# 4.      Quality Assurance

## 4.1.      Specific Aspects of Quality Assurance

The Article 3, paragraph 5, of Helsinki Convention states that the Contracting Parties shall ensure that measurements and calculations of emissions from point sources to water and air and of inputs from diffuse sources to water and air are carried out in a scientifically appropriate manner in order to assess the state of the marine environment of the Baltic Sea Area and ascertain the implementation of this Convention. Additionally, HELCOM 16 has adopted a quality assurance policy, according to which:

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

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

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

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

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

Basic principles of quality assurance are also referred in the HELCOM Monitoring and Assessment Strategy adopted by HELCOM 26/2005.

Detailed description of general aspects of QA/QC is described in the HELCOM COMBINE Manual, Part B. General Guidelines on Quality Assurance for Monitoring in the Baltic Sea, available on the HELCOM website:
http://sea.helcom.fi/Monas/CombineManual2/PartB/BFrame.htm

The Contracting Parties are responsible of the quality assurance of the data submitted to HELCOM PLC-Water database.

The laboratories providing data to PLC should have a quality assurance system that follows the requirements of EN ISO/IEC 17025 (EN ISO/IEC, 2005). Participating laboratories are encouraged to endeavour the obtainment of official accreditation for variables on which they report data in accordance with PLC.

All institutes/laboratories should participate in regular (annual) interlaboratory comparison tests. Also reference materials should be used by participating laboratories.

All Contracting Parties have to nominate a national QA contact person responsible for PLC quality assurance.

The national QA contact person will help in ensuring comparability and reliability of analytical data provided by the laboratories in their country. The national QA contact person should:

– provide information about PLC-Water at the national level and guarantee that information on the PLC Guidelines and the QA section of the COMBINE Manual reach the laboratories submitting PLC data;

– co-operate nationally with the laboratories participating in PLC-Water data collection;

– collect the mandatory information from laboratories and report them to PLC-5 Project in the end of 2006.

The PLC-Water laboratories are asked to estimate the uncertainty of their analytical measurements. The estimated uncertainties should be reported for the individual variables in the form expanded measurement uncertainty with a 95% confidence interval together with information on the concentration level.

Quality requirements for the data have not yet been set in many Contracting Parties. For these reasons they are not set for PLC-Water. The Contracting Parties are encouraged to start preparing of quality requirements for analyses concerning control of pollution load.

## 4.2. Interlaboratory Comparison Tests

It is recommended to perform the interlaboratory comparison tests according to the ISO/IEC Guide 43.

In conducting the interlaboratory comparison tests for PLC is essential, that:

– the test material is identical as much as possible with the matrices (e.g. riverine water and waste water) to be analysed within PLC-Water;

– different concentration levels of each variable in each matrix are included in the test and they are adequate to the concentrations of the samples collecting in PLC-Water, in particular adequate to the concentrations of metals and mercury in riverine waters;

– the test material is homogenous and stable for the duration of the testing period and homogeneity and stability of the material is tested;

– the participating laboratories use the analytical methods, which intend to apply for PLC-Water.

Interlaboratory comparison tests can be found in the internet:

QUASIMEME: http://www.quasimeme.marlab.ac.uk/

Eurofins: http://eurofins.dk EPTIS: http://www.eptis.bam.de/

Department of Applied Environmental Science, Stockholm University: http://www.itm.su.se/

Finnish Environment Institute: http://www.environment.fi (look under "Finnish Environment Institute (SYKE)"

## 4.3. Validation of PLC-Water data

The national QA contact persons are responsible for validation of the data to be used in the calculation of the aggregated data to be submitted to HELCOM PLC-Water Database

The validation of the data should be carried out at the national level based on information on:
- accreditation status (recommended for laboratories),
- measurement uncertainty (mandatory for laboratories),
- limit of detection/quantification (mandatory for laboratories),
- use of reference material (recommended for laboratories),
- use of control charts (mandatory for laboratories),
- participation in laboratory intercomparison test (recommended for laboratories).

In case that some of the mandatory validation information is missing, the data should be flagged in the PLC-Water database.

## 4.4.      Examples for Estimating Measurement Uncertainty

**Example A:   BOD in waste water – results of internal quality control and of a CRM**
(Magnusson et al, 2004:
http://www.nordicinnovation.net/nordtestfiler/tec537.pdf)

Estimation of uncertainty was based on the results obtained in internal quality control and in analysis of the CRM

| Step | Action | Example: BOD in wastewater |
|------|--------|----------------------------|
| 1 | Specify Measurand | BOD in wastewater, measured with EN1899-1 (method with dilution, seeding and ATU). The demand on uncertainty is ± 20 %. |
| 2 | Quantify $u(Rw)$<br><br>A control sample<br><br>B possible steps not covered by the control sample | A: The control sample, which is a CRM, gives an s = 2.6 % (=$u(R_w)$) at a level of 206 mg/L $O_2$. s = 2.6 % is also when setting the control chart limits.<br><br>B: The analysis of the control sample includes all analytical steps after sampling |
| 3 | Quantify method and laboratory bias | The CRM is certified to 206 $\pm$5 mg/L $O_2$ (at 95 % confidence level) The average result of the control chart is 214.8. Thus, there is a bias of 8.8 mg/L = 4.3 %.<br><br>The $s_{bias}$ is 2.6 % (n=19 (the number of batches)<br><br>The $u(Cref)$ is 5 mg/L / 1.96 = 1.2 % |

| 4 | Convert components to standard uncertainty $u(x)$ | $u(R_w) = 2.6\ \%$ |

$$u(bias) = \sqrt{bias^2 + \frac{s_{bias}^2}{\sqrt{n}} + u(Cref)^2}$$

$$= \sqrt{4.3^2 + \left(\frac{2.6}{\sqrt{19}}\right)^2 + 1.2^2} = 4.5\ \%$$

| 5 | Calculate combined standard uncertainty, $u_c$ | $u_c = \sqrt{2.6^2 + 4.5^2} = 5.2\ \%$ |

| 6 | Calculate expanded uncertainty, $U = 2 \cdot u_c$ | $U = 2 \cdot 5.2 = 10.4 \approx 10\ \%$ |

**Example B:  BOD in waste water – results of internal quality control and interlaboratory comparison results** (Magnusson et al, 2004: http://www.nordicinnovation.net/nordtestfiler/tec537.pdf)

Estimation of uncertainty was based on the results obtained in internal quality control and in participating in Interlaboratory comparisons.

| Step | Action | Example: BOD in wastewater |
|------|--------|----------------------------|
| 1 | Specify Measurand | BOD in wastewater, measured with EN1899-1 (method with dilution, seeding and ATU). The demand on uncertainty is ± 20 %. |
| 2 | Quantify $u(Rw)$<br><br>A control sample<br><br>B possible steps not covered by the control sample | A: The control sample, which is a CRM, gives an $s$ of 2.6 % at a level of 206 mg/L $O_2$. s = 2.6 % is also used as s when setting the control chart limits.<br><br>B: The analysis of the control sample includes all analytical steps after sampling |
| 3 | Quantify Method and laboratory bias<br>Data from the Table above (results of interlaboratory comparisons) | $RMS_{bias} = 3.76$<br>$$u(Cref) = \frac{s_R}{\sqrt{n}} = \frac{7.9}{\sqrt{22.3}} = 1.67$$ |
| 4 | Convert components to standard uncertainty $u(x)$ | $u(R_w) = 2.6\ \%$<br><br>$$u(bias) = \sqrt{RMS_{bias}^2 + u(Cref)^2} =$$<br>$$\sqrt{3.76^2 + 1.67^2} = 4.11\%$$ |
| 5 | Calculate combined standard uncertainty, $u_c$ | $u_c = \sqrt{2.6^2 + 4.11^2} = 4.86\ \%$ |

| 6 | Calculate expanded uncertainty, $U = 2 \cdot u_c$ | | $U = 2 \cdot 4.86 = 9.7 \approx 10\ \%$ |

**Table 4.1:** BOD - results from interlaboratory comparisons.

| Exercise | Nominal value | Laboratory result | Bias | $s_R$ | Number of labs |
|---|---|---|---|---|---|
| | mg/L | mg/L | % | % | |
| 1 | 154 | 161 | + 4.5 | 7.2 | 23 |
| 2 | 219 | 210 | - 4.1 | 6.6 | 25 |
| 3 | 176 | 180 | +2.3 | 9.8 | 19 |
| $\overline{X}$ | | | +0.9 | 7.87 | 22.3 |
| $RMS_{bias} = \sqrt{\sum (bias)^2 / n}$ | | | 3.76 | - | - |

## 4.5.    Recommended limits of quantification (LOQ)

It is recommended when analyzing river waters that each laboratory use analytical methods which do not have LOQ higher than the LOQ listed below:

AOX          - 10 µg/l

BOD          - 0.5 mg/l

$COD_{cr}$          - 5 mg/l

TOC          - 0.5 mg/l

$NH_4$          - 10 µg/l

$NO_{23}$-N          - 20 µg/l

$N_{tot}$          - 50 µg/l

$P_{PO4}$          - 5 µg/l

$P_{tot}$          - 10 µg/l

Cd          - 0.01 µg/l

Cr          - 0.05 µg/l

Cu          - 0.05 µg/l

Ni          - 0.05 µg/l

Pb          - 0.05 µg/l

Zn          - 0.5 $\mu$g/l

Hg          - 0.005 $\mu$g/l

Min.Oil     - 0.1 mg/l


## 4.6.      Values under the limit of quantification

It is important to distinguish between the limit of detection (LOD – the lowest detectable amount of a compound) and the limit of quantification (LOQ – the lowest quantifiable amount of a compound). The LOQ is usually significantly larger than the LOD (2-4 times).

There has been much diversity in the way in which the limit of detection of an analytical system is defined. Most approaches are based on multiplication of the within-batch standard deviation of results of blanks by a factor. Further information can be found in the ISO/WD 13530:2003 "Water quality - Guide to analytical quality control for water analysis" or in the chapter B.4.2.3. of the HELCOM Combine Manual:
http://sea.helcom.fi/Monas/CombineManual2/PartB/BFrame.htm

In PLC-5 the LOQ and not the LOD is used to assign a numeric value when handling low-level data. If measured concentrations are below LOQ, the estimated concentration should be calculated using the equation:

Estimation = (100%-A) x LOQ, where A=percentage of samples below LOQ. This approach is equivalent to a modified proposal listed in the guidance document on monitoring adopted by EU under the IPPC Directive.


## 4.7.      Technical Notes on the Determination of Variables in Rivers and Waste Water

This chapter includes technical notes for determination of some variables. For PLC-Water, analysis should be made using unfiltered samples.

The well tested and documented European or international standard methods (ISO or EN) or the methods based on these standards are highly recommended to use.

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

Particularly in the case of nutrients, mercury and metal analysis, a satisfactory blank control is necessary. Therefore, it is important to control the blank daily, for reproducibility and con-stancy over a longer time. The blank should include all analytical pre-treatment procedures, including the addition of the same quantities of chemical substances for the sample.

For calibration purpose in general the working standard should be prepared from the stock standard solution for every batch of samples.

Apart from manual methods, various automated methods are in use. The analyst has to be aware of the effects of the different analytical conditions in automated analysis which might affect accuracy.

***Adsorbable organically bound halogens (AOX)***

AOX is a mass parameter that includes many substances which are adsorbable and contain halogens. It is a general term pertaining mainly to industrial chemicals. Halogens (usually chlorine but also fluoride, bromine, and iodine) envelop organic substances so as to disallow breakdown by bacteria, which means these substances are often not easily degradable. Adsorption tendency most often goes hand in hand with a tendency to accumulate in organisms (bio-accumulativeness).

For the determination of AOX in surface and waste water samples it is strongly recommended following ISO 9562:2004 "Water quality - Determination of adsorbable organically bound halogens (AOX)", formerly EN 1485.

### Biological Oxygen Demand (BOD)

For the determination of BOD in waste water samples it is strongly recommended following ISO 5815:2003 "Water quality - Determination of biochemical oxygen demand after n days (BODn) - Part 1: Dilution and seeding method with allylthiourea addition" and in surface water samples " - Part 2: Method for undiluted samples".

ISO 5815-1:2003 is applicable to all waters having biochemical oxygen demands greater than or equal to 3 mg/l of oxygen (the limit of quantification) and not exceeding 6 000 mg/l of oxygen.

ISO 5815-2:2003 specifies determination of the biochemical oxygen demand of waters of undiluted samples. It is applicable to all waters having biochemical oxygen demands greater than or equal to 0,5 mg/l of oxygen (the limit of quantification) and not exceeding 6 mg/l of oxygen.

During the first meeting of the Fifth Baltic Sea Pollution Load Compilation (HELCOM PLC-5, 1/2005) it was decided to report $BOD_5$ instead of $BOD_7$. If $BOD_7$ is measured, a conversion factor ($BOD_5 = BOD_7/1.15$ ) should be used in order to calculate $BOD_5$.

### Nutrients

### Orthophosphate

Sample bottles of plastic or glass can be used for collecting of samples. Bottles and laboratory glass and plastic ware are recommended to be soaked in hydrochloric acid (ca 2 mol/l) and rinsed carefully with distilled water. The analyses should be carried out as soon as possible. If the samples are not analysed within one day, they have to be preserved. The samples should be stored cold (4 $^{\circ}$ C) and dark.

The molybdenium blue method with ascorbic acid as reductant is recommended to use for determination of orthophosphate.

## Total Phosphorus

Sample bottles of plastic or glass can be used for collecting of samples. Bottles and laboratory glass and plastic ware are recommended to be soaked in hydro chloric acid (ca 2 mol/l) and then rinsed carefully with distilled water. The analyses should be carried out as soon as possible. If the samples are not analysed within one day they have to be preserved. The samples should be stored cold (4 $^{\circ}$C) and dark.

Digestion with potassiumperoxodisulfate is recommended. For analyses of waste waters with high content of organic matter a more powerful oxidation method (with nitric-sulfuric acid) can be necessary. A quality control sample can be prepared of e.g. Na-$\beta$-glycerophosphate or thiaminepyrophosphate chloride.

## Ammonium

Sample bottles of plastic or glass can be used for collecting  of samples. Samples for determination of ammonia should not be preserved. Waste waters shall be determined within one day and river waters within three days. The samples should be stored cold  (4 $^{\circ}$C) and dark.

The indophenol blue method is recommended to use. High concentrations of ammonium can be determined by sample dilution. The Nessler method is not recommended to use.

## Nitrate

Sample bottles of plastic or glass can be used for collecting of samples. The analyses should be carried out as soon as possible.  If the samples are not analysed within one day they have to be preserved.  The samples should be stored cold (4 $^{\circ}$C) and dark.

The cadmium reduction method is recommended to use for analyses. It is necessary to check the capacity of the reductor (at least 90 % exchange) systematically. The salicylate method is not recommended to use because of many interfering effects.

## Total Nitrogen

Sample bottles of plastic or glass can be used for collecting of samples. The analyses should be carried out as soon as possible. If the samples are not analysed within one day they have to be preserved. The samples should be stored cold (4 $^{\circ}$C) and dark.

For analyses of river water it is recommended to use a method based on oxidation with peroxodisulfate and reduction of nitrate with cadmium reductor. Especially for analyses of industrial waste waters the modified Kjeldahl method with Dewarda´s alloy or the Kjeldahl method and the determination of nitrate separately is recommended to use.

The quality control sample can be prepared of e.g. EDTA, glycine or 4-nitroaniline.

## Metals

The basis for the reliable measurement of low concentration of metals in water is to avoid contamination risk in sampling, in preservation of samples and in measurement of samples.

For sampling of river waters bottles of polypropylene, polyetene, teflon or resistant glass (e.g. Pyrex) it is recommended to use. The bottle and the cap should be prepared from colourless material.

Bottles, glass and plastic ware and containers have to be cleaned  soaking them in nitric acid (ca 7 mol/l) at least for one day and then be rinsed with deionized water at least three times.

For determination of ultra trace level it is necessary to follow more extensive cleaning procedure. If the laboratory collect both river and waste waters it is necessary to use separate samplers and bottles for sampling of low content and high content of metals. It is essential to maintain a clean environment during analyses. Reagents of highest possible purity and deionized water shall be used for preparation of solutions. If low content of metals are to be determined, a field blank shall be analysed as well.

The samples which contain particulate material shall be digested with nitric acid in a closed vessel under pressure.

During sample pre-treatment for trace metal analysis is important to eliminate particles that can contaminate the samples or the sample containers from the laboratory environment.

For measurement of metals atomic absorption spectroscopy (AAS flame or flameless), inductive plasma-optical emission (ICP-OES) or inductive coupled plasma-mass spectroscopy (ICP-MS) can be used. For measurement of low contents of metals in rivers ICP/MS or anodic stripping voltametry (ASV) are highly recommended to be used.

Calibration is important to carry out correctly. Commercial stock solutions are available for preparation of calibration solutions. The calibration should be performed with a blank solution and 4-5 calibration solutions for an appropriate concentration range. Need for recalibration has to be checked by measuring a quality control sample once in 20 determinations. In order to reduce the effect of chemical and physical interferences (i.e. high contents of chlorides) the method of standard addition can be applied. Interference effects can also be reduced by gradual dilution or by addition of different chemical modifiers into the sample. If both high and low contents of metals are determined in the same batch, there should be a blank sample between the samples in order to reduce the memory effect.

### Mercury

Eliminating of contamination is important also in analysis of mercury, too.

Good quality of plastic or resistant glass bottles should be used for collecting of samples.

The cleaning procedure for bottles and glass and plastic ware is like that presented for metals. All chemicals should be of a highest quality or a special quality for analyses of mercury. The quality of the reagents should be checked. A field blank shall be collected as well.

Chemical digestion procedure with e.g. potassium permanganate and potassium peroxodisulfate should be carried out before determination of mercury.

Mercury can be detected with cold vapour atomic absorption. For measurement of low contents of mercury (pg/l) enrichment on gold or fluorescence technique is highly recommended to be used.

The blank control chart has to be prepared for identifying contamination during analyses.

### Mineral oil

For the determination of mineral oil hydrocarbons in surface and waste water samples it is strongly recommended following ISO 9377-2:2000 "Water quality - Determination of hydrocarbon oil index - Part 2: Method using solvent extraction and gas chromatography" published in October 2000.

### Total Organic Carbon (TOC)

Oxidation of TOC in water to carbon dioxide can take place by combustion, by the addition of an appropriate oxidant, by UV radiation or any other high-energy radiation.

For the determination of TOC in surface and waste water samples it is strongly recommended following ISO 8245:1999 "Water quality -- Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)", identical to EN 1484:1997

Further information are described in Annex B-17 of the HELCOM COMBINE Manual: http://sea.helcom.fi/Monas/CombineManual2/PartB/BFrame.htm