IAEA Marine Environment Laboratories

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E. IMAP MONITORING GUIDELINES FOR QUALITY ASSURANCE

E-1. Monitoring Guidelines/Protocols for Analytical Quality Assurance for IMAP Common Indicators 13, 14, 17, 18 and 20

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1 Guidelines for Analytical Quality Assurance

1.1 Introduction

The Data Quality Assurance (DQA) programme jointly organised and implemented by MED POL and IAEA/MESL since 1986, provided assistance to several Mediterranean laboratories for improving the quality of their monitoring data. As a result, data generation in the Mediterranean basin has been greatly improved in quantity and quality since the early stages of the MED POL Programme. However, there is room for improvement, because important differences still exist in data quality among different Mediterranean laboratories. If the quality of analytical data is not assured, information on contaminant concentrations variations (both in space and time) and on the biological effects of pollutants may be misleading, resulting to erroneous measures to improve the quality of the marine environment. Therefore, the generation of quality assured data is the key component of a marine pollution monitoring programme. Consequently, the Data Quality Assurance programme is a key component of the UNEP/MAP IMAP.

The Data Quality component involves three groups of stakeholders in an ascending order: i) the national laboratories responsible for the collection, analysis and reporting of data; ii) the IMAP users (i.e. MEDPOL Focal Points and national IMAP competent laboratories); iii) IMAP Info System (the UNEP/MAP - INFO/RAC; MED POL). The four Protocols of this Monitoring Guidelines elaborates the Analytical Quality Assurance for IMAP Common Indicators 13, 14, 17, 18 and 20 regarding the following steps of the analytical procedure chain: a) sample collection); b) sample processing; c) determination of hydrographic parameters; d) determination of key nutrients and chlorophyll *a* in water column; as well as determinations of contaminant in relevant matrices and evaluation of their biological effects and d) reporting of monitoring data.

The responsibility of IMAP competent laboratories within the Quality Assurance system is to ensure consistent measurements and accurate analytical data complying with international standards in terms of scientific/analytical QA and within its specific field (ca. chemistry and biology). Therefore, the objective of the Protocols of this Guideline is to assist laboratories working in implementation of IMAP Pollution Cluster to produce analytical data of the required quality. The guideline intends also to help establishing or improving quality assurance management in the laboratories concerned.

1.2 Technical note for a Quality Assurance scheme

The schemes for Quality Assurance and Control of Data for MED POL Monitoring Database and IMAP (Pilot) Info System have been established in two levels. On the first level there is a monitoring data Quality Assurance and Quality Control (QA/QC) for each IMAP Common Indicator; on the second level there is a full Database Quality Management and Reporting Schemes. Quality Assurance addresses the activities the laboratory undertakes to provide confidence that quality requirements will be fulfilled, whereas Quality Control describes the individual measures which are used to actually fulfil the requirements (EURACHEM, 2014[1]).

The main attributes to be fulfilled in view of obtaining "quality data" are completeness, accuracy, consistency, accessibility, timeliness and validity (UNEP/MAP, 2019a[2]), EURACHEM, 2014):

i) Completeness refers to the fact that provided information should include both data (i.e. the parameter of interest) and associated metadata (i.e. environmental information);
ii) Accuracy refers to the degree to which the result of a measurement approaches to a reference value and it is usually studied as two components: trueness and precision. Trueness is expressed quantitatively in terms of bias and precision is usually expressed by statistical parameters which describe the spread of results, typically the standard deviation.
iii) Consistency refers to the attribute of being able to produce results with the same level of performance over time indifferently of external constrains, extending to any type of data (e.g., data and associated metadata);
iv) Accessibility refers to a user's ability to access or retrieved data stored within a database;
v) Timeliness refers to the requisite of the data to be reported in a timely manner, to ensure the maximization of the value of the collected data from a user's perspective; and
vi) Validity refers to the fact that the data quality concept is a fit-for-purpose target and should comply with certain conditions to serve their expected use. These conditions are the Data Control to be defined in accordance to each parameter (UNEP/MAP, 2019a).

It has to be emphasized that Quality Assurance (QA) applies to all aspects of analytical procedures (sampling, sample pre-treatment, analysis and reporting) (ICES, 2004a[3], Annex XXXXVII). Therefore, it is of

[1] EURACHEM Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, (2nd ed. 2014). B. Magnusson and U. Ornemark (eds.) ISBN 978-91-87461-59-0. Available from www.eurachem.org."

2 UNEP/MAP (2019a) UNEP/MED WG.467/13. Schemes for Database Quality and Quality Assurance and Quality Control (QA/QC) of data related to pollution

3 ICES (2004a). ICES Techniques in Marine Environmental Sciences, No 35. Chemical measurements in the Baltic Sea: Guidelines on quality assurance

paramount importance to establish an integrated data Quality Assurance system for each IMAP Common Indicator.

The Quality System has to be provided in a Quality Manual that needs to be maintained and up-to date. In case the laboratory has an accreditation for the specific analyses (nutrients, chl-a, trace elements, organic contaminants, biomarkers) in relevant matrices (sediment/biota/seawater as appropriate), it has to follow the procedures described in the Quality Manual. If the laboratory is not accredited for such analytical determinations, it has to prepare an Internal Standard Operational Procedures (SOPs) for the analytical methods used, which has to be followed in every relevant laboratory activity, in order to establish an internal Quality Assurance. Guidance on the procedures to follow in order to establish a Quality System in an analytical laboratory can be found in the ISO Standard 17025 "General Requirements for the Technical Competence of Calibration and Testing Laboratories" (2017[4]).

A very important part in the organisation of the laboratory is the necessary documentation related to analysis, which includes a clear description of the analytical methods (CIs 13, 14, 17, 18 and 20); a strict keeping of laboratory journals; keeping of instrument journals; laboratory protocols for sample identification; and clear labelling of samples, reference materials, chemicals, reagents, volumetric equipment, stating date, calibration status, concentration or content as appropriate and signature of the person responsible for the analysis (ICES, 2004a, ICES, 2004b[5]).

The QA systems includes the participation of laboratories in interlaboratory comparison exercises (ILCs) and/or Proficiency Tests (PTs) procedures to ensure a known long-term stability of the laboratory's performance, the use of reference materials, and the documentation required (ICES/OSPAR, 2018a[6], Annex VII).

Therefore, under Technical Note for a Quality Assurance Scheme, this Guideline elaborates the following four Protocols to support efforts of national laboratories that are responsible for IMAP implementation:

- Protocol on QA in sample collection;
- Protocol on QA in sample processing;
- Protocol on QA in the determination of hydrographical parameters, dissolved oxygen, pH, nutrients, chlorophyll *a* and contaminants in relevant matrices and biomarker evaluation;
- Protocol on QA in reporting of data.

1.2.1 Protocol on QA in sample collection

Quality Assurance for sample collection includes the following principal elements (ICES, 2004a):

i) A knowledge of the purpose of the investigation is essential to establish the required data quality, which has to be defined by the Contracting Parties;
ii) Provision and optimization of appropriate facilities and sampling equipment;
iii) Selection and training of staff for the sampling task in question;
iv) Establishment of definitive directions for appropriate collection, preservation, storage, and transport procedures to maintain the integrity of samples prior to analysis;
v) Use of suitable Standard Operating Procedures (SOPs) with appropriate Quality Control for sample handling to prevent uncontrolled contamination and/or loss of the determinant in the sample, as well as collection of field blanks; Standard Operating Procedures is a set of step-by-step instructions compiled by an organization to help workers carry out routine operations. SOPs aim to achieve efficiency, quality output and uniformity of performance, while reducing miscommunication and failure to comply with industry regulations
vi) Preparation and use of written instructions, sampling protocols and sampling logs, so that sample collection data can be traced to the relevant samples and vice versa

Field blanks are used to estimate contamination of a sample during the collection and transportation procedure. A field blank is a sample that is prepared in the field to evaluate the potential for contamination of a sample by site contaminants from a source not associated with the sample collected (for example air-borne dust or organic vapors which could contaminate a seawater, sediment or biota sample). Deionized and organic-free water is taken to the field in sealed containers. The water is poured into the appropriate sample containers at pre-designated locations at the site. The containers are preserved according to the procedures for seawater, sediment and biota samples, are transported in the laboratory for analysis (USEPA, 2017[7]).

In case the laboratory has an established quality system for sampling sediment/biota/seawater samples, it has to follow the relevant Standard Operational Procedures. In case the laboratory is accredited for sampling it has to follow its Quality Manual.

Before embarking on a cruise/field trip to collect samples, the following preparations have to be made:

4 ISO/IEC 17025:2017. General requirements for the competence of testing and calibration laboratories

5 ICES (2004b). Biological monitoring: General guidelines for quality assurance. Ed. by H. Rees. ICES Techniques in Marine Environmental Sciences, No. 32.

6 ICES/OSPAR (2018a). CEMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 6: Determination of metals in sediments – analytical methods.

7 USEPA (2017). Field sampling quality control: operational procedure. SESDRPROC-011-05.

i) Setting a sampling strategy, including sampling sites, water depths, kind and number of samples to be collected;
ii) Decide on the sampling methods to be applied and make sure that involved staff is familiar with them;
iii) Cleaning and purification of all equipment, containers and tools to be used for sample collection, pre-treatment and storage;
iv) Preparation for the collection of field blanks;
v) Identification of samples: clear understanding of the information to be recorded on each sample container
vi) Preparation of reagents to be used during sampling;
vii) Preparation of a detailed sampling protocol (such as use of equipment, pre-treatment, blank determination, recording, sample splitting if required, etc.);
viii) Distribute responsibilities to staff.

During sampling the following procedures need to be applied:

i) Implementing the sampling protocol;
ii) Maintaining sample's integrity by using appropriate sampling procedures
iii) Collecting field blanks;
iv) Record all necessary relevant information (such as time, sea condition, sediment characteristics, water turbidity, temperature, etc.);
v) Avoiding sample contamination handling samples according to relevant IMAP Guidelines;
vi) Making sure that all samples/sub-samples are properly identified;
vii) Applying appropriate pre-treatment to samples as required;
viii) Storing and preserving samples and blanks according to the sample preservation and storage protocol, making sure that sample characteristics are not altered;
ix) Maintaining a record of all activities that demonstrates an unbroken control over the sample from collection to its final disposition.

1.2.2 Protocol on QA in sample processing

Quality Assurance for sample processing before analysis includes the following principal elements (ICES, 2004a, Annex XXXXVII):

i) A knowledge of the purpose of the investigation is essential to establish the required data quality;
ii) Provision and optimization of appropriate laboratory facilities and equipment for processing and pre-treatment of samples;
iii) Selection and training of staff for the laboratory task in question;
iv) Use of suitable pre-treatment procedures prior to the analysis of samples, to prevent uncontrolled contamination and loss of the determinant in the samples;
v) Validation of appropriate processing methods to ensure that sample processing will not alter the measurement of the analyte under investigation;
vi) Conduct of regular intra-laboratory checks on the accuracy of routine measurements related to IMAP Common Indicators 13, 14, 17, 18 and 20, including sample processing using Certified Reference Materials (CRMs), to assess whether the processing methods used are remaining under control, and document results on control charts (A CRM is a Reference Material accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realisation of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence);
vii) Preparation and use of written instructions, laboratory protocols, laboratory journals, etc., so that specific data can be traced to the relevant samples and vice versa.

Sample processing as presented in relevant IMAP Guidelines, includes:

i) CI13: Seawater: storage (freezing -20 °C);
ii) CI14: Seawater: - Chlorophyll *a* (pre-filtration (mesh size > 200 µm), filtration (Glass fibre filter GF/F), filter storage (freezing -80 °C)); - salinity (storage);
iii) CI17: Sediment samples: sieving for grain size (< 2 mm and < 63 µm), freeze drying (if appropriate), weighting and storage; Biota samples: measuring length, sex and weight (fresh), dissection to collect appropriate tissue (muscle from fish and whole body for bivalves, freeze drying (if appropriate), weighting and storage; Seawater samples: filtration (0.7 µm GF/F pre combusted glass fiber filters of organic contaminants and 0.45 µm polycarbonate filters for the analysis of heavy metals (except mercury)), filtrate preservation and storage, SPM drying, weighting and storage;
iv) CI18: For all biota samples: measuring length, sex and weight (fresh), eviscerated weight, liver weight, gonad weight and dissection to collect appropriate tissue. For fish samples: taking liver samples for the evaluation of lysosomal membrane stability (LMS), muscle for the evaluation of AChE activity, blood cells from the caudal vein for the evaluation of Micronuclei frequency. Also, there is a need to undertake the following measurements: Fulton's condition factor (K), gonadosomatic index (GSI), Liver Somatic Index (LSI), as well as a storage at -80 °C. For bivalves, a dissection is needed to remove gills for the evaluation of AChE activity and Micronuclei frequency, whilst Haemolymph cells and digestive gland are needed for the evaluation of LMS);

v) CI20: For seafood samples: measuring length, sex and weight (fresh), dissection to collect appropriate tissue, freeze drying, weighting and storage.

In case the laboratory has an accreditation for these processes it has to follow the procedures described in the relevant Quality Manual. If the laboratory is not accredited for such processes, it has to prepare internal Standard Operational Procedures for sample processing, which has to be followed in every relevant laboratory activity, in order to establish an internal Quality Assurance.

All sample processing procedures for sediment, biota, seafood and seawater, should be performed under the same Quality Assurance and with the same requirements as other parts of the analytical chain. Therefore sieving, drying, weighting and storage methods used in the laboratory have to be validated following the appropriate methodology presented in ISO Standard 17025 (2017).

1.2.3 Protocol on QA in the determination of hydrographical parameters, dissolved oxygen, pH, nutrients, chlorophyll *a* and contaminants in relevant matrices and biomarker evaluation

Quality Assurance in the determination of hydrographical parameter, dissolved oxygen, pH, nutrients, chlorophyll *a* and contaminants in appropriate matrices (sediment, marine biota tissues, and seawater) and biomarker evaluation in molluscs and fish, includes the following elements:

i) A knowledge of the purpose of investigation is essential to establish the required data quality;
ii) Provision and optimization of appropriate laboratory facilities and analytical equipment;
iii) Selection and training of staff for the analytical task in question;
iv) Use of suitable procedures during the analysis of samples, to prevent uncontrolled contamination and loss of the determinant in the samples;
v) Validation of appropriate analytical methods to ensure that chemical measurements are of the required quality to meet the needs of the investigations, according the Contracting Parties decision on this matter; For biomarkers, use of appropriate analytical methods as described in the Protocols specific for the analysis of the different biomarkers to ensure that measurements are of the required quality to meet the needs of the investigations;
vi) Conduct of regular intra-laboratory checks on the accuracy of routine measurements, by the analysis of appropriate reference materials for contaminants analysis and blind samples for biomarker determination, to assess whether the analytical methods are remaining under control. Intra-laboratory checks should be continuously performed and the results documented and interpreted using control charts. Immediate corrective action should be undertaken in case intra-laboratory checks indicate a data quality problem;
vii) Yearly participation in inter-laboratory quality assessments (proficiency testing schemes) to provide an independent assessment of the laboratory's capability of producing reliable measurements. Corrective action should be undertaken by concerned laboratories in case inter-laboratory checks indicate a data quality problem;
viii) Quality Assurance responsible staff needs to keep records on the calibration of the equipment used for chemical analysis;
ix) Quality Assurance responsible staff needs to verify that the analysts in charge to the specific analysis follow the analytical methods validated in the laboratory, taking into consideration the following IMAP Monitoring Guidelines: A-1. Monitoring Guidelines/Protocols for sampling and determination of Hydrographic Physical and Chemical Parameters; A-2. Monitoring Guidelines for sampling and determination of key nutrients and chlorophyll a in seawater; B-1. Monitoring Guidelines/ Protocols for sampling and determination of contaminants in sediment; B-2. Monitoring Guidelines/ Protocols for sampling and determination of contaminants in in marine biota; B-3. Monitoring Guidelines/ Protocols for sampling and determination of contaminants in seawater; C-1. Monitoring Guidelines/ Protocols for sampling and determination of biomarkers in marine molluscs (such as *Mytilus sp*.) and fish (such as *Mullus barbatus*; D-1. Monitoring Guidelines/ Protocols for sampling and determination of contaminants in seafood.
x) The preparation and use of written instructions, laboratory protocols, laboratory journals, etc., so that specific analytical data can be traced to the relevant samples and vice versa.

For hydrographic parameters specifically obtained with CTD probes the main QC/QA scheme is related to the calibration and traceability of sensors that mostly depend on the manufacturer. It is important to build this information in the QA. Detailed description of the steps for QA in the determination of hydrographic parameter by CTD are presented in ICES Guidelines, (2004a) (Annex XXXXVII).

For contaminants analysis, LOD and LOQ are method validation parameters that are defined in each laboratory and depend on many things (equipment used, the analytical method, blanks, sample matrix, concentrations of interfering compounds and on the mass of sediment/biota taken for analysis). When reporting monitoring data, laboratories have to include information on concentration values < LOQ and concentration values < LOD, as indicated in the Data

Dictionaries on contaminants concentrations (UNEP/MED WG.467/8)[8].

For metal analysis in sediment, achievable LOQs for Cd and Pb using ICP-MS are 0.01 and 0.2 mg kg^{-1} dry weight (d.w.) respectively, while Cd and Pb LOQs with AAS are 0.5 and 5 mg kg^{-1} (d.w.) respectively. In biota, LOQs of 5 µg kg^{-1} wet weight (w.w.) for Cd, 10 µg kg^{-1} (w.w.) for Hg and 20 µg kg^{-1} (w.w.) for Pb are also achievable. Therefore, every element in each matrix should have its own Quality Control and Quality Assurance (QC/ QA) scheme including relevant LOD and LOQ. A CRM should be included in each batch to confirm that measuring instrument is operating correctly. Detailed description of the steps for QA in the analysis of metals are presented in (ICES/OSPAR (2018a) (Annex VII).

For organic contaminant's analysis (PCBs, HCB, chlorinated pesticides and PAHs), Quality Assurance should include: i) extraction efficiency and clean-up; ii) calibrant and calibration; iii) system performance; iv) long-term stability; v) use of internal standards; and vi) frequent participation in interlaboratory proficiency testing schemes

For chlorinated compounds in sediments LOQs of 0.1 µg kg^{-1} (d.w., sediment fraction < 2mm) for individual PCBs are achievable. Detailed description of the steps for QA in the analysis of chlorinated compounds are presented in ICES/OSPAR (2018b[9]) (Annex XV) and HELCOM (2012a[10]) (Annex XVI).

For polycyclic aromatic hydrocarbons (PAHs), achievable LOQ for each individual component in sediments using GC-MS are 2 µg kg^{-1} (d.w.), while for biota, LOQs of 0.05 to 0.5 µg kg^{-1} (w.w.) for individual PAH compounds are achievable. Detailed description of the steps for QA in the analysis of PAHs in sediments and biota are presented in ICES/OSPAR (2018c[11]) (Annex XVII) and HELCOM (2012b[12]) (Annex XVIII).

For all analyses a procedural blank should be measured with each sample batch and should be prepared simultaneously using the same chemical reagents and solvents (if appropriate) as for the samples. The procedural blank is also very important in the calculation of limits of detection and limits of quantification for the analytical method. In addition, A CRM or an in-house Quality Control Material developed by a laboratory for its own internal use (ISO Guide 80, 2014[13]) should be analysed within each sample batch. Ideally, stability tests should have been undertaken to show that the reference material yields consistent results over time. The analysis of the reference material is primarily intended as a check that the analytical method is under control and yields acceptable precision, but a certified reference material (CRM) of a similar matrix should be analysed periodically in order to check the method bias.

For biomarkers determination (CI18) (i.e. LMS, MNi frequency, AChE activity and SoS), an intra-laboratory programme for the evaluation of blind mollusc and fish samples should be organized and the labs should be involved in the intercalibration activity of the different biomarkers. These activities will ensure that all the labs involved in the programme collect comparable biomarker data. In addition to the elements above described, a laboratory staff should be specifically designated as responsible of the biomarker determination QA, whilst a Biomarker Analysis Register must be created to register the required information. This Register needs to contain the Animals Collection Reports as provided in Monitoring Guidelines/Protocols for Sampling and Sample Preservation of Marine Molluscs (such as *Mytilus sp*.) and Fish (*Mullus barbatus*) for IMAP Common Indicator 18 (UNEP/MED WG. 509/27) and all the information concerning the biomarker determination and reporting.

Validation of analytical methods

Validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled (ISO/IEC 17025). According to EURACHEM, (2014), the performance characteristics commonly evaluated during method validation include:

i) Selectivity, is the extent to which the method can be used to determine particular analytes in mixtures or matrices without interferences from other components of similar behavior
ii) Analytical sensitivity is the change in instrument response which corresponds to a change in the measured quantity (for example an analyte concentration), i.e. the gradient of the response
iii) Working Range of the method is the interval over which the method provides results with an acceptable uncertainty. The lower end of the working range is bounded by the limit of quantification LOQ. The upper end of the

[8] UNEP/MAP (2019b). UNEP/MED WG.467/8. IMAP Pilot Info System and related Quality Assurance Issues; Data Standards and Data Dictionaries; MAP Data Management Policy.

[9] ICES/OSPAR (2018b). CEMP Guidelines for Monitoring Contaminants in Sediments. Technical Annex 2: technical annex on the analysis of PCBs in sediments

[10] HELCOM (2012a). Manual for marine monitoring in the COMBINE programme. Annex B-13, Appendix 2. Technical note on the determination of chlorinated biphenyls in marine sediment

[11] ICES/OSPAR (2018c). CEMP Guidelines for monitoring contaminants in sediments. Technical Annex 3: Determination of parent and alkylated PAHs in sediments

[12] HELCOM (2012b). Manual for marine monitoring in the COMBINE programme. Annex B-13, Appendix 1. Technical note on the determination of Polycyclic Aromatic Hydrocarbons (PAHs) in sediment

[13] ISO GUIDE 80:2014. Guidance for the in-house preparation of quality control materials (QCMs)

working range is defined by concentrations at which significant anomalies in the analytical sensitivity are observed.

iv) Limit of Detection (LOD) of an analytical method is the smallest concentration (the smallest amount) that the analyst can expect to detect with a given degree of confidence. The limit of detection, defined in terms of either concentration (cL) or amount (qL), is related to the smallest measure of response (xL) that can be detected with reasonable certainty in a given analytical method (IUPAC, 1978[14]). According to this definition, the detection limit in chemical analysis is given by:

$$cL(\text{or } qL) = k \cdot Sb/b$$

where Sb = standard deviation of the blank and b = sensitivity (the slope of the standard curve). A value of k = 3 is strongly recommended by IUPAC.

v) Limit of Quantification (LOQ) is the smallest amount or the lowest concentration of a substance that is possible to be determined by means of a given analytical procedure with the established accuracy, precision, and uncertainty. LOQ should be estimated by using the proper standard measurement or standard sample. In practice, LOQ is calculated by most conventions to be the analyte concentration corresponding to the obtained standard deviation of blank samples multiplied by a factor, k =5, 6, 10, based on "fitness for purpose" criteria (EURACHEM, 2014).

vi) Trueness of a measurement is an expression of how close the mean of an infinite number of results (produced by the method) is to a reference value. Since it is not possible to take an infinite number of measurements, trueness cannot be measured. However, a practical assessment of the trueness can be expressed in terms of bias. A practical determination of bias relies on comparison of the mean of the results (x) from the candidate method with a suitable reference value (ref x).

v) Precision of a measurement is a measure of how close results are to one another. It is usually expressed by statistical parameters which describe the spread of results, typically the standard deviation (or relative standard deviation), calculated from results obtained by carrying out replicate measurements on a suitable material under specified conditions.d.

vi) Uncertainty is not a performance characteristic of a particular measurement procedure, but is a property of the results obtained using that measurement procedure, is also a part of the validation procedure. According to EURACHEM (2014) “uncertainty is an interval associated with a measurement result which expresses the range of values that can reasonably be attributed to the quantity being measured. An uncertainty estimate should take account of *all recognized effects* operating on the result. The uncertainties associated with each effect are combined according to well-established procedures

vii) Ruggedness (robustness) of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters. Ruggedness provides an indication of the method’s reliability during normal usage

Detailed guidelines for the full validation of analytical methods can be found in ISO Standard 17025 (2017), ICES (2004a) (Annex XXXXVII), EURACHEM Guide (2014) and EURACHEM/CITAC 2016[15]).

Internal laboratory Quality Control

After developing an analytical system suitable for producing analytical results of the required accuracy, it is important to establish a continuous control over the system and to show that all causes of errors remain the same in routine analyses Therefore continuous quantitative experimental evidence must be provided in order to demonstrate that the stated performance characteristics of the method chosen remain constant (ICES 2004a), by regularly analysed alongside the samples Certified Reference Materials (CRMs), or an in-house Quality Control Material, which have been checked against a relevant CRM. The CRMs or QCMs should be of similar chemical composition, physical properties, and contaminant concentrations as the samples under investigation.

The ICES Marine Chemistry Working Group (MCWG) regularly publishes comprehensive list of suitable CRMs for marine monitoring programmes including certified determinant concentrations. Further information on CRMs can be obtained from the COMAR database (i.e “The international database for certified reference materials” at https://www.comar.bam.de).

The International Atomic Energy Agency (IAEA) produces a variety of CRMs in different matrices, characterised for analytes belonging to one of the following groups: Radionuclides, Trace Elements and Methyl Mercury, Organic Compounds and Stable Isotopes (https://www.iaea.org/services/laboratory-services/analytical-reference-materials-for-laboratories). The on-line catalogue for the available CRMs can be found in the webpage https://nucleus.iaea.org/sites/ReferenceMaterials/SitePages/Home.aspx

A list of CRMs for heavy metals and organic contaminants in marine matrices (sediment, biota, seawater) prepared by ICES (2004a) are presented in Annex XXXXVII, (pages 43-44). A list of available

[14] IUPAC. 1978. Nomenclature, symbols, units and their usage in spectrochemical analysis - II. Spectrochimica Acta, Part B, 33: 242

[15] EURACHEM/CITAC Guide: Guide to Quality in Analytical Chemistry: An Aid to Accreditation (3rd ed. 2016). ISBN 978-0-948926-32-7.

Reference Materials (RMs) and Certified Reference Materials (CRMs) prepared by IAEA (Marine Environmental Studies Laboratory) are presented in Annex XXXXVIII.

The means to demonstrate that the stated performance characteristics of the method chosen remain constant over time, is the completion of Analytical Quality Control Charts (AQCC). An Analytical Quality Control Chart example is the X-Chart, which can be applied using appropriate Certified Reference Materials for heavy metals and organic contaminants in marine matrices (sediment, biota and seawater). A guidance to use simple X-Charts, as well as other methods to perform internal laboratory Quality Control, is provided in ICES (2004a), including the following:

i) Select an appropriate Certified Reference Material (CRM) to be analysed on a regular basis with environmental samples;
ii) Analyse the CRM at least ten times for the given determinant. The analyses should be done on different days spread over a period of time to ensure that the full range of random errors (for within- and between-batch analyses) is covered. This enables a calculation of the total standard deviation (*st*);
iii) Calculate the mean value (x), the standard deviation (*st*), and the following values: $x + 2st$, $x - 2st$, $x + 3st$, $x - 3st$. Use these data to produce the plot.

If the data for the CRM follow a Normal distribution, 95% of them should fall within $x \pm 2st$ (between the Upper Warning Limit and Lower Warning Limit) and 99.7% should fall within $x \pm 3st$ (between the Upper Control Limit (UCL) and Lower Control Limit (LCL)) (Figure 1). However, if one result falls outside the warning limits, the analyst should not doubt the result or take any action provided that the next result falls within the warning limits. Also, if the results on more than 10 successive occasions fall on the same side of the X line (either between X and UWL or X and LWL) then the analyst needs to check the analytical procedure to determine the cause of this error.

For Biomarkers internal Quality Control, and to demonstrate that the analytical method applied is fit for the purpose of the investigations to be carried out, the Biomarkers QA responsible staff shall organise, at least once a year, a biomarker determination using blind samples from control and polluted sites; the results will be reported in the Biomarker Analysis Register. It would be convenient that labs collect extra biological material during monitoring surveys to be used later in time as Biomarker internal Quality Control Material as CRM are not available.

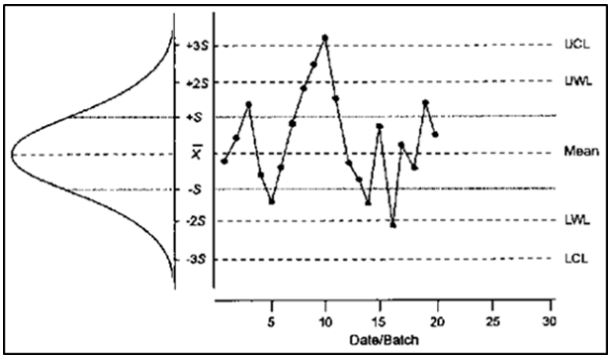

Figure 1. Analytical Quality Control Chart

External Quality Control

The use of validated analytical methods and the performance of internal routine quality control ensures the generation of reliable measurements results within the laboratory. However, laboratories have also to demonstrate that their results are comparable with the results provided by other Mediterranean laboratories participating in implementation of the Integrated Monitoring and Assessment Programme of UNEP/MAP. Therefore laboratories should also participate in external quality assessment processes, i.e. Interlaboratory Comparisons and/or Proficiency Tests, organised by IAEA/MESL or other international/regional organisations, which provide an independent means to detect possible undiscovered sources of errors, demonstrating thus that the analytical quality control of the laboratory is effective.

Proficiency Tests for the determination of heavy metals and organic contaminants (petroleum hydrocarbons including (PAHs), polychlorinated biphenyls (PCBs) and several organochlorine (OC) pesticides) in sediment and biota (fish or bivalves) have been regularly organised since 1986 by UNEP/MAP – MED POL with the collaboration of the IAEA (Marine Environmental Studies Laboratory). In the framework of IMAP participation of designated laboratories in the yearly organised PTs for all target contaminants is mandatory.

The Proficiency Test for the determination of nutrients and chlorophyll-a in seawater has been piloted in the scope of QUASIMEME with assistance of UNEP/MAP – MED POL. However, considering a necessity of comprehensive assistance to support implementation of IMAP Common Indicators 13 and 14, related proposal for inter-calibration proficiency testing and training courses for nutrients and chlorophyll-a will be prepared.

In Proficiency Tests for biomarkers identical sub-samples (test materials) from a uniform homogenized and stable bulk material (sediment, biota or seawater) are sent to the participating laboratories, which are requested to analyse the sample independently of each another. The participating laboratories have to use the methods described in the related protocols as provided in the Guidelines for biomarker analysis CI18 of marine molluscs (such as *Mytilus sp*.) and fish (such as *Mullus barbatus*) (IMAP Monitoring Guidelines: Chapter C Monitoring Guidelines for CI18). The protocols also describe in detail the equipment, the materials, the

chemicals and methodologies to be used in the different biomarker analysis. (ICES, 2010[16], 2011[17]; OSPAR, 2013[18]; Viarengo et al., 2000[19]).

Considering the results of the intercalibration exercise realised in the initial phase of the MEDPOL biomonitoring programme, as well as similar international monitoring programmes (e.g. the EU Funded Research Programme realized in 1998 "The Biological Effects Quality Assurance in Monitoring Programmes (BELQUAM)"; Project "Biological Effects of Environmental Pollution in marine coastal ecosystems" (BEEP) supported by EU in 2002; Background document and technical annexes for biological effects monitoring of OSPAR Commission, as updated in 2013), the inter-calibration testing is proposed to guarantee the comparability of the biomarkers data as provided in document (UNEP/MAP WG. 492/6[20], Annex XXXXIX). Due to the differences in the methodologies used for the collection of the data for different biomarkers, the intercalibration activities are elaborated separately for the four different biomarker analysis' (i.e. for Intercalibration of Lysosomal Membrane Stability (LMS); Intercalibration of Micronuclei frequency (MNi); Intercalibration of Acetylcholinesterase activity (AChE) and Intercalibration of Stress on Stress (SoS)).

Follow-up actions

Proficiency Tests provide objective information on the performance of laboratories in the determination of contaminants, nutrients and biomarkers, indicating issues that need to be taken into consideration in order to improve performance, if necessary. Therefore, laboratories (especially those with unsatisfactory performance) should use the results of the Proficiency Tests for identifying the causes of their unsatisfactory performance in view of correcting them.

Laboratories that face data quality problems but are not in the position to resolve them internally should request external assistance for the identification and solution of potential causes of unsatisfactory performance. It is important to underline that good quality of data can only be achieved if the laboratory is strongly dedicated to improving its performance, as a continuous process.

It should also be noted, that during the last 30 years UNEP/MAP – MED POL with the assistance of the IAEA (MESL) organizes the hand-on training course to assist the Mediterranean laboratories to improve their analytical performance on the determination of heavy metals and organic contaminants in sediment and marine biota samples, complementary to the proficiency testing.

In the future, these training courses should primary target laboratories showing unsatisfactory performance. Following experience in organization of the training courses for IMAP Common Indicator 17, UNEP/MAP – MEDPOL has prepared the proposal of training course aimed at strengthening of the analytical capacities of IMAP competent laboratories to implement IMAP Common Indicator 18 as provided in UNEP/MAP WG. 492/6 (Annex XXXXIX).

1.2.4 Protocol on QA in Reporting of Data

Data quality assurance requires a proper design of functions to ensure a smooth flow of the monitoring process, which starts with the sample collection and ends with the data reporting in the appropriate format (UNEP/MAP 2019b). Therefore, reporting of data and metadata of IMAP is an important task, which has to be implemented by National Laboratories guaranteeing the traceability of the datasets (ICES, 2004a).

A UNEP/MAP Monitoring Guideline on Reporting Monitoring Data for IMAP Common Indicators 13, 14, 17, 18 and 20, as provided in IMAP Monitoring Guidelines, Chapter F. Monitoring Guidelines for Reporting) provides detail reporting templates for IMAP Common Indicators 13, 14, 17, 18 and 20. The objective of this Monitoring Guideline is to assist the laboratories working in marine monitoring to report analytical data in line with the content, format and structure of the database and relationship between its different elements as requested in relevant IMAP Data Standards (DSs) and Data Dictionaries (DDs).

An important insight into the data flows for QA in marine pollution monitoring is to ensure, as much as possible, that the generated data at each process is quality assured by two or more persons, which might not have participated in the chain of analytical procedure (e.g. sampling, processing, analysis and reporting). This means that if solely a person participated in the sample processing and analytical determinations, he/she should not be the solely person performing the reporting/registry QA for the entire process. This is applicable to all the processes including the final reporting from IMAP users (i.e. MEDPOL Focal Points and national IMAP competent laboratories) to IMAP Info System, which should be checked by a second staff member. In brief, the person(s) that does the operations could not be the same that performs the quality assurance (QA) for a given process and data reporting (UNEP/MAP, 2019a).

[16] ICES. 2010. Report of the ICES\OSPAR Workshop on Lysosomal Stability Data Quality and Interpretation (WKLYS), 13–17 September 2010, Alessandria, Italy. ICES CM 2010/ACOM:61

[17] ICES. 2011. Report of the Study Group on Integrated Monitoring of Contaminants and Biological Effects (SGIMC), 14–18 March 2011, Copenhagen, Denmark. ICES CM 2011/ACOM:30

[18] OSPAR 2013. Background document and technical annexes for biological effects monitoring, Update 2013

[19] Viarengo, A.; Lafaurie, M.; Gabrielides, G.P.; Fabbri, R.; Marro, A., Roméo, M., 2000. Critical evaluation of an intercalibration exercise undertaken in the framework of the MED POL biomonitoring program. Mar. Environ. Res. 49, 1-18

[20] UNEP/MAP 2021. UNEP/MED WG.492/6. Monitoring Guidelines/Protocols for IMAP Common Indicator 18:

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For analytical data for IMAP Common Indicators 13, 14, 17, 18 and 20, Quality Assurance information, such as inter-laboratory performance results should be included in the Reporting Templates, with automatic flagging of categories according to the QA information (z scores). The UNEP/MAP document on Quality Assurance / Quality Control (UNEP/MAP, 2019a) proposes five Data QA categories to be flagged in the Reporting Templates for IMAP Common Indicators of Ecological Objectives 05 and 09:

- Category A (CI13, CI14, CI17, CI20). Laboratories/Contracting Parties reporting successful Proficiency testing ($|z| \leq 2$) and/or accreditation for the chemical or parameter analysed; metadata completed and timely submitted (max 2 years delay).
- Category A (CI18). Laboratories/Contracting Parties reporting successful Proficiency testing and/or accreditation for the biomarkers or parameter analysed; metadata completed and timely submitted (max 2 years delay).
- Category B (CI13, CI14, CI17, CI20). Laboratories/ Contracting Parties reporting Proficiency testing for the chemical or parameter analysed ($2<|z|<3$) and/or accreditation; metadata completed and timely submitted (max 2 years delay).
- Category B. (CI18). Laboratories/ Contracting Parties reporting Proficiency testing for the biomarkers or parameter analysed and/or accreditation; metadata completed and timely submitted (max 2 years delay).
- Category C. Laboratories/ Contracting Parties with no participation in Proficiency testing (for the last 2 years); metadata completed and timely submitted. It also could include scientific literature with full QA reported.
- Category D. Laboratories/ Contracting Parties with no participation in Proficiency testing (for the latest 5 years); metadata completed but not timely submitted. It also includes scientific literature without QA specifically reported.
- Category E. Laboratories/ Contracting Parties with gross reporting errors, although might be completed and timely submitted.

The 'flagging quality' scheme based on the Database QA and Reporting Procedures will help to develop an accurate assessment with known source of uncertainty, as well as to boost the national capabilities and resources to fit the requirements.

The IMAP Info System includes Data Controls (i.e. algorithms to set the range of acceptable values), such as:

- Minimum and maximum values allowed for a parameter;
- Valid concentration range of the parameter;
- Limit if Detection (LOD);
- Limit of Quantification (LOQ)

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