Annex

Description of important parameters for the determination of POPs in air, human blood and breast milk

The following section is, to a large extent, is taken from the recommendations for POPs analysis developed under the UNEP/GEF project "Assessment of Existing Capacity and Capacity Building Needs to Analyse POPs in Developing Countries".

Before the start of any POPs analysis, an adequate study design has to be established to ensure that the sampling and subsequent analysis will meet the objectives of the study. All activities should be conducted by trained professionals, according to a well-designed plan and using internationally or nationally approved methods, carrying out the same method each time over the time span of the programme. It should be understood that mistakes in sampling or analysis as well as reporting or storage of data or any deviation from standard operational procedures can result in meaningless data or even programme-damaging data. Before initiation, the study design has to be discussed between and approved by all involved actors including the data users.
Laboratories may adopt published methods for sample extraction, clean up, and analysis, and have to validate them within the laboratory. The most basic requirements are:

- The laboratory must be able to prove competence for infrastructure, instrumentation, and well-trained staff to conduct specific analyses;

- Validation of the analytical methods including in-house methods;

- Standard Operating Procedures (SOPs) for the validated methods, including all the laboratory equipment and consumables;

- Quality criteria for quality assurance and quality control (QA/QC) described in the SOPs, *e.g.,* analysis of blank samples, use of reference materials, signal/noise ratio, and sensitivity of the analytical system.

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.

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.

1.1 General sampling procedures

General sampling procedures include:

- Preparation of sampling equipment(s), eventually shipment of samplers;

- Establishment of criteria for acceptance of samples at the laboratory;

- Establishment of standard operation procedures for sampling;

- Establishment of quality assurance procedures, *e.g.,* field blanks, chain-of-custody;

- Establishment of field blank procedures.

1.2 Infrastructure and set-up

With respect to sampling indispensable requirements include:

- Equipment: Adequate sampling instruments according to the type of matrix and POP;

- Materials: Sampling instrumentation that is analyte-compatible, including utensils, containers, *etc.* (stainless steel-glass, never plastic);

- Personal protection: Those in charge of the sampling must wear adequate protection outfits depending on the type of samples they will work;

- Sample blanks: These allow for the assessment of potential contamination;

- Preservation: Samples and sample blanks are preserved according to matrix and type of POP requirements;

- Transportation: Adequate transportation that minimizes the possibility to contaminate the sample, ensuring its integrity and conservation until it reaches the laboratory in charge of the analysis;

- Availability of "*in situ*" monitoring equipment: To measure relevant environmental parameters according to each environment. The environmental conditions should be registered;

- Geo-referencing and photographic registers: Availability of GPS to locate sampling sites with precision and ensure future location of the site;

- Standardized protocol: Well-established sampling procedures have to be applied. Such sampling protocols have been developed by institutions or organizations such as ASTM (American Society for Testing and Materials), EC (European Commission), US-EPA (Environmental Protection Agency), GEMS (Global Environment Monitoring System), and WHO (World Health Organization);

- Labeling: Unambiguous labels are needed;

- Interview protocol: May be needed for human samples;

- Approval from an ethical committee: May be needed for human samples;

- Interface between sampling personnel and analytical laboratory: Close cooperation is crucial between project planners, the samplers, the analytical laboratory, and data users;

- Training of personnel: Personnel should be sufficiently trained and familiarized with the sampling techniques;

- Storage capacity: The laboratory must have an adequate storage capacity, *i.e.,* refrigerators or freezers at sufficiently low and stable temperatures, to ensure the integrity of the samples. These temperatures should be monitored constantly and documented;

- Waste Treatment: Consideration of suitable treatment/handling of the waste generated during the sampling.

1.3 Standard operating procedure (SOP)

A Standard Operating Procedure (SOP) has to be established for each type of matrix. In these SOPs the following requirements must be addressed:

- The objective of the sampling exercise, including sampling protocols and specifications;

- Sample size in accordance with the analytical requirements and limitations in order to meet regulations or other objectives as given in the study;

- Description and geographic location of the sampling sites, preferentially with GPS coordinates;

- Guidelines for representative samples;

- Criteria for composite samples, *e.g.,* number of sub-samples, homogenization;

- Description of field blank procedures;

- Date, time of the sample taking;

- Conditions during sampling;

- Time intervals between sampling exercises;

- Specifications for the sampling equipment, including the operating, maintenance, and cleaning procedures (glassware can be cleaned by heating the glass to 300 °C over night);

- Identity of the person(s) who has taken the sample;

- Full description of sample characteristics;

- Labelling (sample numbers should be assigned in the protocol and prepared labels taken into the field);

- Labelling of samples (in the field) and sample registration for further follow-up;

- Indication of expected level of POP concentration in the sample;

- Any additional observation that may assist in the interpretation of the results;

- Quality assurance procedures to prevent cross-contamination.

The SOP should also contain a section with details on personal protective equipment that must be worn and listing of other safety concerns as appropriate.

1.4 Sub-contracting a sampling laboratory

No general recommendation can be given with respect to who should perform the sampling. For certain matrices, *e.g.,* human blood, a specialist, *i.e.,* medical doctor or nurse, has to take the sample. 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 established quality assurance and quality control (QA/QC) conditions.

In case a laboratory is 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.

2 Transport and storage

The SOP also includes the requirements for transport and storage. More specifically, these are:

- Transport and storage conditions for each sample matrix including adequate facilities and infrastructure to be provided, *e.g.,* freezers;

- Preservation of integrity of samples during transport (temperature, light, *etc.*);

- Provisions for adequate storage, including:
  - Registry of the performance of refrigerators and freezers, *e.g.,* registration and control of temperature;
  - Availability of automatic power-supply equipment in case of power cuts;
  - There may be limits in storage times, temperature and other conditions;

- Preservation of individual samples for their re-analysis (counter-sample);

- Pre-analytical treatment of the sample: statistical criteria to obtain sub-samples and composite samples (pools) that are representative; homogenization of solids and tissue.

Note: there may be requirements for shipment to be addressed and respected. Especially in the case of international shipment, considerations for transport and customs' clearance must be taken into account since restrictions may exist.

3 Analysis

Key steps to be considered are:

- Procedures and acceptance criteria for handling and preparation of the sample in the laboratory;

- Standard QA/QC procedures must be followed by the laboratory;

•Participation at international intercalibration studies, analysis of certified or laboratory reference materials are essential.

3.1. Set-up and infrastructure

In order to guarantee preservation of the samples, control of potential cross-contamination, standardization of the technique, calibration, and good maintenance of instruments, the requirements listed below are considered indispensable. In general, the laboratory should be clean and safe, well organized, and have adequately trained staff to conduct the analysis. Having implemented the above mentioned measures may allow for accreditation. The requirements include:

- General laboratory environmental conditions should ensure enough laboratory space for each step of the analysis and avoid interference between individual samples. This includes:
  - Physical separation of standards and samples;
  - Expected POP concentration (minimize cross-contamination by separating highly contaminated samples from low contamination samples);
  - Control of temperature and provision of air-conditioning;
  - Availability of extraction hoods;
  - Handling area of inflammable products;
  - Provisions for laboratory waste disposal.

- Ensure and document the custody chain of the sample: verify the integrity and preservation of the samples (maintenance) in terms of temperature, containers, labels, registry, those responsible at each stage, establishment of acceptance criteria (conditions as well as quantity of material, according to analyte and matrix);

- Separation of aliquots: In the case of complementary analysis (for example, fat determinations) prior to the freezing of the sample;

- Selection and validation of the analytical method: Use method validation protocol according to the type of analyte and matrix (selectivity, repeatability, ability to reproduce, extraction efficiency, recovery, detection limit, quantification limit, accuracy). Quality of solvents and reagents (blanks). Clean glass material (avoid cross-contamination). Maintenance and calibration of auxiliary equipment (stoves, scales, test tubes, pipettes, glassware). Protocols and procedures must be clearly described and documented.

3.2. Extraction

There are various methods for extraction, which include Soxhlet, solid phase, liquid-liquid, and pressurized extractions. After extraction, the extract will be concentrated. In order to do so, the technique should be optimized to avoid excessive loss of the analyte. Typically, this step includes: evaporation under vacuum or with nitrogen (Note: control of temperature, flow of nitrogen, and vacuum are essential). Complete drying of the extract should be avoided; the possibility of adding a high boiling compound as a “keeper” may be considered.

- Before or during extraction, water, lipids, proteins, and sulfur should be eliminated. This can be done by:
  - Elimination of water by drying of the sample with sodium sulphate or equivalent demonstrated acceptable drying procedure;

– Elimination of lipids with sulphuric acid or permeation in gels after extraction;
– Denaturation of proteins with oxalate;
– Elimination of sulphur with activated copper or by gel permeation after extraction.

- Purity of extraction solvents is also a major consideration. Only high purity glass distilled solvents should be used;

- Extraction should be standardized with respect to extraction times, type of solvent, and performance of auxiliary equipment;

- Before extraction, internal standards should be added to control the extraction efficiency;

- The recoveries of the extraction standards differ with POP to be analyzed and matrix. Based on current experiences (from international calibration studies) as a general rule:
  – For PCB and pesticides: 80 %-120 % (for tetra- and penta-chlorinated PCB recoveries down to 60 % can be accepted);
  – For PCDD/PCDF: 50 %-130 % (for hepta- and octa-chlorinated PCDD/PCDF 40 %-150 % can be accepted).

- The extracts not used in the analysis can be stored, preferably in glass ampoules, at 20°C.

3.3. Clean-up

Clean-up is done to remove interfering substances/materials from the analyte in order to obtain unambiguous results. Purification should be efficient enough so that the chromatographic retention is not influenced by the matrix (especially when no labelled internal standards are used or no mass-specific detector is available).

Clean-up is performed with various combinations of adsorbents and solvents depending on selectivity, conditioning and column flow. During purification the following aspects need to be controlled or maintained:

- An internal standard is added at a concentration giving a signal/noise ratio of at least 20/1, with fixed concentrations of internal standards from sample to sample in order to obtain adequate response factors;

- Control fraction cut.

3.4. Separation

Separation of POPs is conducted using gas chromatography with electronic capture detector (ECD), mass selective detector (MS detector) or, if available, high-resolution mass spectrometry (HRMS). Other separation techniques, such as high pressure liquid chromatography (HPLC), have not been found adequate.

- In general, an appropriate stationary phase has to be selected and enough peak separation must be achieved to allow accurate quantification (general numeric criteria cannot be given, but the use of capillary columns with lengths of 30-60 m, internal diameters of 0.15-0.25 mm, a film thickness of 0.1-0.3 μm and helium or hydrogen as a carrier gas should ensure sufficient resolution) (note: hydrogen cannot be used together with MS detection);

- Separation of critical pairs of compounds has to be verified, *e.g.,* pairs of PCB 28 and 31, 118 and 149; in dioxin analysis separation of PCDD/PCDF from polychlorinated diphenyl ethers (PCDE) should be checked;

- Helium, compared to nitrogen, gives a better choice to achieve the desired separation of pesticide POPs and PCB. The best carrier gas to achieve the required separation is hydrogen but it has some safety risk. If all the precautions and safety procedures are in place a hydrogen generator may be considered;

- Sample clean-up procedures should be efficient to prevent contamination of the detector;

- For PCB analysis and ECD detection, a minimum of two internal standards - one eluting at the beginning and one at the end of the chromatogram – should be used. It is recommended to also use one PCB congener that elutes in the middle of the chromatogram. Thus, the following three congeners are recommended: PCB #112, #155, and #198. These three congeners are quite stable and typically not found in commercial PCB mixtures. Note: decachlorobiphenyl (PCB #209) is not recommended because it tends to precipitate easily in standard solutions and due to long retention times, the peaks tend to be broad and have tailings. PCB #209 has also been identified in environmental samples and could not be quantified if this congener is selected as an internal standard;

- Adequate handling and preservation of all standards and reference materials;

- Injection:
  - Ensure cleanliness of injector (deactivated glass insert, evaluate activity with an acceptance criterion, for example, for DDE/DDT < 20 %);
  - Verify the split/splitless relation, flows and state of septum;
  - Repeatability must be ensured (for example, criterion < 5 %), and

- Verification of chromatographic conditions include:
  - Resolution, symmetric peak shape;
  - Reproducibility of retention times;
  - Purity of gases;
  - Use of second column of different polarity as confirmation column;
  - Verification of the linear range of the instrument.

- Registration and traceability of services and performance of equipment.

3.5. Identification

The information available to identify the compounds eluted from the gas chromatographic column depends on the type of detector being used. The following criteria may generally be used:

- Retention time should match between sample and internal standard;

- Confirmation of peaks can be performed on a second column with different polarity;

- Matrix spikes (or co-injection) are recommended to verify components and check the quantification;

For HRGC-ECD combinations, the following specific recommendations are given:

- Retention time ± 0.2 min;

For HRGC-MS detection combinations, the following specific recommendations are given:

- Positive identification should be done on isotopic ratios within 20 % of theoretical value;

- For positive identification with MS detection, the retention time of the labelled internal standard to the native compound should be within 3 seconds;

- The use of MS libraries is useful (if full scan).

3.6. Quantification

In general, quantification of the analyte should be done according to the internal standard methodology. For PCDD/PCDF and dioxin-like PCB, typically additional requirements are needed. The following requirements are considered to be indispensable:

- At least one standard representative for the POPs analyte group analyzed should be added at the normal level of quantification;

- For quantification it must be assured that the concentration of the compounds is within the previously determined linear range of the detector (Note: Not necessary when multi-level calibration is performed!);

- Integration: select the baseline level and the adequate signal to noise relation of integration according to the type of sample, verify the general form of the chromatogram, the form of the peaks and manually verify integration;

- Verification that the concentration of blanks is significantly lower than the samples; recommendation: < 10% ;

- Noise should be defined as close as possible to the peak of interest;

- At least 10 data-points should be sampled across a peak for quantification (Note: some instruments do so automatically);

- Calibration:
  - Labelled internal standards are an added value;
  - Multi-point calibrations should be carried out;
  - Daily calibration checks in connection with analyzing a series of samples should be done (for large batches calibration drifts have to be checked during the run);
  - Suitable laboratory reference material should be used to verify the performance.

3.7. Reporting

Data compilation and reporting together with data storage are the final steps in analysis. The report form must include:

- Date, name of the sample and description, method used, the name of staff that has performed analysis, and signature of person in charge of the POPs laboratory;

- Only SI units (International System) should be used and should be verified before clearing the report;

- Clear references to the basis for the concentration must be given, *e.g.,* fresh weight, lipid weight, or volume;

- Data below the LOQ but above the LOD should be reported as "LOD-LOQ", data below LOD as "<LOD";

- Recovery efficiency should be reported;

- Measured or estimated information on the uncertainty in the results should be made available;

- Reporting values should not be corrected for percentage of recovery;

- It should be demonstrated that the blank is 10-times lower than the value that is reported. Reporting values should not be corrected by laboratory blanks (Note: There may be high fluctuations for laboratories' blanks, *e.g.,* for PCB 118). Handling of all blanks needs written documentation; in the case of high laboratory blanks; handling of such cases and justification should be clearly indicated in the SOP;

3.8. Definitions

- Limit of detection and limit of quantification are defined as follows:
  - LOD should be 3 times the noise;
  - LOQ should be 3 times the LOD.

- Results for sum parameters where one or several individual compounds are <LOQ should be reported as intervals with a lower bound limit calculated with the <LOQ set to 0, and the upper bond limit with <LOQ set equal to LOQ.

- There are two methods available to provide information on uncertainty:
  - Quantification of uncertainty for each step
  - Overall uncertainty derived from inter- and intra-laboratory results.

4 Further important issues to consider

4.1. Maintenance of equipment

The maintenance of the analytical equipment is considered as one of the most important aspects in POPs analysis. It is very expensive to have service contracts for all the maintenance and therefore it is important to train the laboratory personnel to do the basic maintenance when the QA/QC results are unacceptable.

Laboratories must arrange for proper training, including basic maintenance, when new equipment is installed in the laboratories.

4.2. Training of laboratory staff

Human resources are crucial for any analytical work. The following specific problems need to be addressed and resolved:

- The lack of skilled laboratory personnel to conduct the analytical work has been identified as one of the critical problems;

- The training requirements. Two levels of training exist:
  – Training of people to follow the analytical procedures and to report the results;
  – Training of people to do troubleshooting and conduct the necessary maintenance when the QA/QC criteria fail;

- Countries with experienced personnel should assist other countries with training of laboratory personnel;

- There is a need in the region for training courses and annual training workshops for the transfer of technology know-how.

4.3. Housing

For POPs analytical laboratories there are certain requirements as to housing. These include:

- Proper environmental conditions (humidity is a most critical factor) for instrumental analysis but also for sample preparation;

- Minimization of vibration (most important for HRMS instruments);

- Temperature control for helium carrier gas used with ECD;

- At certain locations where the incoming air has to be cleaned. Ideally this would involve a well ventilated lab with air pre-filtered through HEPA (HEPA Corporation) and carbon filters. The analysis of blank samples will disclose background interferences, and to identify the influence from the laboratory environment, a small volume of a solvent left in an open Petri dish for a couple of days will catch the compounds in the atmosphere;

- Occupational Health Safety venting;

- Environmentally sound/safe disposal of laboratory wastes and highly contaminated samples must be guaranteed.

5 References

UNEP/GEF POPs Laboratory Project: http://www.chem.unep.ch/pops/laboratory/default.htm

The full text of the guidelines can be downloaded from:
http://www.chem.unep.ch/pops/laboratory/documents.htm