with ultrapure demineralised water to the required salinity. The filtered seawater is dispensed into 1 litre glass bottles. Composite solutions containing the halogenated organic compounds are prepared in methanol. These are used to spike the individual bottles of seawater.

The test materials are assumed to be homogeneous, as each batch is prepared in bulk, thoroughly mixed and spiked to the same concentration level. The test materials are stable for the purposes of the study.

Full salinity test materials (Salinity > 30 psu)				
Determinands	**Units**	**Indication of Concentration Range**	**Constant Error**	**Proportional Error %**
Aldrin	ng/l	1 – 20	0.5	12.5
pp' DDD	ng/l	0.1 - 10	0.5	12.5
pp' DDE	ng/l	0.2 - 10	0.5	12.5
op' DDT	ng/l	0.2 - 20	0.5	12.5
pp' DDT	ng/l	0.2 - 20	0.5	12.5
Dieldrin	ng/l	1 - 20	0.5	12.5
Endosulphan I	ng/l	0.2 - 10	0.2	12.5
Endosulphan II	ng/l	0.1 - 10	0.2	12.5
Endrin	ng/l	1 – 20	0.5	12.5
HCB	ng/l	0.1 - 10	0.2	12.5
HCBD	ng/l	0.2 - 20	0.2	12.5
α HCH	ng/l	0.2 - 20	0.2	12.5
β HCH	ng/l	0.2 - 20	0.2	12.5
γ HCH	ng/l	0.5 - 20	0.2	12.5
δ HCH	ng/l	0.2 - 20	0.2	12.5
Isodrin	ng/l	1 – 20	0.5	12.5
Pentabromodiphenylether	ng/l	0.5 – 20	0.5	12.5
Pentachlorobenzene	ng/l	0.5 – 20	0.5	12.5
1,2,3 TCB	ng/l	1 - 20	0.5	12.5
1,3,5 TCB	ng/l	0.5 - 20	0.5	12.5
1,2,4 TCB	ng/l	1 - 20	0.5	12.5
Trifluralin	ng/l	0.5 - 20	0.5	12.5

Low salinity test materials (Salinity ca. 10 psu)				
Determinands	**Units**	**Indication of Concentration Range**	**Constant Error**	**Proportional Error %**
Aldrin	ng/l	2 – 200	0.5	12.5
pp' DDD	ng/l	1 - 50	0.5	12.5
pp' DDE	ng/l	1 - 50	0.5	12.5
op' DDT	ng/l	1 - 50	0.5	12.5
pp' DDT	ng/l	1 - 50	0.5	12.5
Dieldrin	ng/l	2 - 100	0.5	12.5
Endosulphan I	ng/l	1 - 20	0.2	12.5
Endosulphan II	ng/l	0.5 - 20	0.2	12.5
Endrin	ng/l	2 - 200	0.5	12.5
HCB	ng/l	0.5 - 20	0.2	12.5
HCBD	ng/l	2 - 50	0.2	12.5
α HCH	ng/l	2 - 50	0.2	12.5
β HCH	ng/l	1 - 50	0.2	12.5
γ HCH	ng/l	2 - 50	0.2	12.5
δ HCH	ng/l	1 - 50	0.2	12.5
Isodrin	ng/l	2 - 200	0.5	12.5
Pentabromodiphenylether	ng/l	2 – 100	0.5	12.5
Pentachlorobenzene	ng/l	2 - 100	0.5	12.5
1,2,3 TCB	ng/l	2 - 50	0.5	12.5
1,3,5 TCB	ng/l	2 - 50	0.5	12.5
1,2,4 TCB	ng/l	5 - 100	0.5	12.5
Trifluralin	ng/l	2 - 50	0.5	12.5

AQ-6 Volatile organochlorines in seawater (VOCs)

The seawater is collected from the Eastern Atlantic Ocean and filtered to remove bacteria and other particles. The filtered seawater is dispensed into 1 litre glass bottles. Composite solutions containing the volatile organochlorine compounds are prepared in methanol. These are used to spike the individual bottles of seawater. Glass beads are added to the chlorinated volatile organic compounds test materials to raise the headspace in order to prevent volatilisation of the spiking solution.

The test materials are assumed to be homogeneous, as each batch is prepared in bulk, thoroughly mixed and spiked to the same concentration level. The test materials are stable for the purposes of the study.

(Salinity > 30 psu)				
Determinands	**Units**	**Indication of Concentration Range**	**Constant Error**	**Proportional Error %**
Carbon tetrachloride	µg/l	0.2 - 10	0.1	12.5
Chloroform	µg/l	0.5 - 20	0.1	12.5
1,2 Dichloroethane	µg/l	1 - 10	0.1	12.5
Dichloromethane	µg/l	0.2 – 10	0.1	12.5
Trichlorothene	µg/l	0.2 - 20	0.1	12.5
1,1,1 Trichloroethane	µg/l	0.2 - 10	0.1	12.5
1,1,2 Trichloroethane	µg/l	1 - 20	0.1	12.5
Tetrachloroethene	µg/l	0.2 - 10	0.1	12.5
Trichloromethane	µg/l	0.2 - 10	0.1	12.5

AQ-7 Pentachlorophenol (PCP) in seawater

PCP is offered as a separate determinand as it is usually determined by a different method and requires a separate volume of sample.

The seawater used to prepare these test materials is collected from the Eastern Atlantic Ocean and is filtered to remove bacteria and other particles. The filtered seawater is dispensed into 1 litre glass bottles. Composite solutions containing pentachlorophenol are prepared in methanol. These are used to spike the individual bottles of seawater.

The test materials are assumed to be homogeneous, as each batch is prepared in bulk, thoroughly mixed and spiked to the same concentration level. The test materials are stable for the purposes of the study.

(Salinity > 30 psu)					
Determinands	**Units**	**Indication of Concentration Range**		**Constant Error**	**Proportional Error %**
		Typical Minima	**Typical Maxima**		
Pentachlorophenol	ng/l	20	200	10	12.5

AQ-8 Triazines and organophosphorus compounds in seawater

The AQ-8 studies include both full salinity seawater test materials and low salinity test materials. The same constant errors and proportional errors are used for both types of test material. The concentration ranges differ for the full salinity and low salinity test materials.

The seawater is collected from the Eastern Atlantic Ocean and filtered to remove bacteria and other particles. The low salinity test materials are prepared by diluting the filtered seawater with ultrapure demineralised water to the required salinity. The filtered seawater is dispensed into 1 litre glass bottles. Composite solutions containing the triazines and organophosphorus compounds are prepared in methanol in bulk. An ampoule containing the composite solution is provided along with a bottle of filtered seawater and participants are asked to dilute the spiking solution with the seawater on the day of analysis to avoid stability problems.

The homogeneity of the test materials is assumed, as the materials are prepared in bulk. The test materials are stable for the purposes of the study.

Full salinity test materials (Salinity > 30 psu)				
Determinands	**Units**	**Indication of Concentration Range**	**Constant Error**	**Proportional Error %**
Alachlor	ng/l	2 – 200	1	12.5
Atrazine	ng/l	5 - 200	1	12.5
Azinphos ethyl	ng/l	5- -200	1	12.5
Azinphos methyl	ng/l	5 - 200	1	12.5
Chlorfenvinphos	ng/l	5 - 200	1	12.5
Chlorpyriphos	ng/l	2 – 200	1	12.5
Coumaphos	ng/l	2 - 100	1	12.5
Demeton	ng/l	5 - 200	1	12.5
Diazinon	ng/l	5 - 200	1	12.5
Dichlorvos	ng/l	2 - 200	1	12.5
Dimethoate	ng/l	5 - 100	1	12.5
Diuron	ng/l	5 - 200	1	12.5
Fenchlorphos	ng/l	2 - 200	1	12.5
Fenitrothion	ng/l	2 - 200	1	12.5
Fenthion	ng/l	5 - 200	1	12.5
Irgarol 1051	ng/l	2 - 200	1	12.5
Isoproturon	ng/l	2 – 200	1	12.5
Malathion	ng/l	5 - 200	1	12.5
Omethoate	ng/l	5 - 200	1	12.5
Parathion ethyl	ng/l	5 - 200	1	12.5
Parathion methyl	ng/l	5 - 200	1	12.5
Simazine	ng/l	5 - 200	1	12.5
Triazophos	ng/l	10 - 500	1	12.5

Low salinity test materials (Salinity ca. 10 psu)				
Determinands	**Units**	**Indication of Concentration Range**	**Constant Error**	**Proportional Error %**
Alachlor	ng/l	20 - 500	1	12.5
Atrazine	ng/l	20 - 500	1	12.5
Azinphos ethyl	ng/l	20 - 500	1	12.5
Azinphos methyl	ng/l	20 - 500	1	12.5
Chlorfenvinphos	ng/l	20 - 500	1	12.5
Chlorpyriphos	ng/l	20 - 500	1	12.5
Coumaphos	ng/l	20 - 500	1	12.5
Demeton	ng/l	50 - 500	1	12.5
Diazinon	ng/l	20 - 500	1	12.5
Dichlorvos	ng/l	20 - 500	1	12.5
Dimethoate	ng/l	20 - 500	1	12.5
Diuron	ng/l	50 - 500	1	12.5
Fenchlorphos	ng/l	20 - 500	1	12.5
Fenitrothion	ng/l	20 - 500	1	12.5
Fenthion	ng/l	20 - 500	1	12.5
Irgarol 1051	ng/l	50 - 500	1	12.5
Isoproturon	ng/l	20 - 500	1	12.5
Malathion	ng/l	20 - 500	1	12.5
Omethoate	ng/l	50 - 500	1	12.5
Parathion ethyl	ng/l	20 - 500	1	12.5
Parathion methyl	ng/l	20 - 500	1	12.5
Simazine	ng/l	20 - 500	1	12.5
Triazophos	ng/l	50 - 500	1	12.5

AQ-11 Chlorophyll a *in seawater*

Chlorophyll test material will be prepared from cultures of Isochrysis galbana in natural seawater. A known volume of the culture, grown at the RIVO Institute in Yerseke, the Netherlands, will be diluted with natural seawater. The bulk with diluted culture is mixed continually during the sub-sampling process. The diluted samples are then filtered through a Whatman GF/F, 47 mm filter paper. The resulted damp filter papers are wrapped in aluminium foil, inserted into a numbered cryovial and immediately transferred into a -80˚C freezer until the day of dispatch.

Chlorophyll *a* in seawater is inherently patchy and it can be difficult to obtain a homogeneous sample. The sequence in which the test materials are filtered is recorded and samples are selected at regular intervals for homogeneity testing. The homogeneity testing is completed before the test materials are dispatched.

The test materials are shipped to participants on frozen (-80℃) cool packs.

Determinand	**Units**	**Indication of Concentration Range**	**Constant Error**	**Proportional Error %**
Chlorophyll *a*	µg/l	0.1 - 10	0.05	12.5
Chlorophyll *b*	µg/l	0.01 - 0.2	0.01	12.5
Chlorophyll *c*	µg/l	0.02 - 0.5	0.01	12.5
Pheopigments	µg/l	0.02 - 1	0.01	12.5

AQ-12 Organotins in seawater

The seawater is collected from the Eastern Atlantic Ocean and filtered to remove bacteria and other particles. In total 3 seawater samples spiked with low concentrations of organotins will be sent within each exercise.

Determinands	Units	Indication of Concentration Range	Constant Error	Proportional Error %
Tributyltin (TBT)	µg/kg	0.001 – 0.1	0.1	12.5
Dibutyltin (DBT)	µg/kg	0.02 – 0.2	0.1	12.5
Monobutyltin (MBT)	µg/kg	0.02 – 2	0.1	12.5
Triphenyltin (TPT)	µg/kg	0.02 – 0.5	0.1	12.5
Diphenyltin (DPT)	µg/kg	0.02 – 0.5	0.1	12.5
Monophenyltin (MPT)	µg/kg	0.05 - 1	0.1	12.5

AQ-13 PAHs in seawater

The seawater is collected from the Eastern Atlantic Ocean and filtered to remove bacteria and other particles. In total 3 seawater samples spiked with low concentrations of PAHs and a standard solution will be sent within each exercise.

Determinands	Units	Indication of Concentration Range	Constant Error	Proportional Error %
Acenapthene	µg/l	1 – 50	1	12.5
Acenaphtylene	µg/l	1 – 10	1	12.5
Anthracene	µg/l	1 – 200	1	12.5
Benzo[a]pyrene	µg/l	1 – 200	1	12.5
Benzo[b]fluoranthene	µg/l	1 – 500	1	12.5
Benzo[g,h,i]perylene	µg/l	1 – 500	1	12.5
Benzo[k]fluoranthene	µg/l	1 – 200	1	12.5
Fluoranthene	µg/l	1 – 500	1	12.5
Indeno(1,2,3-cd)pyrene	µg/l	1 – 200	1	12.5
Phenanthrene	µg/l	1 - 500	1	12.5
Naphtalene	µg/l	1 – 200	1	12.5

3.3 Sediment Test Materials

The sediment test materials are divided into four groups: MS-1 trace metals, MS-2 chlorinated organics, i.e. chlorobiphenyls and organochlorine pesticides, MS-3 polycyclic aromatic hydrocarbons (PAHs) and MS-6 organotins. A minimum of two test materials is provided for each study.

The sediment test materials cover a range of natural unspiked sandy and silty sediments from open water, estuaries and harbour locations around the North Sea, Eastern Atlantic Ocean and Mediterranean Sea. Although wet sediments constitute a more realistic natural material, previous QUASIMEME Laboratory Performance studies have shown that there was no significant difference in laboratory performance when dry sediments were used compared to wet sediments. Where wet sediments are provided, these are stabilised by sterilisation. The dry sediments are sieved and milled to <0.5 mm and may also be stabilised by sterilisation. Both the wet and dry sediments are divided into representative sub samples.

The level of test material homogeneity is assessed following BCR guidelines (1993)[10]. The dry sediments have been shown to be stable over a number of years when stored at room temperature.

[10] BCR (1993). Guidelines for the production and preparation of BCR Reference Materials Doc BCR/48/93. European Commission DG XII Rue Montoyer 75, Brussels 1049, Belgium.

Dry sediments are considerably less expensive than wet sediments, therefore QUASIMEME will continue to provide these, unless there are specific reasons / requests to provide wet sediments.

MS-1 Trace metals in sediment

Determinands	Units	Indication of Concentration Range	Constant Error	Proportional Error %
Aluminium	%	1 - 10	0.1	12.5
Arsenic	mg/kg	2 - 50	1	12.5
Cadmium	µg/kg	10 - 2000	20	12.5
Chromium	mg/kg	10 - 1000	2	12.5
Copper	mg/kg	1 - 500	1	12.5
Iron	%	0.5 - 10	0.1	12.5
Lead	mg/kg	5 - 500	2	12.5
Lithium	mg/kg	10 - 100	0.1	12.5
Manganese	mg/kg	100 - 2000	0.1	12.5
Mercury	µg/kg	50 - 2500	10	12.5
Nickel	mg/kg	5 - 100	1	12.5
Scandium	mg/kg	1 - 20	0.1	12.5
Zinc	mg/kg	20 - 1500	2.5	12.5
TOC	%	0.2 - 10	0.1	12.5
Inorganic carbonate	%	0.05 - 10	0.05	12.5

MS-2 Chlorinated organics in sediment

Determinands	Units	Indication of Concentration Range	Constant Error	Proportional Error %
CB 28	µg/kg	0.1 - 50	0.025	12.5
CB 31	µg/kg	0.1 - 50	0.025	12.5
CB 52	µg/kg	0.1 - 50	0.025	12.5
CB 101	µg/kg	0.2 - 50	0.025	12.5
CB 105	µg/kg	0.1 - 10	0.025	12.5
CB 118	µg/kg	0.1 - 50	0.025	12.5
CB 138[11]	µg/kg	0.2 - 50	0.025	12.5
CB 153	µg/kg	0.2 - 50	0.025	12.5
CB 156	µg/kg	0.05 – 5	0.025	12.5
CB 180	µg/kg	0.1 – 50	0.025	12.5
pp' DDD	µg/kg	0.1 – 20	0.025	12.5
pp' DDE	µg/kg	0.1 – 10	0.025	12.5
op' DDT	µg/kg	0.02 – 5	0.025	12.5
pp' DDT	µg/kg	0.1 – 10	0.025	12.5
Dieldrin	µg/kg	0.1 – 10	0.025	12.5
HCB	µg/kg	0.05 - 20	0.025	12.5
HCBD	µg/kg	0.1 – 10	0.025	12.5
α HCH	µg/kg	0.02 – 1	0.02	12.5
β HCH	µg/kg	0.05 – 2	0.025	12.5
γ HCH	µg/kg	0.05 - 2	0.025	12.5
Transnonachlor	µg/kg	0.01 - 2	0.025	12.5
TOC	%	0.2 - 10	0.02	12.5

[11] CB 138 is equivalent to CB 138 and CB 163

MS-3 PAH in sediment

Determinands	Units	Indication of Concentration Range	Constant Error	Proportional Error %
Acenaphthene	µg/kg	2 - 500	0.1	12.5
Acenaphthylene	µg/kg	2 - 100	0.2	12.5
Anthracene	µg/kg	2 - 500	0.1	12.5
Benzo[a]anthracene	µg/kg	10 - 1500	0.1	12.5
Benzo[a]fluorene	µg/kg	10 - 1000	0.5	12.5
Benzo[a]pyrene	µg/kg	10 - 1500	0.1	12.5
Benzo[b]fluoranthene	µg/kg	10 - 1500	0.5	12.5
Benzo[e]pyrene	µg/kg	10 - 1500	0.2	12.5
Benzo[g,h,i]perylene	µg/kg	10 - 1500	0.2	12.5
Benzo[k]fluoranthene	µg/kg	10 - 1000	0.1	12.5
Chrysene	µg/kg	10 - 1500	0.2	12.5
Dibenz[a,h]anthracene	µg/kg	5 - 500	0.05	12.5
Dibenzo[a,i]pyrene	µg/kg		0.5	12.5
Dibenzothiophene	µg/kg	2 - 200	0.1	12.5
3,6 Dimethylphenanthrene	µg/kg	1 - 500	0.5	12.5
Fluoranthene	µg/kg	20 - 3000	0.2	12.5
Fluorene	µg/kg	2 - 300	0.1	12.5
Indeno[1,2,3 cd]pyrene	µg/kg	10- -1500	0.2	12.5
2 Methylphenanthrene	µg/kg	5 - 1000	0.5	12.5
1 Methylpyrene	µg/kg	2 - 500	0.5	12.5
Naphthalene	µg/kg	10 - 1500	0.5	12.5
Perylene	µg/kg	10 - 500	0.2	12.5
Phenanthrene	µg/kg	10 - 2000	0.5	12.5
Pyrene	µg/kg	10 - 3000	0.2	12.5
Triphenylene	µg/kg	20 - 3000	0.5	12.5
TOC	%	0.2 - 10	0.02	12.5

MS-6 Organotins in sediment

Determinands	Units	Indication of Concentration Range	Constant Error	Proportional Error %
Tributyltin (TBT)	µg/kg	1 – 500	0.1	12.5
Dibutyltin (DBT)	µg/kg	1 – 500	0.1	12.5
Monobutyltin (MBT)	µg/kg	2 – 500	0.1	12.5
Triphenyltin (TPT)	µg/kg	0.1 – 100	0.1	12.5
Diphenyltin (DPT)	µg/kg	0.1 – 100	0.1	12.5
Monophenyltin (MPT)	µg/kg	0.1 – 100	0.1	12.5

3.4 Biota Test Materials

The biota test materials are divided into eight groups: BT-1 trace metals, BT-2 chlorinated organics, i.e. chlorobiphenyls and organochlorine pesticides, BT-3 non-ortho CBs, PCDFs and PCDDs, BT-4 polycylcic aromatic hydrocarbons (PAHs), BT-5 toxaphene, BT-7 amnesic shellfish poisoning toxins, BT-8 organotins and BE-1 imposex and intersex in marine snails. The test materials for BT-1 to BT-5 are discussed below. The test materials for BE-1 are described on page 27.

The test materials for BT-1 to BT-8 are collected from contaminated waters from open water and coastal locations around the North Sea and Mediterranean, and include e.g. plaice, cod, mussels, shrimps, flounder and tuna. All materials are homogenised and sterilised by autoclaving. The use of wet tissues by QUASIMEME is unique for the purposes of the Laboratory Performance studies, and allows participants to analyse determinands in a test material matrix similar to a natural sample.

The level of test material homogeneity is assessed following BCR guidelines (1993)[12]. The test materials have been shown to be stable for a number of years when stored at room temperature.

A minimum of two test materials is provided for each study. In BT-1, BT-2 and BT-3, normally one shellfish and one fish test material is provided. The fish tissue test material is either a muscle or liver, however fish liver is not used more than once per group in any year. A series of wet tissue test materials are supplied for BT-5, which can be used to test the methods of separation and quantification of toxaphene congeners. BT-2, BT-3 and BT-5 are provided as separate studies because:

- the matrix type and concentration ranges are very different for each group and attempting to provide one common matrix will result in an unacceptable compromise
- this enables participants to tailor their requirements without having to pay for additional materials or assessments which are not needed

In BT-7 and BT-8 a minimum of two testmaterials is provided for each study. The content of the testmaterials can vary, but normally two shellfish materials will be sent for each exercise.

BT-1 Trace metals in biota

The BT-1 studies include fish and shellfish test materials. For some determinands, a different constant error is applied for shellfish test materials to that used for fish tissue. The concentration ranges for shellfish, fish muscle and fish liver are given separately.

Shellfish				
Determinands	**Units**	**Indication of Concentration Range**	**Constant Error**	**Proportional Error %**
Arsenic	mg/kg	0.5 – 5	0.02	12.5
Cadmium	µg/kg	50 – 500	20	12.5
Chromium	µg/kg	50 – 5000	20	12.5
Copper	µg/kg	500 – 5000	100	12.5
Lead	µg/kg	100 – 1000	5	12.5
Mercury	µg/kg	20 – 100	20	12.5
Nickel	µg/kg	200 – 2000	20	12.5
Selenium	µg/kg	200 – 1000	10	12.5
Silver	µg/kg	5 – 50	5	12.5
Zinc	mg/kg	10 – 100	2	12.5
Ash weight	%		0.1	12.5
Dry weight	%		0.1	12.5
Total Lipid	%		0.1	12.5
Extractable Lipid	%		0.1	12.5

[12] BCR (1993). Guidelines for the production and preparation of BCR Reference Materials Doc BCR/48/93. European Commission DG XII Rue Montoyer 75, Brussels 1049, Belgium.

Fish muscle				
Determinands	**Units**	**Indication of Concentration Range**	**Constant Error**	**Proportional Error %**
Arsenic	mg/kg	1 - 10	0.02	12.5
Cadmium	µg/kg	1 - 50	1	12.5
Chromium	µg/kg	50 - 500	20	12.5
Copper	µg/kg	100 - 1000	100	12.5
Lead	µg/kg	10 - 50	5	12.5
Mercury	µg/kg	20 - 1000	20	12.5
Nickel	µg/kg	10 - 200	20	12.5
Selenium	µg/kg	200 - 2000	10	12.5
Silver	µg/kg	0.5 - 50	1	12.5
Zinc	mg/kg	2 - 10	2	12.5
Ash weight	%		0.1	12.5
Dry weight	%		0.1	12.5
Total Lipid	%		0.1	12.5
Extractable Lipid	%		0.1	12.5

Fish liver				
Determinands	**Units**	**Indication of Concentration Range**	**Constant Error**	**Proportional Error %**
Arsenic	mg/kg	1 - 5	0.02	12.5
Cadmium	µg/kg	5 - 1000	1	12.5
Chromium	µg/kg	20 - 1000	20	12.5
Copper	µg/kg	2000 - 10000	100	12.5
Lead	µg/kg	10 - 1000	5	12.5
Mercury	µg/kg	20 - 100	20	12.5
Nickel	µg/kg	20 - 1000	20	12.5
Selenium	µg/kg	200 - 5000	10	12.5
Silver	µg/kg	20 - 1000	1	12.5
Zinc	mg/kg	10 - 50	2	12.5
Ash weight	%		0.1	12.5
Dry weight	%		0.1	12.5
Total Lipid	%		0.1	12.5
Extractable Lipid	%		0.1	12.5

BT-2 Organics in biota

The BT-2 studies include fish and shellfish test materials. The same constant errors and proportional errors are used for all test materials. The concentration ranges for fish liver are given separately to those for shellfish and fish muscle.

Fish muscle and shellfish				
Determinands	**Units**	**Indication of Concentrations**	**Constant Error**	**Proportional Error %**
CB 28	µg/kg	0.05 - 5	0.025	12.5
CB 31	µg/kg	0.03 – 3	0.025	12.5
CB 52	µg/kg	0.05 - 5	0.025	12.5
CB 101	µg/kg	0.1 - 20	0.025	12.5
CB 105	µg/kg	0.05 – 10	0.025	12.5
CB 118	µg/kg	0.2 - 20	0.025	12.5
CB 138[13]	µg/kg	0.3 - 30	0.025	12.5
CB 153	µg/kg	0.4 - 40	0.025	12.5
CB 156	µg/kg	0.03 -10	0.025	12.5
CB 180	µg/kg	0.05 - 5	0.025	12.5
pp' DDD	µg/kg	0.1 - 10	0.025	12.5
pp' DDE	µg/kg	0.3 - 30	0.025	12.5
op' DDT	µg/kg	0.01 - 1	0.025	12.5
pp' DDT	µg/kg	0.1 – 10	0.025	12.5
Dieldrin	µg/kg	0.20 - 20	0.025	12.5
HCB	µg/kg	0.02 - 5	0.025	12.5
HCBD	µg/kg		0.025	12.5
α HCH	µg/kg	0.05 - 5	0.02	12.5
β HCH	µg/kg	0.05 - 5	0.025	12.5
γ HCH	µg/kg	0.05 - 5	0.025	12.5
Transnonachlor	µg/kg	0.02 - 10	0.025	12.5
Total Lipid	%		0.1	12.5
Extractable Lipid	%		0.1	12.5

Fish liver				
Determinands	**Units**	**Indication of Concentrations**	**Constant Error**	**Proportional Error %**
CB 28	µg/kg	5 – 50	0.025	12.5
CB 31	µg/kg	1 - 10	0.025	12.5
CB 52	µg/kg	10 – 100	0.025	12.5
CB 101	µg/kg	30 – 300	0.025	12.5
CB 105	µg/kg	10 - 100	0.025	12.5
CB 118	µg/kg	30 – 300	0.025	12.5
CB 138[13]	µg/kg	60 - 600	0.025	12.5
CB 153	µg/kg	100 - 1000	0.025	12.5
CB 156	µg/kg	3 - 40	0.025	12.5
CB 180	µg/kg	20 - 200	0.025	12.5
pp' DDD	µg/kg	10 - 100	0.025	12.5
pp' DDE	µg/kg	50 - 500	0.025	12.5
op' DDT	µg/kg	0.1 – 2	0.025	12.5
pp' DDT	µg/kg	0.3 - 10	0.025	12.5
Dieldrin	µg/kg	10 - 100	0.025	12.5
HCB	µg/kg	5 - 50	0.025	12.5
HCBD	µg/kg	0.1 – 5	0.025	12.5
α HCH	µg/kg	0.5 - 5	0.02	12.5
β HCH	µg/kg	0.5 - 5	0.025	12.5
γ HCH	µg/kg	0.2 - 5	0.025	12.5
Transnonachlor	µg/kg	3 - 40	0.025	12.5
Total Lipid	%		0.1	12.5
Extractable Lipid	%		0.1	12.5

[13] CB 138 is equivalent to CB 138 and CB 163.

BT-3 Non-ortho CBs, PCDFs & PCDDs in biota

The BT-3 studies include fish and shellfish test materials. The same constant errors and proportional errors are used for all test materials. The concentration ranges for fish liver are given separately to those for shellfish and fish muscle.

Fish muscle and shellfish				
Determinands	**Units**	**Indication of Concentrations**	**Constant Error**	**Proportional Error %**
CB 77	ng/kg	10 - 100	0.02	12.5
CB 126	ng/kg	1 - 20	0.02	12.5
CB 169	ng/kg	0.2 - 5	0.02	12.5
2,3,7,8 TCDF	ng/kg	0.03 - 5	0.02	12.5
1,2,3,7,8 PeCDF	ng/kg	0.03 - 1	0.02	12.5
2,3,4,7,8 PeCDF	ng/kg	0.05 – 3	0.02	12.5
1,2,3,4,7,8 HxCDF	ng/kg	0.01 – 0.2	0.02	12.5
1,2,3,6,7,8 HxCDF	ng/kg	0.01 – 0.2	0.02	12.5
2,3,4,6,7,8 HxCDF	ng/kg	0.01 – 0.5	0.02	12.5
1,2,3,7,8,9 HxCDF	ng/kg		0.02	12.5
1,2,3,4,6,7,8 HpCDF	ng/kg	0.02 – 0.5	0.02	12.5
1,2,3,4,7,8,9 HpCDF	ng/kg		0.02	12.5
OCDF	ng/kg	0.02 - 0.5	0.02	12.5
2,3,7,8 TCDD	ng/kg	0.01 – 0.5	0.02	12.5
1,2,3,7,8 PeCDD	ng/kg	0.02 – 1	0.02	12.5
1,2,3,4,7,8 HxCDD	ng/kg	0.02 – 0.2	0.02	12.5
1,2,3,6,7,8 HxCDD	ng/kg	0.03 - 1	0.02	12.5
1,2,3,7,8,9 HxCDD	ng/kg	0.02 – 0.5	0.02	12.5
1,2,3,4,6,7,8 HpCDD	ng/kg	0.05 - 5	0.02	12.5
OCDD	ng/kg	0.05 - 5	0.02	12.5
Total TEQ (Dr.CALUX)	ng/kg		0.1	12.5
Total Lipid	%		0.1	12.5
Extractable Lipid	%		0.1	12.5

Fish liver				
Determinands	**Units**	**Indication of Concentrations**	**Constant Error**	**Proportional Error %**
CB 77	ng/kg	500 – 4000	0.02	12.5
CB 126	ng/kg	200 - 2000	0.02	12.5
CB 169	ng/kg	30 - 300	0.02	12.5
2,3,7,8 TCDF	ng/kg	10 - 100	0.02	12.5
1,2,3,7,8 PeCDF	ng/kg	3 - 20	0.02	12.5
2,3,4,7,8 PeCDF	ng/kg	3 - 20	0.02	12.5
1,2,3,4,7,8 HxCDF	ng/kg	1 - 10	0.02	12.5
1,2,3,6,7,8 HxCDF	ng/kg	1 - 10	0.02	12.5
2,3,4,6,7,8 HxCDF	ng/kg	1 - 10	0.02	12.5
1,2,3,7,8,9 HxCDF	ng/kg		0.02	12.5
1,2,3,4,6,7,8 HpCDF	ng/kg	1 - 10	0.02	12.5
1,2,3,4,7,8,9 HpCDF	ng/kg		0.02	12.5
OCDF	ng/kg	0.2 - 2	0.02	12.5
2,3,7,8 TCDD	ng/kg	3 - 30	0.02	12.5
1,2,3,7,8 PeCDD	ng/kg	0.2 - 2	0.02	12.5
1,2,3,4,7,8 HxCDD	ng/kg		0.02	12.5
1,2,3,6,7,8 HxCDD	ng/kg	2 - 20	0.02	12.5
1,2,3,7,8,9 HxCDD	ng/kg	0.5 – 5	0.02	12.5
1,2,3,4,6,7,8 HpCDD	ng/kg	3 - 20	0.02	12.5
OCDD	ng/kg	3 - 20	0.02	12.5
Total TEQ (Dr.CALUX)	ng/kg		0.1	12.5
Total Lipid	%		0.1	12.5
Extractable Lipid	%		0.1	12.5

BT-4 PAH in shellfish

Determinands	Units	Indication of Concentration Range	Constant Error	Proportional Error %
Acenaphthene	µg/kg	0.5 – 100	0.2	12.5
Acenaphthylene	µg/kg	0.2 – 5	0.2	12.5
Anthracene	µg/kg	0.2 – 10	0.2	12.5
Benzo[a]anthracene	µg/kg	0.2 - 10	0.2	12.5
Benzo[a]fluorene	µg/kg		0.5	12.5
Benzo[a]pyrene	µg/kg	0.2 – 5	0.2	12.5
Benzo[b]fluoranthene	µg/kg	0.2 - 10	0.2	12.5
Benzo[e]pyrene	µg/kg	0.2 - 10	0.2	12.5
Benzo[g,h,i]perylene	µg/kg	0.2 – 5	0.2	12.5
Benzo[k]fluoranthene	µg/kg	0.2 - 5	0.2	12.5
Chrysene	µg/kg	0.2 - 20	0.2	12.5
Dibenz[a,h]anthracene	µg/kg	0.2 – 2	0.1	12.5
Dibenzo[a,i]pyrene	µg/kg		0.5	12.5
Dibenzothiophene	µg/kg	0.2 – 5	0.5	12.5
3,6 Dimethylphenanthrene	µg/kg	0.2 - 2	0.5	12.5
Fluoranthene	µg/kg	5 – 50	0.2	12.5
Fluorene	µg/kg	1 - 50	0.2	12.5
Indeno[1,2,3 cd]pyrene	µg/kg	0.2 - 5	0.2	12.5
2 Methylphenanthrene	µg/kg	0.2 – 5	2	12.5
1 Methylpyrene	µg/kg		2	12.5
Naphthalene	µg/kg	1 - 100	0.2	12.5
Perylene	µg/kg	0.1 - 5	0.5	12.5
Phenanthrene	µg/kg	2 - 50	0.2	12.5
Pyrene	µg/kg	1 - 20	0.2	12.5
Triphenylene	µg/kg		5	12.5
Total Lipid	%		0.1	12.5
Extractable Lipid	%		0.1	12.5

BT-5 Toxaphene in biota

Determinands	Units	Indication of Concentrations	Constant Error	Proportional Error %
CHB 26	µg/kg	0.5 – 5	0.1	12.5
CHB 32	µg/kg	0.1 – 10	0.1	12.5
CHB 40	µg/kg	0.1 - 10	0.1	12.5
CHB 41	µg/kg	0.1 - 10	0.1	12.5
CHB 44	µg/kg	0.2 - 10	0.1	12.5
CHB 50	µg/kg	0.2 - 10	0.1	12.5
CHB 62	µg/kg	0.2 - 10	0.1	12.5
Total Toxaphene	µg/kg	1 - 100	0.1	12.5
Total Lipid	%		0.1	12.5
Extractable Lipid	%		0.1	12.5

BT-7 Amnesic Shellfish Poisoning Toxins in biota

Determinands	Units	Constant Error	Proportional Error %
Domoic + Epidomoic Acid	mg/kg	0.1	12.5

BT-8 Organotins in biota

Determinands	Units	Indication of Concentration Range	Constant Error	Proportional Error %
Tributyltin (TBT)	µg Sn/kg	2 - 50	0.1	12.5
Dibutyltin (DBT)	µg Sn/kg	1 - 100	0.1	12.5
Monobutyltin (MBT)	µg Sn/kg	5 - 30	0.1	12.5
Triphenyltin (TPT)	µg Sn/kg		0.1	12.5
Diphenyltin (DPT)	µg Sn/kg		0.1	12.5
Monophenyltin (MPT)	µg Sn/kg		0.1	12.5

BE-1 Imposex and intersex in marine snails

Nucella lapillus and *Littorina littorea* are sampled from contaminated waters from open water and coastal locations around the North Sea and/or Mediterranean. The snails are then placed on a tray, coned 100 times, quartered, coned 100 times continuing until sub-samples of 50 snails are achieved. The sub-samples of snails are then placed in a mesh bag, which is secured by tying. The bags of snails are kept in water prior to being distributed to participants.

The snails are packed with newspaper into polystyrene boxes. Frozen (-20°C) cool packs are placed on top of the newspaper packaging to keep the snails cool. The packages arrive at the participating institutes within two days of the dispatch. Due to a better availability of suitable live test materials the dispatch is scheduled for June.

The measurements requested are:
Nucella lapillus: Shell height, sex, penis length, and vas deferens stage
Littorina littorea: Shell height, sex, female prostrate length, and intersex stage

3.5 Biota and Sediment Test Materials

The **biota** test materials are collected from contaminated waters from open water and coastal locations around the North Sea and/or Mediterranean, and include herring, plaice, salmon, mackerel and dab. All materials are homogenised and sterilised by autoclaving. The use of wet tissues by QUASIMEME is unique for the purposes of the Laboratory Performance studies, and allows participants to analyse determinands in a test material matrix similar to a natural sample.

The level of biota test material homogeneity is assessed following BCR guidelines (1993)[12]. The test materials have been shown to be stable for a number of years when stored at room temperature.

The **sediment** test materials cover a range of natural unspiked sandy and silty sediments from open water, estuaries and harbour locations around the North Sea, Eastern Atlantic Ocean, and/or Mediterranean. Although wet sediments constitute a more realistic natural material, previous QUASIMEME Laboratory Performance studies have shown that there was no significant difference in laboratory performance when dry sediments were used compared to wet sediments. Where wet sediments are provided, these are stabilised by sterilisation. The dry sediments are sieved and milled to <0.5 mm and may also be stabilised by sterilisation. Both the wet and dry sediments are divided into representative sub samples.

Dry sediments are considerably less expensive than wet sediments, therefore QUASIMEME will continue to provide these, unless there are specific reasons / requests to provide wet sediments.

The level of test material homogeneity is assessed following BCR guidelines (1993). Both biota and sediment test materials have been shown to be stable over a number of years when stored at room temperature.

A minimum of two test materials is provided for each study, one shellfish or fish test material and one sediment test material.

BS-1 Brominated Flame Retardants

The BS-1 studies include sediment and biota test materials. A lower constant error is applied for biota test materials, reflecting the lower concentrations of the determinands in the biota test materials.

Sediment				
Determinands	**Units**	**Indication of Concentration Range**	**Constant Error**	**Proportional Error %**
BDE 28	µg/kg	0.01 – 2	0.05	12.5
BDE 47	µg/kg	0.1 – 20	0.05	12.5
BDE 99	µg/kg	0.1 – 50	0.05	12.5
BDE 100	µg/kg	0.01 – 10	0.05	12.5
BDE 153	µg/kg	0.1 – 5	0.05	12.5
BDE 154	µg/kg	0.01 – 5	0.05	12.5
BDE 183	µg/kg	0.1 – 2	0.05	12.5
BDE 209	µg/kg	20 – 200	0.05	12.5
Dimethyl-TBBP-A	µg/kg		0.05	12.5
α HBCD	µg/kg		0.05	12.5
β HBCD	µg/kg		0.05	12.5
γ HBCD	µg/kg		0.05	12.5
Total HBCD	µg/kg	50 – 200	0.05	12.5
TBBP-A	µg/kg		0.05	12.5

Biota				
Determinands	**Units**	**Indication of Concentration Range**	**Constant Error**	**Proportional Error %**
BDE 28	µg/kg	0.001 – 1	0.005	12.5
BDE 47	µg/kg	0.05 – 20	0.005	12.5
BDE 99	µg/kg	0.01 – 10	0.005	12.5
BDE 100	µg/kg	0.005 – 2	0.005	12.5
BDE 153	µg/kg	0.01 – 1	0.005	12.5
BDE 154	µg/kg	0.001 – 1	0.005	12.5
BDE 183	µg/kg	0.001 0.1	0.005	12.5
BDE 209	µg/kg	0.01 - 0.1	0.005	12.5
Dimethyl-TBBP-A	µg/kg		0.005	12.5
α HBCD	µg/kg		0.005	12.5
β HBCD	µg/kg		0.005	12.5
γ HBCD	µg/kg		0.005	12.5
Total HBCD	µg/kg		0.005	12.5
TBBP-A	µg/kg		0.005	12.5

3.6 Development Exercises

3.6.1 Shellfish toxins

DE-10 Diarrhetic Shellfish Poisoning Toxins

Development Exercise for the Chemical Measurement of Diarrhetic Shellfish Poisoning Toxins (DE-10). Extending the programme for the measurement of shellfish toxins, which began in 2003 – 2004, the development exercises will focus on diarrhetic shellfish poisoning (DSP) with the measurement of the okadaic acid group. The test materials will comprise:

- a standard solution containing okadaic acid
- an extract of whole shellfish homogenate
- two shellfish tissue homogenates

Other matrices and determinands may be added to this study in future years.

3.6.2 Organic determinands

DE-11 Alkylphenols in seawater

As a result of the alkylphenol workshop in Berlin 2006, a development exercise is initiated on alkylphenols in seawater. The test materials will comprise:

- A standard solution containing known concentrations of the alkylphenols
- Two SPE-columns spiked with alkylphenols

Other matrices and determinands may be added to this study

DE-12 Alkylphenols in sediment

As a result of the alkylphenol workshop in Berlin 2006, a development exercise is initiated on alkylphenols in sediment. The test materials will comprise:

- A standard solution containing known concentrations of the alkylphenols
- Two homogenized unspiked sediments

DE-13 Passive sampling

Acceptance of the list with the priority substances of the new Water Framework Directive, resulted in a intensive discussion about sampling procedures on organic contaminants like e.g. PAHs. Passive sampling can become an important procedure to measure concentrations of these determinands in seawater. Therefore a development exercise will be offered in the new proficiency testing scheme. The test materials will comprise:

- A standard solution containing known concentrations of PAHs
- Two passive sample strips spiked with PAHs.

Other determinands may be added to this study following the SAG meeting.

4. How to Participate in the QUASIMEME Laboratory Performance Studies

Participation is open to any organisation, world-wide.

1. Consult the enclosed information on the QUASIMEME LP studies, the timetable and the programme.
2. Select the test materials required.
3. Complete the application form (included in this document, or available as an electronic application form from the QUASIMEME website or by e-mail from the QUASIMEME Project Office).
4. Enter the appropriate fee from the table.
5. Send the completed application form to the QUASIMEME Project Office, preferably by e-mail.
6. **DO NOT** send any money with the application form. The QUASIMEME Project Office will invoice your institute within two weeks. Details of how to pay will be provided with the invoice.
7. The invoice should be paid in Euros within **30 days of receipt**.
8. In case of excessive delay in payment of the invoice, additional costs may be charged.

4.1 The Costs for June 2007 to May 2008

Orders received before 30 November 2007 for the 2007 - 2008 QUASIMEME year will be charged using the pricing structure given below. The costs include the test materials, shipment to your institute, replacement test materials through breakage or loss in transit, QUASIMEME helpdesk, assessments and electronic copies of reports, invitation to the QUASIMEME workshops at preferential registration fee, and a copy of the QUEST program. (See section 1.4 for details of the subscription.) Please note that the costs are given in Euros.

A data assessment report, of your data and z-scores will be provided for each study for which you return data, both on paper and electronically. In addition, you will receive the generic LP study report for each study in which you participate. QUASIMEME continues to work towards a paperless programme. The LP study reports including the cover sheets are provided electronically for participants to print their own report, as they require.

Permanent membership of Quasimeme

Laboratories can subscribe annually or choose to subscribe for an indefinite period ("becomes a permanent member of Quasimeme"). We will give an example of the latter. You subscribe to a number of groups in 2007 and indicate that you wish to subscribe for an indefinite period. In 2008, the Quasimeme Project Office will send you in March an overview of the groups and services you have subscribed to. You can add or delete groups as you wish or terminate the participation. If you do not communicate any changes before a stated deadline, we will assume that you will continue the subscription on the same basis and will send you an invoice accordingly. The contract for 2008 will be the basis for the renewal in 2009 and so on. Subscribing for an indefinite period has a number of advantages:

1) You do not have to complete the subscription form every year, you only have to notify us about changes in your participation;
2) We only charge handling fees when you start the subscription for the indefinite period and when changes are made;
3) You will receive a discount of 3% on the subscription fee.

Please tick the appropriate box on the subscription form if you wish to subscribe for an indefinite period.

Costs for the QUASIMEME Laboratory Performance Studies			
Group Number	**Costs per group (Euro, €)**	**Group Number**	**Costs per group (Euro, €)**
AQ-1	550	BT-1	650
AQ-2	650	BT-2	650
AQ-3	600	BT-3	650
AQ-4	575	BT-4	650
AQ-5	425	BT-5	500
AQ-6	400	BT-7	650
AQ-7	400	BT-8	650
AQ-8	425	BE-1	625
AQ-11	650	BS-1	600
AQ-12	500	DE-10	650
AQ-13	500	DE-11	500
MS-1	550	DE-12	550
MS-2	550	DE-13	650
MS-3	550		
MS-6	550		

A discount of 5% of the total amount is applied for laboratories subscribing to 5 or more groups.
A discount of 10% of the total amount is applied for laboratories subscribing to 10 or more groups.

A handling fee of €25 is added to all orders. Customs charges and bank handling charges are for the account of the customer.
VAT (19%) is charged on all orders from Dutch laboratories and on orders from any laboratories in other EU countries if the VAT number is not provided with the order.

In some cases, packages of test materials remaining from development exercises may remain. When available, these packages can be obtained for €450

Under certain circumstances it may be possible to subscribe for one in stead of two rounds. Please contact the Quasimeme project office for more information.

Quasimeme participants may purchase additional test materials. The availability depends on the stock. When an order is made, the Quasimeme Project Office will provide a list of materials from which the customer can make a selection. The order takes effect if the customer confirms the selection of one or more materials. The following costs apply for individual test materials:

1 test material	€175
2 test materials	€150 each
3 test materials	€135 each

We do not permit the purchase of more than 3 of any single test material. QUASIMEME does not supply test materials for ring tests not co-ordinated by QUASIMEME.

If you have any queries please do not hesitate to contact the QUASIMEME Project Office.

4.2 Application Form

QUASIMEME welcomes subscribers at any time during the year. However, to ensure that we can send the first set of test materials on time, please help us by returning your application form before 15 May 2007 where possible, to:

QUASIMEME Laboratory Performance Studies
Wageningen UR, Alterra CWK
P.O. Box 47
6700 AA Wageningen
The Netherlands

Phone: +31 (0) 317 48 65 46 (Direct Line)
Fax: +31 (0) 317 41 90 00
e-mail: Quasimeme@wur.nl

Please type or print the information requested below. An electronic version of this form is available on the QUASIMEME website or by e-mail from the QUASIMEME Project Office.

Group	Number required	Group	Number required	Group	Number required	Group	Number required
AQ-1		AQ-11		BT-1		BE-1	
AQ-2		AQ-12		BT-2		BS-1	
AQ-3		AQ-13		BT-3			
AQ-4				BT-4		DE-10	
AQ-5		MS-1		BT-5		DE-11	
AQ-6		MS-2		BT-7		DE-12	
AQ-7		MS-3		BT-8		DE-13	
AQ-8		MS-6					
Enter total number of Groups selected							
Handling fee						€ 25	
Enter total cost						€	

I wish to participate in the QUASIMEME Laboratory Performance Studies as indicated above. I agree to the conditions as given in this brochure.

Yes, I wish to become a permanent member of Quasimeme ☐

For benefits see Quasimeme brochure page 31

Contact name for **invoice**			
QUASIMEME Laboratory code (if applicable)			
Institute			
Address			
Town / City			
Region / State			
Country			
Telephone number	Fax number		
E-mail address			
VAT no[1].			
Your reference or purchase order number			
Signature:			
Date:			

Delivery address for the test materials and reports, <u>if different from previous page</u>:

[1] The VAT number must be entered for all (non Dutch) EU institutes to avoid VAT being added.

Contact name for delivery of **test materials** and **reports**	
Test material groups	
QUASIMEME Laboratory code (if applicable)	
Institute	
Address	
Town / City	
Region / State	
Country	
Telephone number	
Fax number	
E-mail address	

Contact name for delivery of **test materials** and **reports**	
Test material groups	
QUASIMEME Laboratory code (if applicable)	
Institute	
Street / PO Box no.	
Town / City	
Region / State	
Country	
Telephone number	
Fax number	
E-mail address	

Additional contact names for the QUASIMEME newsletter.

Contact name	E-mail address

4.3 New Determinands and Matrices - Questionnaire

Participants and co-ordinators of national monitoring programmes may request that additional determinands and matrices be incorporated into the QUASIMEME Laboratory Performance (LP) studies. The QUASIMEME Project Office and the Scientific Assessment Group will assess these requests, and where there is sufficient demand every attempt will be made to include them in the programme. A number of determinands and matrices have been proposed for inclusion in the QUASIMEME LP studies. Please indicate whether you would participate in an LP study that included these determinands or matrices. We would like to have as full a response as possible. Please complete this questionnaire, even if you wish to decline the offer, and return the form to the QUASIMEME Project Office. (An electronic version of this form is available on the QUASIMEME website or by e-mail from the QUASIMEME Project Office.)

Name			
Institute Name			
Address			
Country			
Laboratory Code (if applicable)			
Please complete each section	Would participate now (next 12 months)	May participate in the future	Would not participate
Biota			
Methyl-mercury			
Alkyl phenol ethoxylates (endocrine disruptors)			
Oil (total and aliphatic)			
PSP shellfish toxins			
PAH metabolites in fish bile			
Sediment			
Dioxins and planar CBs			
Toxaphene			
Oil (total and aliphatic)			
Bulk properties: carbon, hydrogen, nitrogen, phosphorus, carbonate, water content			
Dredge Spoil			
Trace metals			
Organochlorine compounds			
PAHs			
Oil (total and aliphatic)			
Seawater			
Particle size			
Suspended solids			
Chiral compounds			
Standard solutions			
Trace metals in standard solution			
Chlorinated organics in standard solution			
Comments			
Other studies (please suggest)			

Annex I

Organisation and Structure of the QUASIMEME Laboratory Performance Studies

The QUASIMEME Project Office

The QUASIMEME Project Office at FRS Marine Laboratory, Aberdeen, United Kingdom was established for the EU funded project, QUASIMEME I (1992-1996), and continued to operate as the project co-ordination centre for QUASIMEME from 1996 to 2005, when co-ordination of the project transferred to Wageningen University and Research Centre. A small team runs the QUASIMEME LP studies at Wageningen University and Research Centre. Roles and responsibilities of the QUASIMEME team are outlined in the table below. The contact details for the QUASIMEME Project Office are given on the first page of this document.

Name	Role	Responsibilities
Wim Cofino	Project director	Manager and Scientific Director of the QUASIMEME Laboratory Performance studies.
Steven Crum	Project co-ordinator	Co-ordination and organisation of the QUASIMEME Laboratory Performance studies Preparation of test materials Homogeneity and stability testing Test material dispatch Data assessment and Statistics Quality Assurance
Joop Harmsen	Scientific co-ordinator	Scientific support Development exercises and workshops (seawater and sediment)
Monique Jansen	Project administrator	Maintenance of the QUASIMEME database and subscriptions Data entry and retrieval QUASIMEME finances Data assessment Help desk Secretariat to the QUASIMEME Scientific Assessment Group and Advisory Board.
Arriënne Matser	Project assistance	Test material dispatch Preparation of test materials Data entry and retrieval Data assessment

The QUASIMEME Scientific Assessment Group

The QUASIMEME Scientific Assessment Group (SAG) gives advice on the implementation of the scientific programme to the QUASIMEME Project Office and oversees the data assessments and reports on the results of the Laboratory Performance (LP) studies.

The QUASIMEME Project Manager appoints the members of the SAG, which consist of experts in the field of QA and the assessment of LP studies. The members have experience in the design and operation of LP studies and / or environmental measurements in matrices related to the marine environment. The QUASIMEME Project Director is the chairman of the SAG.

Membership of the SAG is confirmed annually. The membership of the SAG will be sufficient in number and breadth of experience to adequately cover the areas included in the QUASIMEME LP studies. Therefore, the size of this group may change in accordance with the needs of the LP studies. The SAG may recommend specialists to the QUASIMEME Project Director to be invited to contribute to specific QUASIMEME activities as required. The contact details for members of the SAG are given following this section

Terms of Reference of the QUASIMEME Scientific Assessment Group

The terms of reference for the SAG were agreed at the annual SAG meeting, 24 - 25 June 1999, and confirmed annually at Scientific Assessment Group meetings.

The SAG will meet at least annually to advise and assist the QUASIMEME Project Office on:

1. The design of the QUASIMEME LP studies and provision of test materials and protocols.

2. The assessment of the LP studies and study reports.

3. The preparation of documentation, both printed and electronic.

4. The preparation of a progress report to the QUASIMEME Advisory Board which will include:
   - An executive summary of the LP studies for the current year.
   - Recommendations of changes in structure or content of the LP studies.
   - A proposed work programme for future LP studies.

   After presentation to the Advisory Board, a progress report will be published.

5. The SAG will review and make recommendations to the QUASIMEME Project Office on the composition and breadth of expertise which is required to maintain the objective assessment of the programme and the results of the participants' studies.

Membership of the QUASIMEME Scientific Assessment Group

Name	Address	Tel	Fax	E-mail
Prof. Dr Wim Cofino (Chairman)	QUASIMEME Project Director, Alterra, Wageningen University and Research Centre, PO Box 47, 6700 AA Wageningen, The Netherlands	+31 317 486547	+31 317 419000	wim.cofino@ wur.nl
Prof. Dr Jacob de Boer	Vrije Universiteit Amsterdam, Institute for Environmental Studies, Faculty of Earth and Life Sciences, De Boelelaan 1105, 1081 HV Amsterdam, The Netherlands	+31 20 598 7777	+31 20 598 5611	jdeboer@ ivm.vu.nl
Ing. Steven Crum	Alterra, Wageningen University and Research Centre, PO Box 47, 6700 AA Wageningen, The Netherlands	+31 317 474346	+31 317 419000	steven.crum@ wur.nl
Dr Ian Davies	FRS Marine Laboratory, Victoria Road, Aberdeen, AB11 9DB, United Kingdom	+44 1224 295468	+44 1224 295511	daviesim@ marlab.ac.uk
Miss Ulla Eriksson	Department of Applied Environmental Sciences, Stockholm University, S-106 91 Stockholm, Sweden	+ 46 8 674 7175	+ 46 8 674 7637	ulla.eriksson@ itm.su.se
Dr Joop Harmsen	Alterra, Wageningen University and Research Centre, PO Box 47, 6700 AA Wageningen, The Netherlands	+31 317 486432	+31 317 419000	joop.harmsen@ wur.nl
Dr Philipp Hess	Marine Institute, Rinville, Oranmore, Galway, Republic of Ireland	+353 91 387246	+353 91 387201	Philipp.hess@ marine.ie
Ing. Ton van der Zande	National Institute for Coastal and Marine Management/RIKZ, Kerklaan 30, 9751 NN, Haren, The Netherlands	+31 50 533 1301	+31 50 534 0772	a.e.vdzande@ rikz.rws.minvenw. nl
Dr Patrick Roose	Management Unit of the North Sea Mathematical Models, 3e & 23e Linieregimentsplein, 8400 Oostende, Belgium	+32 5970 0131	+32 5970 4935	P.Roose@ mumm.ac.be
Dr David Wells (Advisor)	Manna Associates, Àrdan Gràs, Corsehill, Denside of Durris, By Banchory, Kincardineshire, AB31 6EB, United Kingdom	+44 1330 811007		david@mannadew .plus.com
Dr Gerhard G Rimkus (Correspondence member)	European Commission, DG Health and Consumer Protection, Food and Veterinary Office (FVO), F5.3 Residues, Grange, Dunsany, Co. Meath, Republic of Ireland	+353 46 9061 893	+353 46 9061 703	Gerhard.Rimkus @cec.eu.int
Ms Monique Jansen (Secretariat)	QUASIMEME Project Office, Alterra, Wageningen University and Research Centre, PO Box 47, 6700 AA Wageningen, The Netherlands	+31 317 486546	+31 317 419000	monique.jansen@ wur.nl

The QUASIMEME Advisory Board

The QUASIMEME Laboratory Performance (LP) studies will have an Advisory Board to advise the Project Office and the Scientific Assessment Group on matters relating to external quality assessment in support of environmental measurements related to the marine environment.

QUASIMEME Advisory Board will consist of representatives from organisations to which QUASIMEME participants submit environmental monitoring data:

1. A representative from the Oslo and Paris Commission (OSPAR) to maintain communication with OSPAR, particularly in relation to the QA requirements of the Joint Assessment and Monitoring Programme (JAMP).

2. A representative to maintain communication with the Helsinki Commission (HELCOM), particularly in relation to the QA requirements of the Baltic Monitoring Programme (BMP) and the Coastal Monitoring Programme (CMP).

3. A representative to maintain communication with the International Council for the Exploration of the Sea (ICES).

4. A representative to maintain communication with the Mediterranean Pollution Programme (MEDPOL).

5. A representative of the QUASIMEME Scientific Assessment Group (if not represented by any other member of the Advisory Board).

6. Representatives of national monitoring programmes. Two representatives from national monitoring programmes will be invited based on the national levels of participation in QUASIMEME. Representatives of other national monitoring programmes may request to attend.

7. The QUASIMEME Project Director.

8. A representative to maintain communication with the European Environmental Agency.

9. A representative to maintain communication with the Arctic Monitoring and Assessment Programme (AMAP).

The organisations represented will be responsible for nominating their member of the QUASIMEME Advisory Board. The membership of the QUASIMEME Advisory Board is given following this section.

Terms of Reference of the QUASIMEME Advisory Board

The membership and terms of reference of the Advisory Board were reviewed and revised by the QUASIMEME Scientific Assessment Group, 24 - 25 June 1999, and agreed by the QUASIMEME Advisory Board, 10 - 11 October 1999, and are confirmed annually at Advisory Board meetings.

The Advisory Board will meet at least annually to:

1. Advise on matters relating to the Quality Assurance and Quality Control requirements for the national and international marine monitoring programmes and to provide links with these programmes.

2. Provide information and advice on the list of determinands required for the national and international monitoring programmes, the matrices and the concentration ranges. Where lists of studies in the current LP studies are being revised, the Board shall indicate the relative priority of the studies to be undertaken.

3. Advise on the level of performance required for specific monitoring programmes in terms of precision and bias for each determinand - matrix combination.

4. Receive and comment on the Progress Report of the QUASIMEME LP studies.

5. Appoint the chairman of the Advisory Board and review the membership of the Advisory Board.

6. Review and revise the terms of reference of the Advisory Board, as necessary.

7. Advise on the management of the QUASIMEME LP studies.

Membership of the QUASIMEME Advisory Board

Name	Address	Tel	Fax	E-mail
Dr David Wells (Chairman)	Manna Associates, Àrdan Gràs, Corsehill, Denside of Durris, By Banchory, Kincardineshire, AB31 6EB, United Kingdom	+44 1330 811007		david@mannadew.plus.com
Prof. Dr Wim Cofino (QUASIMEME Project Director)	Alterra, Wageningen University and Research Centre, PO Box 47, 6700 AA Wageningen, The Netherlands	+31 317 486547	+31 317 419000	wim.cofino@wur.nl
Ms Monique Jansen (Secretariat)	QUASIMEME Project Office, Alterra, Wageningen University and Research Centre, PO Box 47, 6700 AA Wageningen, The Netherlands	+31 317 486546	+31 317 419000	monique.jansen@wur.nl
Ms Julie Gillin (ICES)	Manager of the Data Centre, ICES, H.C Andersen Boulevard 44 – 46, DK1553 Copenhagen V, Denmark	+45 3338 6712	+45 33 93 4215	julie@ices.dk
Dr Elisabeth Sahlsten (HELCOM)	SMHI Oceanographic Services, Nya Varvet 31, V. Frölunda, 426 71, Sweden	+46 31 751 8990	+46 31 751 8980	elisabeth.sahlsten@smhi.se
Dr Patrick Roose (OSPAR)	Management Unit of the North Sea Mathematical Models, 3e & 23e Linieregimentsplein, 8400 Oostende, Belgium	+32 5970 0131	+32 5970 4935	P.Roose@mumm.ac.be
Vacancy (MEDPOL)				
Dr Jarle Klungsoyr (AMAP)	AMAP Secretariat, Strøømsveien 96, PO Box 8100 Dep., N-0032 Oslo, Norway	+47 22 57 34 00	+47 22 67 67 06	Jarle.klungsoeyr@imr.no
Colin Allchin (UK NMCAQC)	CEFAS, Remembrance Avenue, Burnham-on-Crouch, Essex CMO 8HA,United Kingdom	+44 1621 787200	+44 1621 784989	colin.allchin@cefas.co.uk
Dr Peter Lepom (Federal Environmental Agency)	Federal Environmental Agency, FG II 3.6, POB 33 00 22, D14191 Berlin, Germany	+49 30 8903 2689	+49 30 8903 2965	peter.lepom@uba.de
Dr. Daniel Cossa (EEA)	Laboratory of Biogeochemistry of Metalic Contaminants Department Biogeochemistry and Ecotoxicology Institut Français de Recherche pour l'Exploitation durable de la Mer (IFREMER) BP 21105, 443311Nantes cedex 03, France			Daniël.cossa@ifremer.fr
Dr Richard Emmerson (Correspondence member – OSPAR)	OSPAR, New Court, 48 Carey St, London WC2A 2JE, United Kingdom	+44 171 242 9927	+44 171 430 2999	secretariat@ospar.org
Mr Juha-Markku Leppänen (Correspondence member – HELCOM)	Professional Secretary, Helsinki Commission, Katajanokanlaituri 6B, FIN 00160 Helsinki, Finland	+358 9622 02227	+358 9622 02239	juha-markku.leppanen@helcom.fi
Dr. Franco Giovanardi	Instituto Centrale Per la Ricerca, Via di Casalotti, 300 00166 ROMA, Italy	+39 06 61570 401	+39 06 61561 906	f.giovanardi@icram.org

QUASIMEME Collaborators[i]

	Contact	Address	Tel.	Fax	E-mail
Aqueous test material preparation	Mr Steven Crum	Alterra, Wageningen University and Research Centre, PO Box 47, 6700 AA Wageningen, The Netherlands	+31 317 474346	+31 317 419000	steven.crum @wur.nl
Nutrient test material homogeneity and stability testing	Mr Marc Knockaert	Management Unit of the North Sea Mathematical Models, 3e & 23e Linieregimentsplein, 8400 Oostende, Belgium	+32 59 242058	+32 59 700 131	m.knockaert @mumm.ac.be
Sediment test material preparation including homogeneity and stability testing	Dr Stephen de Mora	International Atomic Energy Agency (IAEA), Marine Environment Laboratory, 4 Quai Antoine 1er, BP800, MC98012, Monaco	+377 9797 7236	+377 9797 7276	S.de_Mora@ iaea.org
Sediment test material preparation including homogeneity and stability testing	Bram Eijgenraam	WEPAL, Dreijenplein 10, 6703 HB Wageningen,The Netherlands	+31 317 482349/48 2339	+31 317 485666/ 483766	Info.wepal@ wur.nl
Biological test material: preparation	Peter Korytar	Wageningen Imares, Institute for Marine Resources & Ecosystem Studies PO Box 68, 1970 AB IJmuiden, The Netherlands	+31 255 56 4607	+31 255 564 644	Peter.korytar @wur.nl
Biological test material: homogeneity and stability testing Organotin compounds	Drs Jan Willem Wegener	Vrije Universiteit Amsterdam, Institute for Environmental Studies, De Boelelaan 1105, 1081 HV Amsterdam, The Netherlands	+31 20 598 9517	+31 20 598 5611	Jan.willem. wegener@ ivm.vu.nl
Isochrysis galbana culture for Chlorophyll *a* analyses.	Dr. Pauline Kamermans	Wageningen Imares, Institute for Marine Resources & Ecosystem Studies, PO Box 77, 4400AB Yerseke, The Netherlands	+31 113 672302	+31 113 573477	Pauline. Kamermans @wur.nl
Imposex/Intersex test materials	Dr Ian Davies	FRS Marine Laboratory, PO Box 101, Victoria Road, Aberdeen, AB11 9DB, United Kingdom	+44 1224 295468	+44 1224 295511	daviesim@ marlab.ac.uk
Shellfish toxin test material preparation, including homogeneity and stability testing	Dr Phillip Hess	Marine Institute, Rinville, Oranmore, Galway, Republic of Ireland	+353 91 387246	+353 91 387201	philipp.hess@ marine.ie

	Contact	Address	Tel.	Fax	E-mail
QUASIMEME database	Dr Paul McKay	R&D Software, 8 Mearns Walk, Stonehaven, Aberdeenshire, AB39 2DG, United Kingdom	+44 1569 763543		paul@mckay.demon.co.uk
Imposex/Intersex test materials	Johan Jol	Wageningen Imares, Institute for Marine Resources & Ecosystem Studies, PO Box 77, 4400AB Yerseke, The Netherlands	+31 113 67 2308	+31 255 56 4644	Johan.jol@wur.nl

[i] The list of QUASIMEME collaborators is correct at time of printing (March 2007). Collaborators may change during the year.

Annex II

The Z-Scores

A z-score [1] is calculated for each participant's data for each matrix / determinand combination which is given an assigned value. The z-score is calculated as follows:

$$z\text{-score} = \frac{\text{Mean from Laboratory} - \text{Assigned Value}}{\text{Total Error}}$$

It is emphasized that in many interlaboratory studies the between-laboratory standard deviation obtained from the statistical evaluation of the study is used as 'total error' in the formula above. In Quasimeme the total error is estimated independently taking the needs of present-day international monitoring programs as starting point. For each determinand in a particular matrix, a proportional error (PE) and a constant error (CE) have been defined. The total error depends on the magnitudes of these errors and on the assigned value:

$$\text{Total Error} = \frac{\text{Assigned Value} \times \text{Proportional Error (\%)}}{100} + 0.5 \times \text{Constant Error}$$

The values for the PE and CE are set by the Scientific Assessment Group and are monitored annually. The values are based on the following criteria:

Consistency of the required standard of performance to enable participating laboratories to monitor their assessment over time.

Achievable targets in relation to the current state of the art and the level of performance needed for national and international monitoring programmes.

The assessment is based on ISO 43 as z-scores. The QUASIMEME model is designed to provide a consistent interpretation over the whole range of concentration of analytes provided, including an assessment where Left Censored Values (LCVs) are reported.

The proportional error is set at 6% for nutrients and for standard solutions, and 12.5% for all other matrices. This applies to all determinands. The constant error has been set for each determinand or determinand group (e.g. chlorinated biphenyls). This value was initially set to reflect the limit of determination, but is at present more closely related to the overall laboratory performance. The magnitude of the CE is set to provide a constant assessment in terms of z-score regardless of concentration. Therefore at low concentrations the level of accuracy required to obtain a satisfactory z-score is less stringent than at a high concentrations.

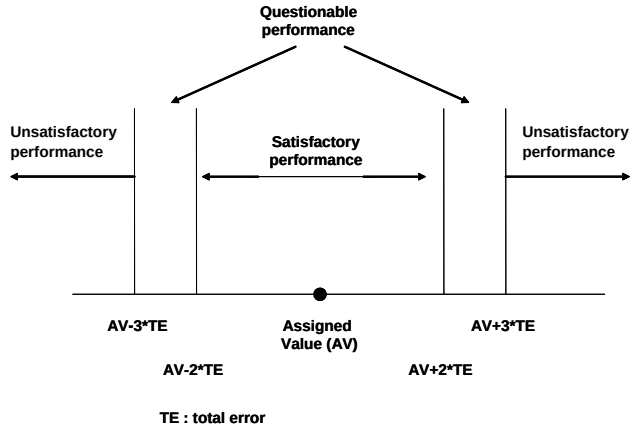
The performance of the laboratories is examined in detail when the total error exceeds 50% of the consensus concentration. If there is good agreement between the laboratories, i.e. the criteria to set an assigned value are met, the CE may be revised to a lower value reflecting the performance of laboratories for this measurement at lower concentrations. These revisions are undertaken at the time of the assessment and ratified by the Scientific Assessment Group. In making any adjustments to the CE an overall assessment of performance at these lower concentrations over a number of different rounds is reviewed. This provides evidence of a long-term trend of improved performance rather than a single set of data. When the agreement is judged to be insufficient, no assigned value is established. In such cases an indicative value is given.

Following usual practices e.g. ISO 43, the z-scores can be interpreted as follows for laboratories which take part in Quasimeme to assure the quality of their data for use in international marine monitoring programmes:

[1] International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories. M Thompson, R Wood, Journal of AOAC International Vol. 76, No. 4, 1993

|z| < 2 Satisfactory performance
2 < |z| < 3 Questionable performance
|z| > 3 Unsatisfactory performance

The following figure illustrates the interpretation of the z-scores:



|z| > 6 frequently points to gross errors (mistakes with units during reporting, calculation or dilution errors, and so on).

It is not possible to calculate a z-score for left censored values (LCV's). Quasimeme provides a simple quality criterion:

LCV/2 < (concentration corresponding to |z|=3): LCV consistent with assigned value
LCV/2 > (concentration corresponding to |z|=3): LCV inconsistent with assigned value, i.e. LCV reported by laboratory much higher than numerical values reported by other laboratories.

Annex III

List of abbreviations

BDE = Brominateddiphenylether
CB = Chlorobiphenyl
CHB = Chlorobornane
DDD = Dichlorodiphenyldichloroethane
DDE = Dichlorodiphenyldichloroethylene
DDT = Dichlorodiphenyltrichloroethane
HBCD = Hexabromocyclododecane
HCB = Hexahlorobenzene
HCBD = Hexachlorobutadiene
HCH = Hexachlorocyclohexane
HpCDD = Heptachlorodibenzodioxin
HpCDF = Heptachlorodibenzofuran
HxCDD = Hexachlorodibenzodioxin
HxCDF = Hexachlorodibenzofuran
OCDD = Octachlorodibenzodioxin
OCDF = Octachlorodibenzofuran
PAHs = Polycyclic aromatic hydrocarbons
PeCDD = Pentachlorodibenzodioxin
PeCDF = Pentachlorodibenzofuran
TBBP-A = Tetrabromobisphenol A
TCB = Trichlorobenzene
TCDD = Tetrachlorodibenzodioxin
TCDF = Tetrachlorodibenzofuran
TEQ = Toxic equivalent
TOC = Total organic carbon

Costs for the QUASIMEME Laboratory Performance Studies 2007-2008			
Group Number	**Costs per group (Euro, €)**	**Group Number**	**Costs per group (Euro, €)**
AQ-1	550	BT-1	650
AQ-2	650	BT-2	650
AQ-3	600	BT-3	650
AQ-4	575	BT-4	650
AQ-5	425	BT-5	500
AQ-6	400	BT-7	650
AQ-7	400	BT-8	650
AQ-8	425	BE-1	625
AQ-11	650	BS-1	600
AQ-12	500	DE-10	650
AQ-13	500	DE-11	500
MS-1	550	DE-12	550
MS-2	550	DE-13	650
MS-3	550		
MS-6	550		

A discount of 5% of the total amount is applied for laboratories subscribing to 5 or more groups.
A discount of 10% of the total amount is applied for laboratories subscribing to 10 or more groups.

A handling fee of €25 is added to all orders. Customs charges and bank handling charges are for the account of the customer.
VAT (19%) is charged on all orders from Dutch laboratories and on orders from any laboratories in other EU countries if the VAT number is not provided with the order.

In some cases, packages of test materials remaining from development exercises may remain. When available, these packages can be obtained for €450

Under certain circumstances it may be possible to subscribe for one in stead of two rounds. Please contact the Quasimeme project office for more information.

Quasimeme participants may purchase additional test materials. The availability depends on the stock. When an order is made, the Quasimeme Project Office will provide a list of materials from which the customer can make a selection. The order takes effect if the customer confirms the selection of one or more materials. The following costs apply for individual test materials:

1 test material	€175
2 test materials	€150 each
3 test materials	€135 each

We do not permit the purchase of more than 3 of any single test material. QUASIMEME does not supply test materials for ring tests not co-ordinated by QUASIMEME.

Terms of participation

The QUASIMEME LP Studies will be open and available to any organisation for the purposes of providing support to laboratories' QA programme through participation in external quality assurance scheme.

The LP Studies is financed by the participants. Each participant will be required to pay a fee for each group of test materials required. The list of test materials in each group and the scale of charges for the current year are given on this site and in the brochure which can be downloaded. The cost of the LP Studies includes the following:

(i) Provision of the test materials delivered to the stated address.
(ii) Provision of information and/or protocols to carry out that study.
(iii) Provision of the QUEST (QUASIMEME Electronic Storage and Transfer system) programme and instructions for installing and use. Any updates necessary will be provided free of charge.
(iv) An assessment of the study and a report giving the details of the exercise.
(v) Provision of a helpdesk and support relating to any matters of the LP Studies. Where a laboratory experiences long term difficulty on a particular analysis then the participant will be advised to contact an expert in that field to help with the solution to the problem.
(vi) Access to the restricted part of the interactive website.
(vii)QUASIMEME newsletter and bulletin and other publications of QUASIMEME.
(viii)Invitation for attendance at all QUASIMEME workshops. Currently the cost of travel and accommodation is to be covered by the participant. QUASIMEME may require a registration fee to cover the cost of the conference facilities and invited speakers.

Each test material will be provided in a suitable container, correctly labelled with the hazard warning and international codes for transport. It will be homogeneous and stable for the duration of the study period and sufficient for the determination. The amount of material required will be determined by the Scientific Assessment Group. In most cases there will be sufficient material for multiple determination. Also the test material may be stable for considerable period beyond the study period. Additional test material will only be provided when a participant can demonstrate that the amount provided was inadequate. Additional test material will be charged on a pro rata basis. Only one set of data will be assessed from each participant for each test material, except where replicate data are specifically requested. Sharing test materials between participants is positively discouraged due to the high risk of contamination or handling effects on the sample. Each participant should request a separate set of test materials.

The subscription year for the LP Studies shall operate from June 1 to May 30 the following year. There will be four study periods in one subscription year.

The QUASIMEME Project Office will issue a list of test materials to be offered within the LP Studies for the following subscription year at least three months prior to the commencement of that year. Participants will be requested to complete the list of the groups of test materials required, and to return this to the QUASIMEME Project Office at least one month prior to the commencement of the subscription year. The test materials for any period will be dispatched within one month of the commencement of that period. Participants may request test material at any time. The QUASIMEME Project Office will normally despatch the material within one month of receipt of the order if it is during the study period. However, the project office cannot guarantee the timely arrival of the test material if it is ordered after the normal despatch date of that study period.

All participants will be notified of the dispatch of all test materials and an acknowledgement of receipt of these test materials will not normally be required. If no reply is received, it will be assumed by the Project Office that the goods have been received in good condition. It is the responsibility of the participant to notify the Project Office if there is any damage or if the test materials are not received. Participants will be invoiced for all orders received after the first set of test material has been dispatched that year.

A damaged test material will be replaced free of charge, provided that the participant provides details of the damage. However, where a history of damaged goods occur with shipment to any one location the project office may require evidence of the damage in order to claim for that damage in transit with the shipping agent.

The fees and annual subscription will be due, following receipt of the participant's request and dispatch of the first set of test material, regardless of whether the participant submits data on that study. Data will be regarded as submitted for assessment if it is delivered to the QUASIMEME Project Office, in the stated format, on or before the

deadline of that study period. Data which arrive after the deadline may not be included in the assessment.

No subscription fee will be returned to any participant except, in unforeseen circumstances where a study has been cancelled and the test material is not issued. Once a test material has been issued it will not be accepted for return and full payment of the material will be due.

Please type or print the information requested below and e-mail it to the Quasimeme Project office: Quasimeme@wur.nl

Group	Number required	Group	Number required	Group	Number required	Group	Number required
AQ-1		AQ-11		BT-1		BE-1	
AQ-2		AQ-12		BT-2		BS-1	
AQ-3		AQ-13		BT-3			
AQ-4				BT-4		DE-10	
AQ-5		MS-1		BT-5		DE-11	
AQ-6		MS-2		BT-7		DE-12	
AQ-7		MS-3		BT-8		DE-13	
AQ-8		MS-6					
Enter total number of Groups selected							
Handling fee						€ 25	
Enter total cost						€	

I wish to participate in the QUASIMEME Laboratory Performance Studies as indicated above. I agree to the conditions as given in this brochure.

Yes, I wish to become a permanent member of Quasimeme ☐

For benefits see Quasimeme brochure page 31

Contact name for **invoice**			
QUASIMEME Laboratory code (if applicable)			
Institute			
Address			
Town / City			
Region / State			
Country			
Telephone number	Fax number		
E-mail address			
VAT no[1].			
Your reference or purchase order number			
Signature:			
Date:			

Delivery address for the test materials and reports, <u>if different from previous page</u>:

Contact name for delivery of **test materials** and **reports**	
Test material groups	

[1] The VAT number must be entered for all (non Dutch) EU institutes to avoid VAT being added.

QUASIMEME Laboratory code (if applicable)	
Institute	
Address	
Town / City	
Region / State	
Country	
Telephone number	
Fax number	
E-mail address	

Contact name for delivery of **test materials** and **reports**	
Test material groups	
QUASIMEME Laboratory code (if applicable)	
Institute	
Street / PO Box no.	
Town / City	
Region / State	
Country	
Telephone number	
Fax number	
E-mail address	

Additional contact names for the QUASIMEME newsletter.

Contact name	E-mail address

4.3 New Determinands and Matrices - Questionnaire

Participants and co-ordinators of national monitoring programmes may request that additional determinands and matrices be incorporated into the QUASIMEME Laboratory Performance (LP) studies. The QUASIMEME Project Office and the Scientific Assessment Group will assess these requests, and where there is sufficient demand every attempt will be made to include them in the programme. A number of determinands and matrices have been proposed for inclusion in the QUASIMEME LP studies. Please indicate whether you would participate in an LP study that included these determinands or matrices. We would like to have as full a response as possible. Please complete this questionnaire, even if you wish to decline the offer, and return the form to the QUASIMEME Project Office. (An electronic version of this form is available on the QUASIMEME website or by e-mail from the QUASIMEME Project Office.)

Name			
Institute Name			
Address			
Country			
Laboratory Code (if applicable)			
Please complete each section	Would participate now (next 12 months)	May participate in the future	Would not participate
Biota			
Methyl-mercury			
Alkyl phenol ethoxylates (endocrine disruptors)			
Oil (total and aliphatic)			
PSP shellfish toxins			
PAH metabolites in fish bile			
Sediment			
Dioxins and planar CBs			
Toxaphene			
Oil (total and aliphatic)			
Bulk properties: carbon, hydrogen, nitrogen, phosphorus, carbonate, water content			
Dredge Spoil			
Trace metals			
Organochlorine compounds			
PAHs			
Oil (total and aliphatic)			
Seawater			
Particle size			
Suspended solids			
Chiral compounds			
Standard solutions			
Trace metals in standard solution			
Chlorinated organics in standard solution			
Comments			
Other studies (please suggest)			

Confidentiality

QUASIMEME is designed to provide support to laboratories for accreditation and for submission of data to monitoring, research or contracted organizations. It is the full responsibility of the laboratory to disclose information on its participation in QUASIMEME and its performance as part of their quality system. While QUASIMEME gives full support to each laboratory's quality system, it is not in itself part of that system. In setting this standard of confidentiality, QUASIMEME can maintain a position of impartiality. Support can be given to a laboratory which may require assistance to improve performance on a particular study, e.g. using the marine network to allow a participant to contact an experienced laboratory who can provide guidance and specific information. Each laboratory should decide when, and if, it wishes to declare the performance of any specific laboratory study.

The following statements refer to data and information.

1. All reports and information on the performance of individual participants will remain confidential within the QUASIMEME Project Office and the QUASIMEME Scientific Assessment Group. QUASIMEME will not convey any information on data generated by a specific participant in the laboratory performance study to any third party. This also includes the transfer of information from one participant to another within the LP Studies.

2. All individual sets of data generated by each participant for the QUASIMEME LP Studies including information on the assessment of performance of the laboratory such as Z-scores or comments on laboratory performance shall remain the property of the laboratory submitting these data. Information on a specific laboratory may only be used by that laboratory.

3. All general comments made by the QUASIMEME assessors on any study, where there is no specific reference to any identifiable laboratory, shall remain the property of the QUASIMEME LP Studies and be available for publication.

4. QUASIMEME positively encourages each participant to make full use of their own study performance results in support of their Quality System for chemical measurements.

Openness

The overall information on the progress of the QUASIMEME LP Studies will be published in reports and in the open literature. No participant will be identified in these publications. Full use of these reports and papers may be made with due acknowledgement to the LP Studies.