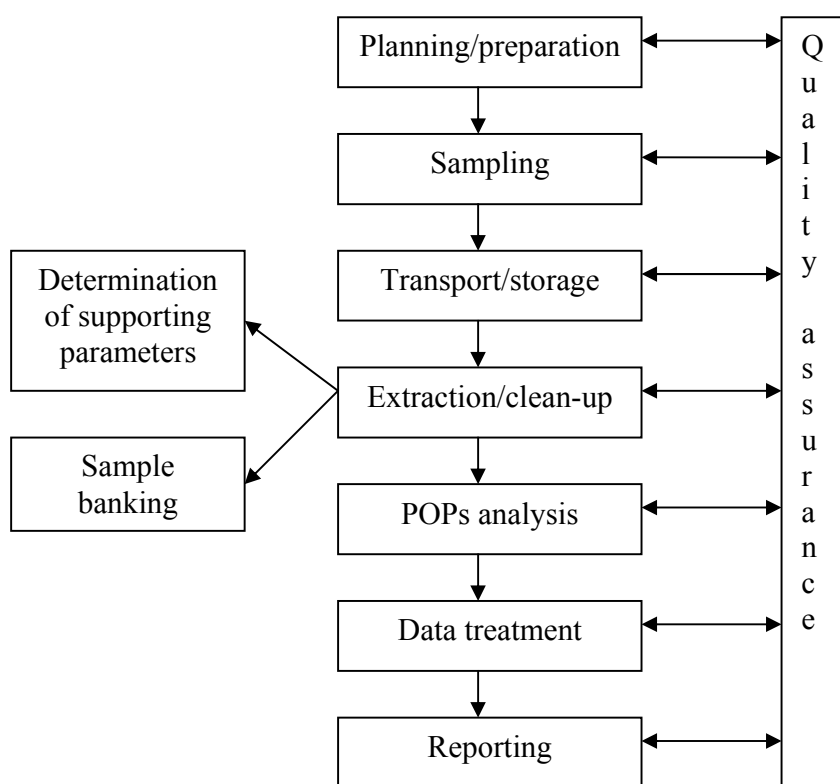


## 5 ANALYTICAL METHODOLOGY

Before starting new measurements, it is important to investigate whether there are any other monitoring activities going on in the region. It may be global programmes, such as the WHO GEMS/Food, regional programmes such as AMAP, or national monitoring activities. Another source of information is the reports from the recent global assessment of PTS. It is assumed that the GMP shall, at least partly, be based on existing activities, and co-operation with those is essential. This may also influence the strategy for the chemical analyses, and if the methods used in on-going projects are good enough, those can be applied also for the GMP.

The process to determine the concentrations of the POPs in a sample include a series of events as outlined in Figure 5.1.



**Figure 5.1. The different stages of the process to determine POPs concentrations.**

This document will not provide a detailed description of the methods to be used for the determination of POPs. It will not even prescribe the use of specific methods that have been described for this purpose, as that would delay the development and acceptance of new, improved methods. The intention is to give a general description of the analytical procedures, and to provide references on how this is done in other monitoring programmes. It is, however, essential that the methods used are validated to give comparable data within the programme.

Some of the major operations in the process will be briefly discussed in this chapter, and, in Annex on Analysis, there is a detailed description of the parameters important for the process.

## 5.1 Sampling

The aim of any sampling activity is to obtain a sample that can serve the objective of the study. In this activity it is considered indispensable to ensure the representativeness and integrity of the sample during the entire sampling process. Additionally, quality requirements in terms of equipment, transportation, standardization, and traceability are indispensable. It is important that all sampling procedures are agreed upon and documented before starting a sampling campaign.

The analyte, matrix, sampling site, time or frequency, and conditions should be determined depending on the objective of the sampling. In case of human samples it may also be necessary to use a suitable interview form.

Although it may be too expensive to get full accreditation for sampling, Quality Assurance and Quality Control (QA/QC) procedures for sampling should be put in place.

No general recommendation can be given with respect to who should perform the sampling. For certain matrices, *e.g.*, human blood, there is no doubt that a specialist, *i.e.*, medical doctor or nurse, has to take the sample. In addition, for human samples, ethical considerations have to be respected. There are pros and cons for sub-contracting a laboratory specialist in sample taking. Sub-contracting the sampling can be an advantage to the laboratories that don't have the required personnel and equipment, but the laboratory must be sure that the sampling was taken under established quality assurance and quality control (QA/QC) conditions. In case an external organization will be sub-contracted to take the sample, it is recommended that the analytical laboratory establishes and provides the sampling protocol. Those in charge of the sampling process must apply security seals, as well as follow the preservation criteria to guarantee the integrity of the sample during transportation.

## 5.2 Extraction and clean-up

The appropriately prepared sample can be extracted by any one of a number of techniques. The main points to consider are to allow adequate time of exposure of the solvent system in the sample matrix and to limit sample handling steps, *i.e.* avoid filtration steps by using Soxhlet or semi-automated systems (*e.g.* pressurized fluid extractors, EPA method 3545A). Extractions can also be accelerated by the use of ultrasonication. Cross contamination from residues left behind by high levels of POPs in other samples is a concern at this stage and equipment must be thoroughly cleaned and checked from batch to batch.

Purity of extraction solvents is also a major consideration. Only high purity glass distilled solvents should be used. Internal standards should be added to the sample as early as possible in the process.

If the results are reported on a lipid weight basis, the determination of the lipid content in the sample is critical. From this aspect the choice of solvents is crucial, and has been discussed in a recent article (Jensen *et al.*, 2003). If the whole sample is not used for the extraction, the remaining part can be frozen and stored for future control analysis, or analysis of other substances. Likewise the extracts not used in the analysis can be stored, preferably in glass ampoules, at -20 °C.

Isolation steps can be relatively straightforward for low lipid samples such as air. Generally small Silica gel or Florisil columns (either prepared in the lab or pre-purchased) will suffice. The purpose of this step is to remove co-extractive pigments and to separate non-polar PCB (plus

p,p'-DDE) from more polar POPs (HCH, most chlordanes, dieldrin/endrin). This is achieved by applying the extract in a small volume of non-polar solvent and fractionating by eluting with hexane followed by one or two other elutions of increasing polarity. Alumina is not recommended because of possible dehydrochlorination of some POPs, e.g. 4, 4'-DDT.

For the human samples a lipid removal step must be included. This can be achieved using size exclusion or gel permeation chromatography (GPC) either in automated systems, using high pressure liquid chromatography (HPLC) columns or by gravity flow columns. The advantage of GPC is that it is non-destructive while the disadvantage is a requirement for large volumes of solvent (low pressure or gravity systems) or expensive columns (HPLC). Lipid removal using sulfuric acid washing or sulfuric acid – silica columns is also effective but does result in loss of some analytes such as dieldrin.

Following fractionation on silica or Florisil final extracts are prepared in small gas chromatography (GC) vials for analysis. Addition of a recovery standard to check solvent volume is recommended at this stage. Careful evaporation is required at this step and only high purity compressed gas (usually nitrogen) should be used.

Analytical methodology for PCDD/PCDF and PCB with TEFs differs from those used for routine ortho-PCB and OCPs in that it requires much lower detection limits (typically 10-100 times lower) because guideline limits in food products are in the low pg/kg range, the Provisional Tolerable Monthly Intake being 70 pg/kg body weight (Joint FAO/WHO Expert Committee on Food Additives (JEFCA), 2001). To enforce and control these low concentrations for PCDD/PCDF isotope dilution MS (<sup>13</sup>C-surrogates for all PCDD/PCDF homologue groups), enrichment on carbon to isolate planar compounds, very small final volumes (10-50 µL) for GC-HRMS quantification is used. Methodology for PCDD/PCDF, slightly modified to include the dioxin-like PCB, developed by the US EPA, is well established and validated by numerous inter-laboratory comparisons. This methodology would be recommended for use in a global monitoring programme. Unlike the guidelines for PCB and OCPs, this very specific guidance for the extraction, isolation and quantification steps for PCDD/PCDF is recommended in order to be in compliance with ongoing programmes and compatible with results generated with these methods over the past 10 years.

### 5.3 POPs analysis

Since the 1960s, POPs have been determined using gas chromatography (GC) techniques with electron capture detection (ECD), initially using packed columns. Today the separation has been improved by the use of capillary columns and the selectivity by the use of mass spectrometric detectors (MS). Based on the availability of commonly used instruments for the determination of POPs, three types of laboratories can be identified, as described in Table 5.1.

**Table 5.1 Requirements for the instrumental analysis of POPs**

Laboratory tier	Equipment	Infrastructure needs	Cost (estimates, USD)	Chemicals
3	Basic sample extraction and clean-up equipment, capillary GC/ECD	Nitrogen/air conditioning/ power/personnel specifically trained to operate and troubleshoot equipment problems	Instruments: \$50K Lab equip: \$30K Operation: \$10K/year Personnel: 2 PY	Most PCB and all OCPs except toxaphene
2	Sample extraction and clean-up equipment, capillary GC/LRMS	Helium/air conditioning/ consistent power/ personnel specifically trained to operate and troubleshoot equipment problems	Instruments: \$150K Lab equip: \$50K Operation: \$20K/year Personnel: 3 PY	Most PCB and all OCPs; toxaphene if negative chemical ionization is available
1	Sample extraction and clean-up equipment, capillary GC/HRMS	Helium/air conditioning/ consistent power/high operational costs /personnel specifically trained to operate and troubleshoot complicated instrumentation	Instruments: \$400K Lab equip: \$50K Operation: \$50K/year Personnel: 5 PY	PCDD/PCDF, all PCB, all OCPs except toxaphene

<sup>a</sup> GC/ECD – gas chromatography/electron capture detection

<sup>b</sup> GC/LRMS – gas chromatography/low resolution mass spectrometry

<sup>c</sup> GC/HRMS – gas chromatography/high resolution mass spectrometry

Whereas the above table refers to estimated costs to install and run a POPs laboratory, the following indicative costs for analysis of POPs in various matrices can be given based on information provided through the UNEP/GEF project on POPs Laboratories:

- Costs for analysis of POPs pesticides (9 chemicals) range from USD 100 to USD 1,500 with a central estimate of around USD 150-200;
- Costs for analysis of indicator PCB (6-7 congeners) range from USD 90 to USD 900 with a central estimate of around USD 200;
- Costs for analysis of dioxin-like PCB (12 congeners) range from USD 140 to USD 1100 with a central estimate of around USD 750;

- Costs for analysis of PCDD/PCDF (reported as TEQ) range from USD 500 to USD 2100 with a central estimate of around USD 600-800;
- Costs for analysis of all PCDD, PCDF and PCB that contribute to the WHO-TEQ range from USD 600 to USD 1500 with a central estimate of around USD 950.

It is anticipated that improved analytical methods will be developed over the life of the GMP, and the project should be structured so that these improved techniques can be adopted. There is a need to improve the accuracy and lower the costs of these analyses. Emerging procedures with low environmental impact may become more widely available and accepted. It will be necessary to consider comparability as new methods are developed. This could be achieved by analysis of archived samples and direct comparison of new and old methods. Many laboratories are not currently permitted to analyze human blood and milk samples. Special training will be necessary to handle these samples, considering the danger of infectious diseases.

Quality control and quality assurance are important factors in sampling and analysis. Any method performance must be verified through control tables where optimal operational ranges are defined, and the periodical analysis of certified reference materials, own laboratory reference materials, and blind or divided samples should be included in routine QA/QC. The intercalibration exercises are an essential component in quality assurance of the results and are deemed indispensable in the implementation of a regional laboratory network. A recommendation would be that at least once a year such an intercalibration study is performed for each matrix and persistent organic pollutant of interest to the Region.

Numerous analytical approaches are available for quantifying PCB, and OCPs, as well as PCDD/PCDF by gas chromatography. As with extraction/separation steps only general guidance is required for ortho-substituted PCB and OCPs. Some general guidance on the application of gas chromatographic analysis of ortho-substituted PCB and OCPs is provided in Table 5.2. For PCDD/PCDF and PCB with TEFs, quantification solely by isotope dilution HRMS is recommended and details can be found in standard operation procedures (SOPs) (e.g. EPA method 8290A, EPA method 1613).

### **Table 5.2. Common methods used to analyse POPs**

Old Table 5.3 in here.

HRMS can also be used, of course, for determination of all PCB, including congener-specific determination of non-ortho and mono-ortho substituted PCB (e.g. EPA method 1668) as well as OCPs and indeed would provide a very high level of confidence in the results compared to GC-ECD. However, use of GC-ECD is recommended because of wide availability, relatively low cost, and the substantial knowledge base that exists on the use of this technology for analysis of non-ortho and mono-ortho PCB and OCPs at low ng/g levels or higher in environmental matrices.

## **5.4 Data treatment**

There are a number of parameters that have to be reported together with the analytical results. These include the efficiency of the extraction and clean-up, and the blank values, but the results should not be compensated for these parameters. The uncertainty of the results should also be at

least estimated, but preferably determined, using results from inter- or intralaboratory comparisons.

The lowest concentration at which a compound can be detected (limit of detection, LOD) is defined as that corresponding to a signal three times the noise. The lowest concentration that can quantitatively be determined (limit of quantification LOQ) is three times higher than LOD. Compounds found at levels between LOD and LOQ can be reported as present, or possibly as being present at an estimated concentration, but in the latter case the result has to be clearly marked as being below LOQ. Results below the detection limit are often reported as <"LOD".

There are, however, several statistical techniques for treating censored data when the true detection limit is known, e.g. by using a robust statistic such as the median which is unaffected by small numbers reported as below LOD.

Another method uses an estimate of each unknown concentration based on the empirical expected order statistic (Helsel and Hirsch, 1995). This method fits a log-linear regression of the ranked detected concentrations on rank, and then uses this relationship to predict the value of those concentrations reported as below the limit of detection (Figure 5.2).

Old Figure 5.1 in here.

**Figure 5.2** Example of substitution of concentrations reported as less than LOD, by extrapolation from regression of concentrations from the same annual sample above LOD on rank order. Log-linear regression fitted to data above LOD. Circles = concentrations above LOD, Triangles = substituted values for concentrations reported as below LOD, Squares = LOD/2 – values.

Results may also be reported as being in the interval between a value where the lower limit is based on non-quantifiable peaks set to zero and an upper limit where results below LOQ are set as equal to the LOQ.

In the analysis of complex mixtures, such as PCB, there is always a risk for coeluting peaks in the gas chromatograms, and known interferences should be reported.

## 5.5 Organization of quality control

The organization of the quality control needs special attention in the GMP. Many of the recommendations mentioned above deal with QA/QC measures, but to be able to compare results from different labs and regions (and also for the same laboratory over time) there is a need for overarching activities, such as

- Distribution of standard materials
- Organisation of intercalibrations
- Production of reference materials

## 5.6 References (to be checked)

(I added the following list-HF)

EC (2002): Commission Directive 2002/69/EC of 26 July 2002 laying down the sampling methods and the methods of analysis for the official control of dioxins and the determination of

dioxin-like PCBs in foodstuffs. Official Journal of the European Communities, L 209/5-L 209/14 (dated 6.8.2002)

OECD, various years. OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring (various volumes). OECD Principles on Good Laboratory Practice (as revised 1997–1999), OECD. Available at [www.oecd.org/ehs/](http://www.oecd.org/ehs/)

### 5.6.1 Examples: Analytical Methods for POPs Pesticides

AOAC Official Method 970.52 Organochlorine and Organophosphorous Pesticide Residue Method. General Multiresidue Method. 2005 AOAC International

AOAC Official Method 955.22 Organochlorine and Organophosphorous Pesticide Residue Method. 2005 AOAC International

EPA Method 8081A: Organochlorine Pesticides by Gas Chromatography (and ECD)

ISO 6468 (1996) Water quality – Determination of certain organochlorine insecticides, polychlorinated biphenyls and chlorobenzenes – Gas chromatographic method after liquid-liquid extraction

ISO 10382 (2002): Soil quality – Determination of organochlorine pesticides and polychlorinated biphenyls – Gas-chromatographic method with electron capture detection

EPA Method 8081A: Organochlorine Pesticides by Gas Chromatography (and ECD)

### 5.6.2 Examples: Analytical Methods for PCB

DIN 38414-20 (1996): German standard methods for the examination of water, waste water and sludge - Sludge and sediments (group S) - Part 20: Determination of 6 polychlorinated biphenyls (PCB) (P 20)

EN 12766-1 (2000): Petroleum products and used oils – Determination of PCBs and related products – Part 1: Separation and determination of selected PCB congeners by gas chromatography (GC) using an electron capture detector (ECD)

EN 12766-2 (2001): Petroleum products and used oils – Determination of PCBs and related products – Part 2: Calculation of polychlorinated biphenyl (PCB) content

EN 61619 (2004): Insulating liquids – Contamination by polychlorinated biphenyls (PCBs) – Method of determination by capillary column gas chromatography

EPA Method 1668, Revision A: Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS, United States Office of Water, EPA No. EPA 821-R-00-002, Environmental Protection Agency (4303), December 1999

EPA Method 8080: Organochlorine Pesticides and PCBs

EPA Method 8082: Polychlorinated biphenyls (PCBs) by gas chromatography ([www.epa.gov/epaoswer/hazwaste/test/pdfs/8082.pdf](http://www.epa.gov/epaoswer/hazwaste/test/pdfs/8082.pdf))

EPA Method 8275A: Semivolatile organic compounds (PAHs and PCBs) in soils/sludges and solid wastes using thermal extraction/gas chromatography/mass spectrometry (TE/GC/MS), EPA analytical chemistry guidance SW-846

ISO 6468 (1996) Water quality – Determination of certain organochlorine insecticides, polychlorinated biphenyls and chlorobenzenes – Gas chromatographic method after liquid-liquid extraction

ISO 10382 (2002): Soil quality – Determination of organochlorine pesticides and polychlorinated biphenyls – Gas-chromatographic method with electron capture detection

JIS K 0093 (2002): Testing method for polychlorobiphenyl in industrial water and wastewater

### 5.6.3 Examples: Analytical Methods for PCDD/PCDF and dl-PCB

EPA Method 1613: Tetra-through Octa-Chlorinated Dioxins and Furans by Isotope Dilution HRGC/HRMS, October 1994, ([www.epa.gov/waterscience/methods/1613.pdf](http://www.epa.gov/waterscience/methods/1613.pdf))

EPA Method 8290A: Polychlorinated Dibenzodioxins (PCDDs) and Polychlorinated Dibenzofurans (PCDFs) by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry (HRGC/HRMS), revision 1 January 1998

EPA Method T09: Determination of polychlorinated dibenzo-p-dioxins (PCDDs) in ambient air using high-resolution mass spectrometry (HRGC/HRMS)

EPA Method 8280A: The analysis of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans by high resolution gas chromatography/low resolution mass spectrometry (HRGC/LRMS) (EPA analytical chemistry guidance SW-846)

EPA Method 8290: Polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) by high-resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) (EPA analytical chemistry guidance SW-846)

ISO 18073 (2004): Water quality – Determination of tetra- to octa-chlorinated dioxins and furans – Method using isotope dilution HRGC/HRMS

**From the earlier references I suggest keeping the following ones:**

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Jensen, S., Häggberg, L., Jörundsdottir, H., Odham, G., 2003. A quantitative lipid extraction method for the residue analysis of fish involving nonhalogenated solvents. *J. Agric. Food Chem.* 51:5607-5611.

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Nicholson, M., 1989. Analytical results: how accurate are they? How accurate should they be? *Marine Pollution Bulletin*, 20:33-40.



I would consider keeping these references (although they are not so relevant since they mostly refer to aquatic species or refer to intercalibration studies and their evaluation)

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#### **Web references**

UNEP/GEF POPs laboratory capacity building project

<http://www.chem.unep.ch/pops/laboratory/default.htm>

STAP/GEF workshop report <http://www.unep.org/stapgef/documents/popsJapan2003.htm>

WHO GEMS/Food <http://www.who.int/foodsafety/chem/gems/en/>

UNEP Regional Seas Program <http://www.unep.org/water/regseas/regseas.htm>

National monitoring activities [http://www.chem.unep.ch/gmn/02\\_natpro.htm](http://www.chem.unep.ch/gmn/02_natpro.htm).

Global assessment of PTSs <http://www.chem.unep.ch/pts/default.htm>

US EPA <http://www.nemi.gov>

Japan Environment Agency <http://www.env.go.jp/en/topic/pops/index.html>

ICES <http://www.ices.dk/env>

OSPAR <http://www.ospar.org>

HELCOM <http://www.helcom.fi/Monas/CombineManual2/CombineHome.htm>

International organization

for Standardization <http://www.iso.org>

Association of Official

Analytical Chemists

International <http://www.aoac.org>

Gosstandard [http://www.kanexkrohne.](http://www.kanexkrohne.com/english/Downloadarea/gosstandard_russia.shtml)

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