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JRP-Coordinator	
Name, title, organisation	Rosemarie Philipp, Dr, BAM
Tel:	+49-(0)30-81045893
Email:	rosemarie.philipp@bam.de
JRP website address	http://www.emrp-waterframeworkdirective.bam.de
Other JRP-Partners	
Short name, country	BRML, Romania; IJS, Slovenia; JRC, EC; LGC, UK; LNE, France; PTB, Germany; Syke, Finland; TUBITAK, Turkey; UBA, Germany; ISPRA, Italy
REG-Researcher (associated Home Organisation)	
Researcher name, title (Home organisation Short name, country)	Adriana Gonzalez Gago, Dr HZG, Germany
	Start date: 01 Nov 2011 Duration: 35 months
Researcher name, title (Home organisation Short name, country)	Andrés Rodríguez Cea, Dr UNIOVI, Spain
	Start date: 01 Sept 2012 Duration: 24 months

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1 Executive Summary

Introduction

The Water Framework Directive (WFD) 2000/60/EC establishes a legal framework for protecting the quality of surface and coastal waters in the European Union (EU). According to the WFD, EU Member States are obliged to monitor the ecological status of their inland waters in order to ensure their long-term sustainable use. This project helped to improve the quality and comparability of such monitoring data by developing primary reference methods for selected water pollutants listed in the WFD and by producing new concepts for water reference materials and actual test materials.

The Problem

The WFD and its daughter directives 2008/105/EC (Directive on Environmental Quality Standards (EQS) in the field of water policy) and 2009/90/EC (Directive on technical specifications for chemical analysis and monitoring of water status) specify a list of priority water pollutants together with technical requirements for the chemical analysis and monitoring of water. These priority water pollutants are toxic, persistent and liable to bioaccumulate within the food chain thus endangering a wide range of living organisms. Therefore, EQS values equivalent to the maximum allowable concentration, are very low in the WFD for some of the priority water pollutants, which makes them difficult to monitor at such low levels. EQS values for organic pollutants also refer to the “whole water body”, i.e. including not only the analyte fraction in the dissolved phase but also the fraction bound to colloids and suspended particulate matter (SPM). This poses an additional difficulty during analysis, particularly with non-polar hydrophobic compounds which are strongly adsorbed to particles/SPM.

In 2008 a survey by CEN TC 230 “Water Analysis” stated that there was a lack of methods capable of quantifying several priority water pollutants, such as tributyl tin TBT, pentabrominated diphenyl ethers (PBDEs) and polycyclic aromatic hydrocarbons (PAHs) at EQS levels in whole water samples. Furthermore, CEN TC 230 noted that aqueous reference materials with suspended particulate and colloids were not available. Therefore, in order to improve the monitoring of inland water by EU member states such reference materials are needed for proficiency testing and for in-house Quality Assurance in test laboratories.

The Solution

To address these needs, the project developed reference methods for TBT, PBDE and PAH in whole water samples as well as concepts for aqueous reference materials that mimic natural waters regarding SPM and colloids present in the water phase. The project also conducted a systematic study of the interaction and partitioning of pollutants as knowledge of the distribution, interaction and partitioning of the target analytes in whole water are important for the development of accurate measurement methods.

Impact

The reference methods and materials developed in this project have enabled National Metrology Institutes (NMIs), reference materials producers and/or proficiency testing providers to deliver metrological services for testing labs monitoring real water samples under the WFD. This includes testing lab services such as the production and certification of reference materials, the characterisation of proficiency testing samples, estimation of reference values for proficiency testing schemes and calibration services. Using these services testing labs can now benchmark their routine methods against NMIs via reference materials and/or regular participation in proficiency testing schemes and therefore maintain an appropriate Quality Assurance/Quality control (QA/QC) system in order to perform water monitoring more accurately, efficiently and economically. The result of this is that, water testing and monitoring results are linked to the International System of Units (SI) and the comparability between testing labs has been improved, which in turn ensures long-term reliability and global comparability of water monitoring data obtained under the WFD and supports better decision-making in the field of water management.

In addition to environmental contamination all the priority water pollutants investigated in this project can accumulate within the food chain and endanger humans, therefore their uptake is a serious health issue. However, by providing validated reference (primary) methods and materials for TBT, PBDE and PAH the project has helped to reduce the presence of these compounds, according to the requirements of the WFD, to a level not considered harmful.

2 Project context, rationale and objectives

The control of water pollution is an important issue with a huge impact on human health and the environment. The WFD establishes a legal framework for Community action in the field of water policy with the main goal of good ecological status of all inland and coastal waters by 2015. However, the implementation of the WFD depends on the availability of analytical reference methods and reference materials for the priority water pollutants specified. Comparability of water monitoring measurements within the EU is also required in order to ensure reliable water surveillance as a prerequisite for a fair enforcement of the WFD in all Member States. Further to this, the WFD presupposes the application of ISO/EN 17025 in all test and calibration laboratories and the standard itself requires metrological traceability of measurement results as a fundamental prerequisite for their trueness and comparability.

Therefore to address these issues, primary standards for the priority water pollutants specified in the WFD are needed. Such primary standards would form the practical references for traceability and are, as far as technically possible, linked to the international system of units (SI) – i.e. in the case of chemical analysis, to the unit mol, or mass fractions to the unit kg. The project aimed to provide this traceability by:

- Developing primary reference methods for the analysis of selected priority water pollutants and
- Developing concepts for the production of reference materials for the analysis of selected priority water pollutants.

The requirement for specific reference methods and reference materials is described in more detail below along with a summary of key methods and materials developed in the project,

Primary reference methods

The WFD daughter directive 2008/105/EC specifies a list of 33 priority water pollutants together with EQS values for each substance or substance group. Two types of EQSs were set: annual average concentrations (AA-EQS) for protection against long-term and chronic effects, and maximum allowable concentrations (MAC-EQS) to avoid serious irreversible consequences for eco-systems due to acute exposure in the short-term. EQS values for priority water pollutants also refer to whole water samples, i.e. water with colloidal matter and SPM which can pose an additional difficulty during analysis, particularly as non-polar hydrophobic compounds are strongly adsorbed by particles/SPM.

For some of the organic priority water pollutants EQS values are very low:

- | | |
|---|----------|
| • TBT cation: | 0.2 ng/L |
| • Σ 6 PBDEs (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154): | 0.5 ng/L |
| • selected PAHs, e.g. Σ Benzo(g,h,i)-perylene and Indeno(1,2,3-cd)-pyrene: | 2.0 ng/L |

Therefore, for reliable measurements of such contaminants at these EQS levels, the WFD daughter directive 2009/90/EC demands analytical methods for test laboratories which have a limit of quantification (LOQ) equal to or lower than 30% of the EQS and a relative expanded measurement uncertainty (95% confidence) of 50% or less at EQS. Further to this, EQS and LOQ values refer to the whole water body/samples. Therefore, an evaluation of how the distribution of contaminants between liquid and solid suspended phases afflicts extraction and/or preconcentration methods, is needed.

In 2008 a survey by CEN TC 230 “Water Analysis” stated that there was a lack of applicable internationally standardised methods for several priority water pollutants (i.e. hazardous substances), including TBT, PBDE and PAH. The existing ISO standard 17353:2004 “Water quality – determination of selected organotin compounds – gas chromatographic method” allows quantification of TBT down to a limit of approximately 10 ng/L, but for the analysis of PBDE in water international standards are missing.

International standardised methods for analysing PAH in water samples are available, including ISO 17993:2002 based on high-performance liquid chromatography (HPLC) with fluorescence detection after liquid–liquid extraction and the draft method (ISO 28540:2011) based on gas chromatography–mass spectrometry after either liquid/liquid or solid phase disk extraction. Moreover, EPA Method 525 employing C18 cartridges or C18 disks for PAH extraction is widely used as well. However the LOQs of the above

mentioned methods are well above the WFD requirements. Furthermore, none of the standards addresses the problem of determining the fraction of analytes bound to colloidal matter and SPM in the water phase.

As a result of the CEN TC 230 "Water Analysis" survey the European Commission tasked CEN via Mandate 424 to develop and improve robust field suited standards for routine test laboratories. The ENV08 project worked in parallel to Mandate 424 and supported it by delivering primary reference methods (and associated materials) to link testing laboratories and proficiency testing (PT) providers to the SI. The traceable primary reference methods developed by the project included:

- preparation and characterisation of suitable calibration standards and isotopically labelled spike materials in terms of chemical and isotopic purity for priority water pollutants
- development of extraction and preconcentration methods such as liquid/liquid extraction (LLE), solid phase extraction (SPE), solid phase micro extraction (SPME) and large volume injection to achieve the required LOQs for priority water pollutants
- development of isotope dilution (ID) methods in combination with mass spectrometry (MS) and/or inductively coupled plasma mass spectrometry (ICP-MS) for quantification of priority water pollutants
- development of derivatisation methods for TBT (a priority water pollutant) in order to make the analyte accessible for gas chromatography (GC)
- development and validation of methods for the determination of target analytes (i.e. priority water pollutants) associated with SPM, humic acids (HA) or colloids present in the water phase.

Reference materials

The WFD requires the analysis of whole water samples therefore reference materials with suspended solids and colloidal organic matter are necessary for the quality assurance of testing laboratories involved in water monitoring. However, such reference materials also need to be stable and homogeneous and, prior to the start of the ENV08 project whole water reference materials were not available. Previously PT schemes had to use filtered water samples fortified with a spike of the target analytes in a water-miscible organic solvent to make up for this lack of reference materials.

In order to address this, the ENV08 project conducted a feasibility study for the production of aqueous reference materials containing particulate and/or colloidal organic matter. The aim of the study was to develop concepts for the production of whole water reference materials mimicking the properties of real whole water samples (as closely as possible). The study included:

- an investigation of methods for stabilising the organic target compounds in the water phase
- development of methods for including SPM in whole water reference materials
- investigation of the influence of different container materials on the partitioning of target compounds between the water/SPM phase and container walls
- a homogeneity and stability assessment of potential whole water reference materials.

Knowledge about the distribution of contaminants in different compartments of aquatic systems is also important for the provision whole water reference materials and the validation of analytical methods for whole water samples. The distribution of contaminants determines the transport, fate and possible toxic effects of contaminants, therefore, analytical methods are needed that can accurately determine not only the total mass fraction of priority water pollutants but also the pollutant partitioning between macromolecular, colloidal and particulate phases in aquatic systems. Field flow fractionation (FFF) has been proven to be a powerful tool for size-fractionation and separation of a wide range of particulate, colloidal and macromolecular materials. FFF coupled to ICP-MS (FFF-ICP-MS) is also able to produce elemental size distributions with a great level of detail in the sub-micrometer range without laborious repetitive separation steps such as filtration or centrifugation. However, the potential for FFF techniques in combination with elemental and molecular detectors for fractionation of priority water pollutants in environmental whole water samples had not been fully exploited.

The ENV08 project aimed to develop and validate FFF-based methods for fractionation of priority water pollutants in environmental whole water samples to enable traceable and comparable results, improve understanding of aquatic processes and address the need for reliable analysis of whole water samples (including the provision of whole water reference materials). Information was provided by the project on the total element content and speciation of WFD contaminants i.e. priority water pollutants such as PBDEs and

TBT distributed in environmental water fractions of different size and nature (e.g. suspended organic matter, organic and inorganic-rich colloids). This information provided mass balance quantitative data and also relevant information on the interactions, bioavailability and mobility of such contaminants. The mass balance data was used to help validate the accuracy of the analytical methods developed for the priority water pollutants. Furthermore, the FFF-based methods were used to characterise the particle size distribution of the SPM material used for the preparation of the whole water reference materials. From this it was shown that the size distribution of the SPM material was similar to that typically observed in natural whole water samples.

Objectives

The central aims of the project were the development of primary reference methods and reference materials in support of the WFD. The work focused on three priority water pollutants specified in the WFD; TBT, PBDE and PAH and had the following scientific and technical objectives:

1. Validated primary reference methods based on isotope dilution for the analysis of selected priority water pollutants (TBT, PBDE, PAH) in whole water samples at EQS levels. Methods will have specific LOQs and a relative measurement uncertainty of $\leq 50\%$ at the EQS. Methods will also be traceable to the SI.
2. Systematic study of the interaction and partitioning of pollutants (i.e. water pollutants PBDE, TBT) in environmental aquatic compartments by FFF techniques; in order to provide mass balance data for the size based distribution of pollutants in whole water samples.
3. Development of preparation methods for aqueous whole water reference materials with proven homogeneity, and short and long time stability, which are as close as possible to real water samples, i.e. including suspended particulate and colloidal matter.
4. An intercomparison and workshop for stakeholders to disseminate the primary reference methods and reference materials developed in the project.

3 Research results

3.1. Validated primary reference methods based on isotope dilution for the analysis of selected priority water pollutants (TBT) in whole water samples at EQS levels.

3.1.1. Introduction

In order to achieve objective 1 a reference method for the quantitative determination of TBT in whole water samples was developed and validated. In order to meet the requirements of the WFD, the LOQ of the method needed to be lower than 30 % of EQS (i.e. 0.06 ng/L TBT cation) with a relative expanded measurement uncertainty of less than 50 % at EQS. In addition, the method needed to be applicable to whole water samples as defined in the WFD and have measurement results that were traceable to the SI. This traceability was achieved with a well characterised (in terms of chemical purity) calibration standard.

The accurate quantification of organotin species such as TBT is a challenging due to their instability, which often leads to possible inter-conversion reactions between species, and problems with procedural blanks. Therefore, special attention was given to these issues during method development.

3.1.2. Optimisation of extraction, derivatisation and preconcentration

For this task 3 extraction/ derivatisation methods were studied and optimised: Liquid/liquid extraction (LLE), Headspace Solid Phase Microextraction (HS-SPME) and Solid Phase extraction (SPE). Large volume injection was also tested as a further preconcentration method. The parameters of the derivatisation and extraction step were systematically varied to increase the derivatisation/extraction efficiency and minimise procedural blanks. Cleaning of laboratory glassware was also optimised in order to minimise blank values. MilliQ water, laboratory tap water, commercial mineral water, water with HA and natural waters were used as sample material and the sample volume was typically in the range 100 mL to 1 L. Deuterated TBT was used as the internal standard for most of the experiments unless the isotope dilution calibration method was used. The following parameters were studied:

- **LLE:** type and amount of extraction solvent; type of agitation (shaking, stirring); time of extraction
- **HS-SPME:** fiber materials; addition of ethanol to prevent adsorption at the container walls; time and temperature of equilibration; time and temperature of extraction
- **SPE:** A design of experiment (DOE) was developed with the Design Expert software, taking into account interactions between the factors influencing the SPE procedure, namely type and amount of the stationary phase, elution solvent, sample mass, pH and derivatisation procedure.
- **Derivatisation:** volume and concentration of NaBEt₄; solvent for NaBEt₄; pre-treatment of the NaBEt₄ solution (venting with argon, extraction with organic solvents); type and pH of buffer solution; time of derivatisation
- **Cleaning of glassware:** concentration of cleaning agent (nitric acid); heating at 400 °C
- **Large volume injection:** sample volume and temperature program

An optimised LLE and a derivatisation method for TBT which meets the target LOQ for samples with low dissolved organic carbon (DOC) content and low SPM was developed by the project. Blank values could be reduced by about one order of magnitude. HS-SPME was found to be not precise and reproducible enough to meet the WFD requirements. SPE resulted in tedious and long multi step methods (compared to LLE) potentially resulting in an increased contamination risks and problems with repeatability. Large volume injection further decreases the LOQ of the method; with a sample volume of 1 L or more and ICP detection large volume injection is not necessary to meet the EQS value. The results were summarised in a report and data for the DOE SPE measurements was published in a peer-reviewed paper [1].

3.1.3. Purity assessment of neat calibration standards

Purity assessment of the TBT calibration standard tributyl tinchloride (TBTCl) is difficult since the compound is not accessible to GC with flame ionization detection (GC-FID) due to its low volatility. Therefore, quantitative nuclear magnetic resonance spectroscopy (qNMR) methods were used for purity determination. Several batches of commercial neat TBTCl were purchased and first measured by GC-ICP/MS after

derivatisation. No substantial difference was observed in the organotin compound pattern of the standards. Therefore the least expensive material was selected for further purity assessment. A larger amount was purchased and subjected to ^1H and ^{13}C qNMR. A NIST Certified Reference Material SRM 350b (benzoic acid with certified purity) was used as a quantitation standard in these experiments. ^1H qNMR signals turned out to strongly interfere, therefore a single undisturbed signal suitable for quantification could not be identified. The majority of the ^{13}C signals, although less sensitive than ^1H , were resolved. Quantification of the ^{13}C signal of the TBT $\alpha\text{-CH}_2$ group at 17.5 ppm vs. the 4 CH signal of the NIST SRM at 133.8 ppm yielded a purity of (977.3 ± 5.3) mg/g (6 independent determinations). A full uncertainty budget of the result was established.

Tetrabutyltin was identified as the main (tin) organic impurity but signal intensities were too low for a reliable quantification. Coulometric Karl-Fischer titration was performed to measure the mass fraction of water in the TBTCI sample. However, it turned out to be negligible, i.e. (2.2 ± 0.2) mg/g (two independent determinations). A ^{119}Sn labelled butyltin mix was also purchased and characterised by high resolution GC-ICP/MS (GC-HR-ICP/MS). Derivatisation was done by ethylation with NaBEt_4 . The results confirmed the certified values of the supplier with respect to the isotopic composition of the sample.

3.1.4. Development and in-house validation of an IDMS reference method for quantification of TBT

Measurements by GC-ICP/MS were performed with different internal standards (Tripropyltin (TPrT), deuterated TBTCI, ^{119}Sn labelled TBTCI). Parameters such as GC oven programme and ICP conditions were optimised in order to achieve good peak resolution and intensity. The influence of oxygen added to the argon gas for efficient matrix decomposition in the ICP was systematically studied and capillary columns of different length and polarity were tested in order to decrease GC run-time. A GC method which allows effective separation of 15 naturally occurring OTCs including inorganic tin on a 15 m column was established with a GC run time of approximately 13 min.

An EXCEL sheet for IDMS calculations was developed and a joint validation protocol for the IDMS procedure agreed. The validation protocol considered the following parameters: linearity/working range, limits of detection/quantification, trueness, precision, specificity/selectivity and measurement uncertainty. An uncertainty budget was also established for the IDMS procedure in accordance with the Guide to the Expressions of Uncertainty in Measurement (GUM). The combined standard uncertainty has been estimated by propagation of standard uncertainties of all parameters in the model equation. In addition dedicated software (*Wincert v. 3.11*, *Implex*) was used for the calculation of the derivatives. An Ishikawa diagram has also been established which allows the identification of the predominant uncertainty contributions to the TBT mass fraction at EQS level. A publication of these data in the scientific literature is under preparation.

Further to this, a small intercomparison was performed between the institutes involved in the task. Natural mineral water spiked with TBT at EQS and target LOQ level was distributed as sample material. The intercomparison used an optimised LLE method with ^{119}Sn labelled TBT for extraction and preconcentration, whilst quantification was done using IDMS. The results showed that the requirements of the WFD were met for this relatively simple water matrix (Figure 1).

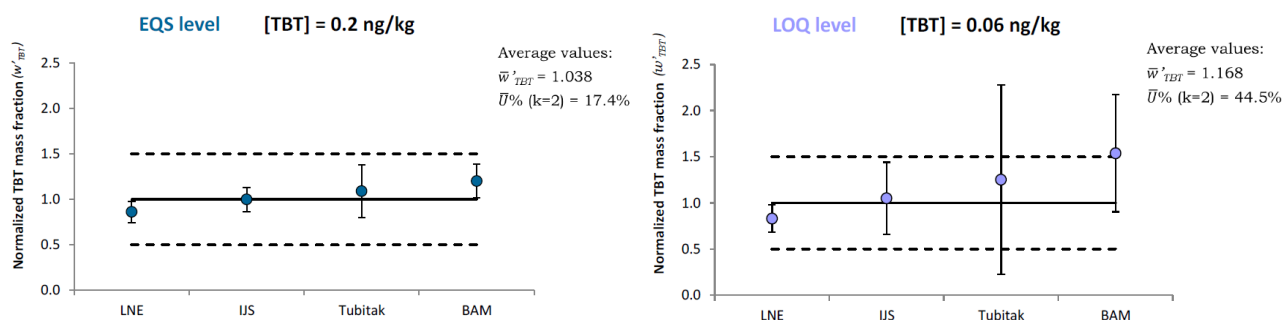


Figure 1: Results of the intercomparison, mineral water spiked with TBT, error bars are expanded uncertainties at 95% confidence interval

3.1.5. Extension of the optimised method(s) for TBT to whole water samples

The methods developed for relatively simple water matrices were tested on complex whole water samples. First experiments showed that the extraction behaviour of TBT strongly depends on the water matrix. Examples are shown in Figure 2.

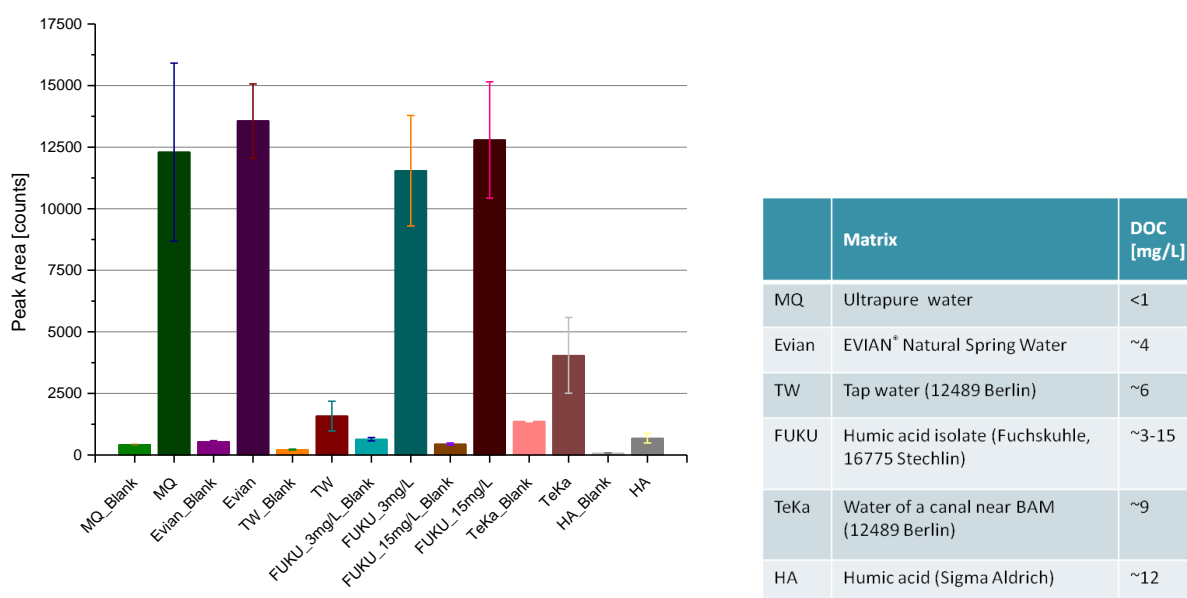


Figure 2: ICP-MS peak areas obtained after extraction of TBT from different water matrices spiked at 1 ng/L (as Sn). Error bars are the standard deviation of three replicates. DOC: dissolved organic carbon

In a systematic approach, methods developed for simple water matrices were tested on whole water samples prepared by spiking HA and/or TBT contaminated SPM to mineral water or tap water. Isotope dilution was applied for the quantification of TBT. The recovery values obtained were in the range of 90 % to 125 %, with repeatability values between 3% and 10 %; the lowest uncertainties achieved were 8% at the concentration level of 0.2 ng/L in mineral water + HA + SPM. The results of these measurements were summarised in a report and as methods, which were presented to stakeholders at the project's final workshop in September 2014 (see section 4.1.4). Two publications on these results are also in preparation.

3.1.6. Determination of TBT by IDMS using GC-MS and –MS/MS

A method for the quantification of TBT in water samples based on IDMS and GC-MS/MS operating in Multiple Reaction Monitoring (MRM) mode was developed. The LOQ of the methodology was 0.04 ng L⁻¹ of TBT cation, which meets the requirements of the WFD. The recovery of TBT at the EQS level was (106.1 ± 4) %. The developed method was used for the quantification of TBT in water samples within the final ENV08 intercomparison (see Section 3.5 and part of objective 4) and the results were in good agreement with those obtained by GC-ICP-MS (Figure 3). The method will be published in the peer-reviewed scientific literature [2, 3].

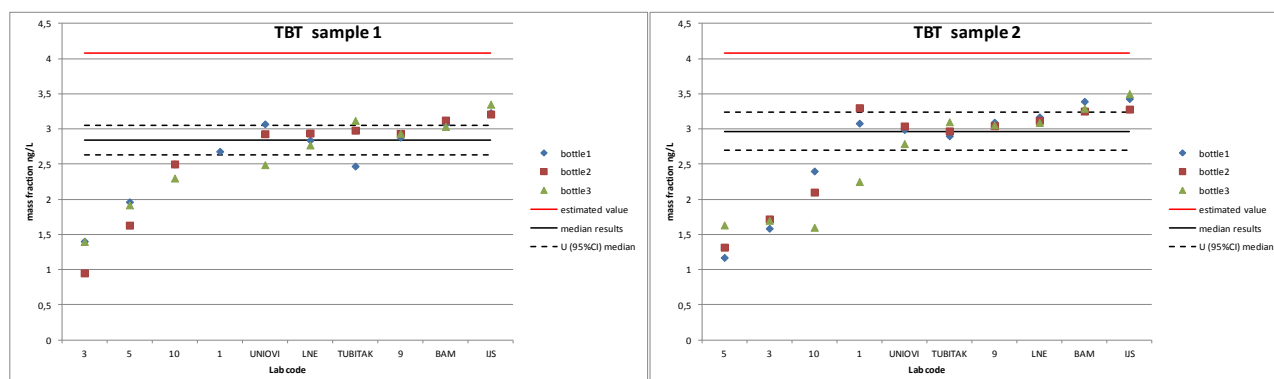


Figure 3: ENV08 intercomparison results for the analysis of TBT. Sample 1 contained HA and SPM, sample 2 only SPM. The UNIOVI result was obtained by GC-MS/MS, the BAM, LNE, TUBITAK and IJS results by GC-ICP-MS.

3.1.7. Conclusion

Objective 1 of the project, a traceable validated reference method capable of quantifying the target priority pollutant TBT at EQS level in whole water samples, was achieved by the project. Two measurement procedures based on LLE and GC-ICPMS or GC-MS/MS were developed and validated for the quantitative determination of TBT in whole water samples containing organic and SPM. Both methods were traceable to the SI and their traceability was established by a purity assessment of the TBT calibrant using ^{13}C qNMR and Karl-Fischer titration. The methods meet the requirements of the WFD with respect to LOQ (≤ 0.06 ng/L) and relative expanded uncertainty at EQS (≤ 50 %) and a description of the methods has been published in peer-reviewed journals [1-3].

The methods were used in the final ENV08 intercomparison (see Section 3.5 and objective 4) and disseminated to stakeholders through the project's final workshop (see Section 4.1.4), via publications [1-3] and via a French PT scheme for organotin analysis (see section 4.3.2). The PT scheme was organised by Institut national de l'environnement industriel et des risques (INERIS) and approximately 15 field laboratories participated. Using the reference method for TBT and its reference values the field laboratories were able to benchmark the performance of their in-house procedures.

3.2. Validated primary reference methods based on isotope dilution for the analysis of selected priority water pollutants (PBDE) in whole water samples at EQS levels.

3.2.1. Introduction

As part of objective 1 a traceable reference method capable of quantifying the target priority pollutant PBDE at EQS level in whole water samples was developed and validated. The PBDE congeners listed in the WFD are PBDE 28 (tri-brominated), 47 (tetra-brominated), 99, 100 (penta-brominated), 153 and 154 (hexa-brominated). EQS of 0.5 ng/L and 0.2 ng/L were established in the WFD for each congener for inland and other surface waters, respectively, with a relative expanded uncertainty of 50 % at the EQS level. The LOQ of the methods used for the water monitoring should also not exceed 30 % of the EQS levels.

Prior to the start of the project, no methods existed which were sensitive enough to reliably determine PBDE in water at such low concentrations, therefore the project chose the most promising methods, used for the determination of PBDE in plastics, following a survey of the (then) current literature. For the determination of PBDE in water, GC coupled to various mass spectrometric techniques such as MS, MS/MS or ICP-MS were chosen as liquid chromatography (LC) produced an unacceptable dilution of the analytes. For the extraction of PBDE from water, LLE as well as SPE using SPE cartridges or SPE discs were used.

As the WFD requires the extraction of PBDE from whole water without prior filtration, (thus containing SPM), all methods faced the challenge of extracting PBDE bound to HA as well as to the particulate phase in the water. The goal was therefore to provide measurement procedures for the determination of PBDE in whole water samples without the separation of SPM and liquid phase.

Furthermore, due to the challenges associated with coastal waters e.g. changes in salinity and SPM content and a lower EQS represent, a specific procedure for the quantification of PBDEs in coastal waters and a field suited method for sampling of coastal waters for PBDE measurements were developed.

3.2.2. Optimisation of the extraction method

Artificial water samples were used for the optimisation of the extraction, separation and detection methods. In a first approach, PBDE 28, 47, 99, 100, 153 and 154 were spiked to tap or mineral water samples. After evaluation of the best method for use with such simple water samples, mineral water was spiked with HA and/or SPM to mimic real water and the best method(s) was then used to determine PBDE in real water samples.

LLE: for the optimisation of the extraction procedure different solvents (hexane, dichloromethane, iso-octane) and solvent mixtures were tested regarding their ability to quantitatively remove the PBDE from water and particulate phase. Hexane or a mixture of hexane and dichloromethane was found to be most effective for extracting PBDE from water samples. However, initial experiments revealed problems with the phase separation when the samples contained a high amount of organic compounds such as HA. A kind of foam (Figure 4) was formed that prevents the water and organic phases from separating.

Different strategies such as the addition of salts or concentrated sulphuric acid were used to overcome the creation of the foam and thus facilitate the phase separation.



Figure 4: Extraction of PBDE congeners from HA containing water samples. Instead of a clear phase separation a kind of foaming can be observed. The foam has to be destroyed or prevented to successfully retrieve the PBDE.

Natural samples may contain a variety of bromine containing compounds that might interfere with the detection of PBDE. These compounds can either be removed during the sample preparation procedure and/or by optimisation of the separation method. For particularly difficult samples which contain brominated compounds that cannot be separated from the analytes with GC, such as shown in Figure 5, a clean-up procedure for the extracts was developed that includes acidic as well as alkaline treatment of the extract and a solid phase purification step using an SPE column containing silica gel impregnated with silver nitrate and a small plug of anhydrous sodium sulphate.

To further improve the detection limit of the measurement procedures the extracts were finally reduced to 100 – 200 μ L with a nitrogen stream.

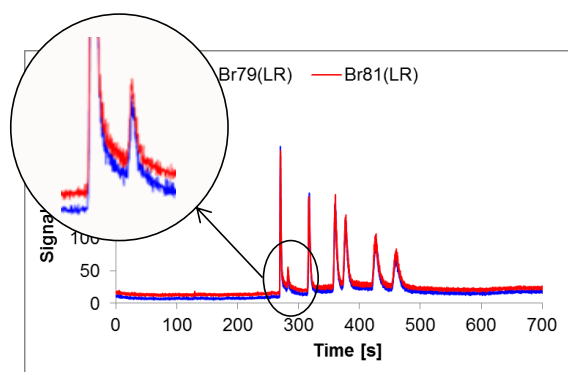


Figure 5: Chromatogram of a real water sample containing an unknown brominated compound which might interfere the evaluation of the peak for PBDE 28.

SPE: SPE was used for the extraction and enrichment of PBDE in water samples and SPE disc extraction was found to be the most suitable approach as columns tend to clog when water samples containing SPM were investigated. The extraction was optimised for the SPE material, disc pretreatment and eluent. The PBDE were most effectively extracted using a C18 disc. Afterwards the PBDEs were recovered from the disc using hexane or a mixture of ethyl acetate and methylene chloride.

3.2.3. Separation and detection of penta-PBDE

GC was used (with a capillary column of different lengths containing 5 % Phenyl – 95 % Dimethylpolysiloxane or a similar composition as the stationary phase) for the separation of the various PBDE congeners from each other and from an interfering matrix. Helium was used as carrier gas and a temperature program was applied varying between 15 min and one hour depending on the complexity of the samples. The selectivity of the separation was tested using mixtures of various PBDE and/or other bromine containing molecules, for example the certified reference material NIST SRM-2257. In Figure 6 the chromatogram achieved using the column DB-5MS (30 m, 0.32 mm, 0.25 μ m) is shown.

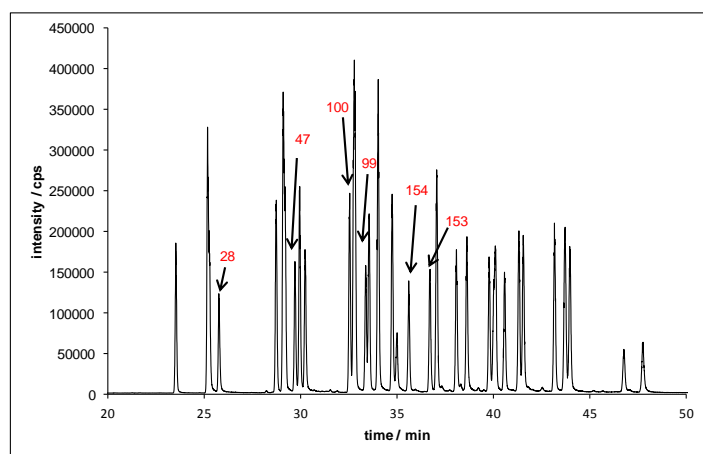


Figure 6: Separation of the PBDE congeners in the reference solution NIST 2257 achieved with GC-ICP-MS. The congeners listed in Annex I of the WFD are labelled in red (Swart et al., submitted [4]).

To achieve the LOQs required in the WFD, the extraction and preconcentration methods were optimised to yield an enrichment factor between 500 and 2000.

An important issue when determining such low concentrations and considering the high enrichment are the blank values. As PBDEs were included in many older computer devices, many laboratories encounter considerable amounts of PBDE 47 in their laboratory environment. Therefore, special measures have to be taken to keep the blank values low such as repeated cleaning of glass ware, use of ultrapure solvents and possibly clean-room conditions.

The PBDE congeners were detected either via their bromine content with ICP-MS or via specific molecule ions and their transition ions with MS/MS. The ions which can be used for identification and quantification are given in Table 1.

Congener	Retention time / min	MRM –quantification (collision energy / V)	MRM –confirmation (collision energy / V)
28	5.43	248.0 → 139.0 (30 V)	405.8 → 245.9 (20 V)
47	6.93	325.8 → 217.0 (30 V)	485.8 → 325.8 (20 V)
100 99	8.14 8.47	403.8 → 296.8 (30 V)	565.7 → 403.8 (20 V)
154 153	9.36 9.79	483.7 → 376.8 (30 V)	645.7 → 485.8 (20 V)

Table 1: Molecule ions and ions created by multiple reaction monitoring (MRM) for the identification and quantification of PBDE congeners with MS/MS (Swart et al., submitted [4]).

3.2.4. Quantification and estimation of measurement uncertainty

The quantification of the PBDE congeners was achieved with ICP-MS detection. As bromine has only two natural isotopes with an isotope ratio of roughly 1:1, exact matching IDMS could not be applied. Therefore, a ratio of 2:1 (m/z 81:79) was chosen for most congeners except PBDE 100 and 154 for which the isotopic enrichment of the spike used was very low. The spike solutions were added prior to any sample preparation to account for losses and species transformation during extraction, enrichment and separation.

For the quantification of the PBDE congeners using MS/MS the samples were spiked with the according congeners isotopically labelled with ^{13}C . The retention times of PBDE with a natural isotopic composition and those labelled with ^{13}C were identical and can thus be used as internal standards. Another approach for the quantitative determination of PBDE was the use of fluorinated PBDE as an internal standard. However care has to be taken that the added fluorinated congeners are clearly separated from all PBDE of interest and that other bromine or fluorine containing compounds do not interfere in the quantification.

Traceability to the SI was achieved by either tracing the isotopically labelled PBDE congeners back to the certified reference material NIST SRM 2257 or to in-house standards gravimetrically prepared from the solid congeners after verification of the purity of the congeners by GC-FID.

Another advantage of IDMS, in account for losses during the sample preparation, is that it can also counterbalance any non-linearity of the detector as long as the spike is fully equilibrated with the analytes in the sample. Therefore, the working range is only limited by the sensitivity of the detector. In contrast the linearity and the limits of the working range play an important role in GC-MS/MS determination. Calibration standards containing the PBDE congeners at different concentration levels were prepared and ^{13}C -labelled PBDE congeners with a constant concentration were added as internal standards to each calibration solution. The calibration was found to be linear over a working range of 0.004 - 100 $\mu\text{g/L}$ (correlation coefficient $r = 0.99$) and the recovery varied from congener to congener but was between 80 and 100 %.

A comprehensive uncertainty budget was prepared for all measurement procedures using the according equation from the GUM. Single IDMS as well as double IDMS was applied in GC-ICP-IDMS. From the results it was concluded that the major contribution to the uncertainty is the accuracy of the determination of the isotope ratio m/z 81:79 in sample-spike blend (both in single and double IDMS) as well as reference-spike blend (in double IDMS). The uncertainty also varied between the congeners. In contrast with GC-

MS/MS, the repeatability followed by the sample preparation influences the uncertainty most. As can be seen in Table 2, the requirement of the WFD to reach an LOQ of (0.15 ± 0.07) ng/L was met by all measurement procedures developed in this project with the exception of the procedure involving LLE followed by GC-EI-MS/MS. As the applied methods are all based on different principles, they provide different and complementing information on the sample constitution and possible interferences.

Congener	GC-ICP- sIDMS / ng/kg		GC-ICP- dIDMS / ng/kg	GC-EI- MS/MS (LLE) / ng/kg	GC-EI- MS/MS (SPE) / ng/kg	GC-ECNI- MS/MS (SPE) / ng/kg
	Inland	Others				
28	0.0500 ± 0.0053	0.0148 \pm 0.0025	0.127 \pm 0.039	N/A	0.0240 ± 0.0008	0.025 ± 0.011
47	0.139 ± 0.023	0.0560 \pm 0.0065	0.111 \pm 0.052	2.17 ± 0.87	0.0260 ± 0.0018	0.150 ± 0.068
99	0.101 ± 0.014	0.0741 \pm 0.0066	0.125 \pm 0.051	3.94 ± 1.58	0.0240 ± 0.0040	0.150 ± 0.068
100	0.314 ± 0.016	0.0473 \pm 0.0094	0.115 \pm 0.045	0.648 ± 0.260	0.0260 ± 0.0014	0.035 ± 0.016
153	0.0650 ± 0.0083	0.0237 \pm 0.0037	0.125 \pm 0.058	0.532 ± 0.270	0.0260 ± 0.0020	0.025 ± 0.011
154	0.407 ± 0.038	0.031 \pm 0.011	0.121 \pm 0.051	0.439 ± 0.220	0.0260 ± 0.0024	0.025 ± 0.011

Table 2: LOQs and according uncertainties achieved with the various measurement procedures for the detection of PBDE in artificial water samples containing HA (Swart et al., submitted [4]).

The accuracy of the developed methods was demonstrated by analyses of natural water samples such as river or lake water as well as sea water fortified with environmentally relevant levels of PBDE. In all cases the recoveries of the spiked priority congeners were close to 100 % and the relative expanded measurement uncertainty was ≤ 50 % at EQS as required by the WFD.

3.2.5. Field sampling procedures suitable for coastal waters

Two different procedures, manual spot sampling and sampling using in situ pumps, were tested as field sampling procedures suitable for coastal waters. Both sample procedures were compared and no significant differences were found between the two procedures. However, the sampling system using in situ pumps was proposed as the best field sampling procedure suitable for coastal waters because it is simpler and allows an easier sampling of large water quantities.

3.2.6. Conclusion

Objective 1 of the project, a traceable validated reference method for PBDEs capable of quantifying the target priority pollutant at EQS level in whole water samples, was achieved. As part of this, extraction methods such as LLE and SPE using discs or cartridges were tested and compared and PBDE congeners contained within a penta-PBDE mix were quantified using GC coupled to ICP-MS and MS/MS, respectively. A complete uncertainty budget was prepared for each method and the results of each method were compared regarding LOQs, uncertainty levels and possible interferences. The requirement of the WFD to reach an LOQ of 0.15 ng/L for PBDE's and this was met by all methods with the exception of LLE followed by GC-EI-MS/MS. However, all methods meet the uncertainty requirement (≤ 50 % at EQS) of the WFD.

None of the methods developed in the project had a clear advantage over others, in terms of quantification of PBDE, i.e. none of the methods were interference free. Therefore the project concluded that choice of method for a given situation would depend on the complexity and contamination level of the samples and the accuracy required. In addition, due to the large number of bromine-containing organic molecules (artificial as well as natural), which might interfere with the various PBDE congeners in real water samples, the project concluded that more than one method may be required. The methods for PBDE and their comparison were summarised in three peer-reviewed papers [4-6].

The PDBE methods were used the final ENV08 intercomparison (see Section 3.5 and objective 4) and disseminated to stakeholders through the project's final workshop (see Section 4.1.4) and publications [4-6].

3.3. Validated primary reference methods based on isotope dilution for the analysis of selected priority water pollutants (PAH) in whole water samples at EQS levels.

3.3.1. Introduction

As part of objective 1 a traceable reference method capable of quantifying the target priority pollutant PAH at EQS level in whole water samples was developed and validated. The WFD specifies 8 individual PAH congeners as priority water pollutants. They are listed in Table 3 below with their EQS values and target LOQs of the analytical method to develop. LOQs for Benzo(g,h,i)perylene and Indeno(1,2,3-cd)pyrene are more than one order of magnitude lower than the LOQs for the other PAHs and therefore particularly challenging.

The relative expanded measurement uncertainty of the reference method should be $\leq 50\%$ at the EQS level and the results should be traceable to the SI.

Compounds		EQS ($\mu\text{g.L}^{-1}$)	Target LOQ of the JRP ($\mu\text{g.L}^{-1}$)
Naphtalene	Naph	2.4	0.72
Anthracene	Ant	0.1	0.03
Fluoranthene	Fluo	0.1	0.03
Benzo(b)fluoranthene	BbF	0.03	0.009
Benzo(k)fluoranthene	BkF		
Benzo(a)pyrene	BaP	0.05	0.015
Benzo(g,h,i)perylene	BghiP	0.002	0.0006
Indeno(1,2,3-cd)pyrene	IndenoP		

Table 3: PAHs from the list of 33 priority substances of the WFD and the required performance criteria

Different extraction methods (LLE, SPE with discs and cartridges) and parameters were tested and optimised for; simple water samples; for SPM; and for whole water samples in order to achieve the highest possible extraction efficiency of PAH. As part of this a common validation protocol was agreed by the project partners and used to compare and validate the performance of the different extraction methods. The following parameters were chosen for the protocol; identification parameters; selectivity; recovery; LOD and LOQ; linearity/working range; trueness; precision and uncertainty.

3.3.2. Measurement methods for PAHs in simple water samples containing HA but no SPM

In order to optimise the extraction method and to implement isotope dilution (primary method) the stability of PAH and the equilibration time for the spiked labelled compounds was studied (Figure 7). The water samples were prepared in brown glass bottles of 1 L. Native PAHs and HA were spiked into the water and the bottles were stored for 24, 48 and 72 hours at 4°C to allow interaction between the matrix and the compounds. Labelled PAHs were spiked shortly before extraction or 24 hours before extraction and all samples were prepared and extracted three times using LLE.

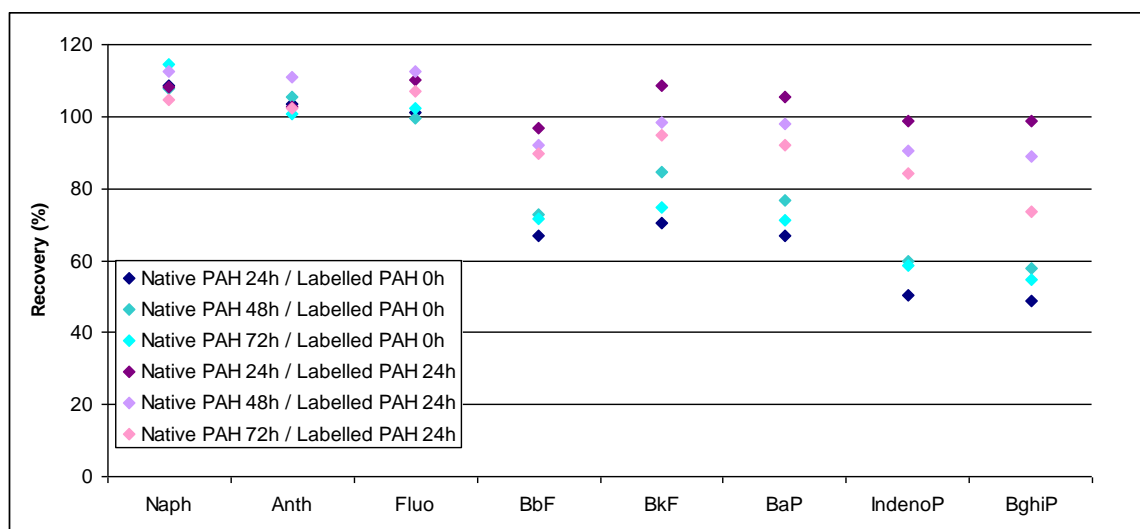


Figure 7: Recovery of PAHs taking into account their residence time in water containing HA

The adsorption effect was more significant for high mass PAHs (benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(123-cd)pyrene and benzo(ghi)perylene): if the labelled PAHs were spiked shortly before the extraction (first three batches in Figure 7), the labelled PAHs do not have enough time for equilibration and the recoveries were only between 50 % and 80 %. The last three batches with an equilibration time of 24h for the labelled compounds gave better results (recoveries between 70 % and 110%) and were independent on the equilibration time of the native PAHs. Further measurements with an equilibration time of 24h for both native and labelled compounds were also performed as there were higher risks of compound degradation after 24h of storage.

Three extraction methods were implemented for the analysis of PAHs in water samples (1L of natural mineral water with or without HA):

1. LLE + ID-GC/MS,
2. SPE with cartridges + isotopic dilution liquid chromatography coupled to tandem mass spectrometry with an atmospheric pressure photo ionisation (ID-LC-APPI-MS²),
3. SPE with disks + ID-GC/MS.

The development and validation experiments demonstrated the limitations of the SPE cartridges extraction i.e. the SPE cartridge was only applicable for water samples containing less than 5 mg/L of HA (as dissolved organic carbon (DOC)) because of clogging problems, while LLE and SPE disks allowed the extraction of water sample up to 10 mg/L of HA (as DOC).

The three methods for PAHs in water sample with and without HA were compared according to a common validation protocol. The three tested methods met the WFD requirements with a relative expanded uncertainty of less than 50% at EQS for all PAH congeners except benzo(ghi)perylene. The required LOQ of the QA/QC Directive was also achieved for most of the analytes for each of the 3 extraction methods, but for indeno(123-cd)pyrene and benzo(ghi)perylene, the LOQ could only be achieved with large volume injection GC/MS. The two most promising extraction methods were therefore, LLE + ID-GC/MS and with SPE with disks + ID-GC/MS. These methods have since been further developed by the project partners in order use them with whole water samples with SPM.

3.3.3. Measurement methods for PAH in SPM

Whole water, as defined in the WFD, includes the dissolved and particulate fraction. PAHs (especially five- and six- rings) are compounds that strongly adsorb to such particles. Therefore, particular attention was required to ensure complete extraction of bound compounds. In water bodies containing high levels of SPM, separate analysis of SPM and of the filtered sample is usually the best option. One challenge was therefore to develop a method capable of quantitative extraction of a low amount of SPM (i.e. up to 500 mg of SPM).

Two extraction methods were tested and optimized for the analysis of PAHs in SPM using 1L samples of natural mineral water spiked with different amounts of SRM NIST 1941b “Organics in Marine Sediment”: the samples were filtered on glass fiber filter (Whatman GF/F) and then extracted with either Microwave Assisted Extraction (MAE) + ID-GC/MS or Pressurised Liquid Extraction (PLE) + ID-GC/MS. It was found that the most volatile PAH congener naphthalene was lost to a large extent in all samples during the method validation of the MAE-GC/MS method and this method could therefore not be validated for this compound.

The required LOQ of the QA/QC Directive could be achieved for most of the analytes. However, for benzo(k)fluoranthene, indeno(123-cd)pyrene and benzo(ghi)perylene, the LOQ was not in agreement for both the MAE-GC/MS and the PLE-GC/MS method. For indeno(123-cd)pyrene and benzo(ghi)perylene this finding is due to the relatively poor sensitivity of the GC/MS measurement method.

The two tested methods met the WFD requirements in term of uncertainty level with a relative expanded uncertainty of less than 50% at EQS level for all congeners except Indeno(123-cd)pyrene and Benzo(ghi)perylene for MAE extraction and benzo(a)pyrene for PLE extraction. Although both methods could be considered as promising for the extraction of PAHs in SPM, PLE extraction turned out to be faster and simpler compared to the MAE extraction. Indeed even if both extraction systems were automated (MAE = carousel of 14 samples, PLE = carousel of 24 samples), the whole MAE extraction procedure includes a separation step of the organic solution from the solid matrix by filtration and this step could cause a loss of analyte, in particular naphthalene. In the PLE extraction this filtration step is absent. Therefore, PLE was decided to be the most promising method for whole water samples.

3.3.4. Measurement methods for PAH in whole water samples including SPM

LLE and SPE disk extraction methods were evaluated on whole water samples (mineral water + different amounts of SPM + different levels of HA). The SPE disk extraction method showed better recoveries than LLE. It was also easier to implement because of the potential automation of the system. For these reasons the SPE disk extraction method was selected and validated on whole water sample.

The LOQs required by the WFD were achieved for most of the compounds with the automatic SPE disc system, except for Indeno(1,2,3-cd)pyrene and Benzo(ghi)perylene (Table 4). Those two compounds have a high retention time, they elute at the end of the chromatogram and GC/MS is not sensitive enough for them. The measurement uncertainties of the SPE disc extraction method met the WFD criterion of $\leq 50\%$ relative expanded uncertainty at EQS level for all PAH congeners.

ng/L	SPE disk	LOQ _{WFD}
Naph	7.1	720
Ant	2	30
Fluo	1	30
BbF	0.7	6
BkF	0.9	3
BaP	0.8	15
BghiP	0.8	0.3
IndenoP	0.8	0.3

Table 4: SPE disk LOQs compared to WFD target LOQs

For further method development two different methods for the quantification of PAH in whole water samples were compared. The methods were (as shown in Figure 8):

1. analysis of combined liquid/SPM phase using SPE disk extraction followed by ID-GC/MS
2. filtration of the whole water sample. Parallel extractions of the filtered water sample using SPE disk and the filter using PLE extraction followed by ID-GC/MS.

Both methods gave comparable results as shown in Figures 9 and 10.

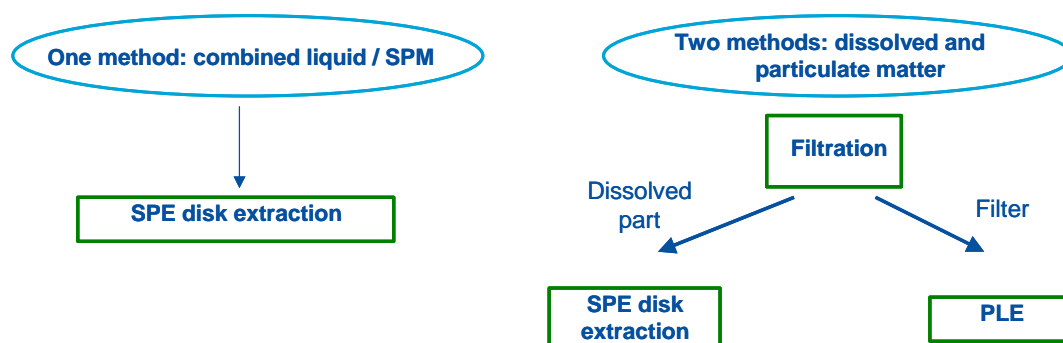


Figure 8: Two different methods to analyse whole water samples

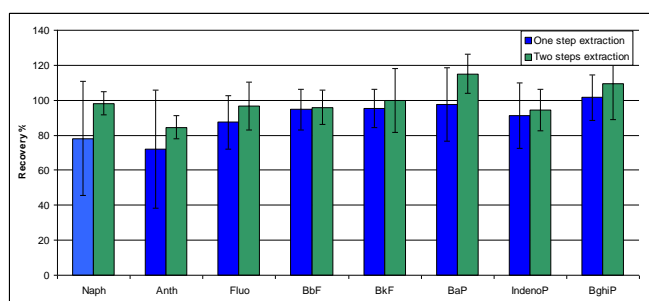


Figure 9: Comparison of two methods to analyse whole water samples: Water sample+160 mg/L SPM PAH IRMM + 5 mg/L HA

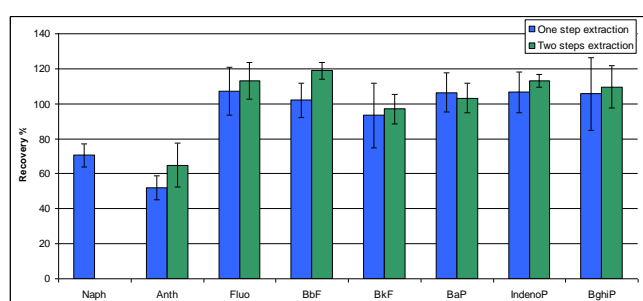


Figure 10: Comparison of two methods to analyse whole water samples: Water sample+400 mg/L SPM Blank IRMM + 5 mg/L HA

There was no significant difference observed between the two methods for measuring PAH. However, as the SPE disk is faster than the two steps extraction (SPE disk + PLE), the one step analysis of the whole water sample by SPE disk was selected as it is more convenient to implement in the laboratory in terms of time and cost.

All partners participated in the ENV08 final intercomparison (see section 3.5 and objective 4). The results for the LLE method and SPE disk methods are displayed in Figures 11 and 12.

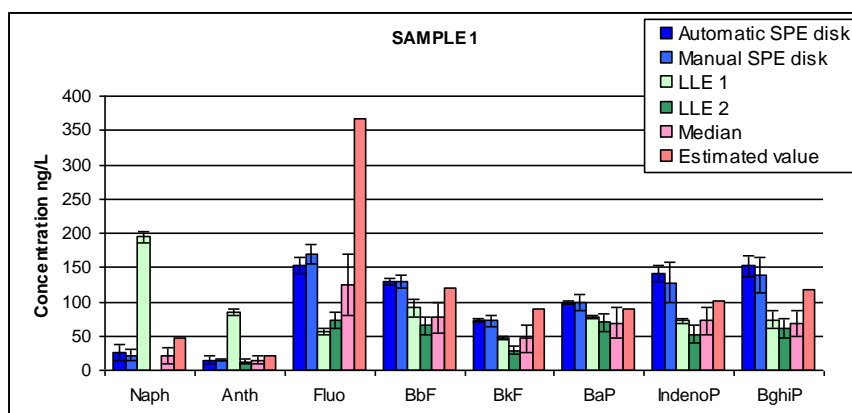


Figure 11: Results of the final ENV08 intercomparison, sample 1 (natural mineral water spiked with HA and SPM (ultra-fine milled soil) contaminated with the target analyte(s)), measurements in triplicate

As shown in Figure 11, the results of the two SPE disk measurements and the results of the two LLE methods do not differ significantly. SPE disk extractions gave higher values than LLE, however, there were

larger differences between the results and the median of all results for naphthalene and anthracene using the LLE method.

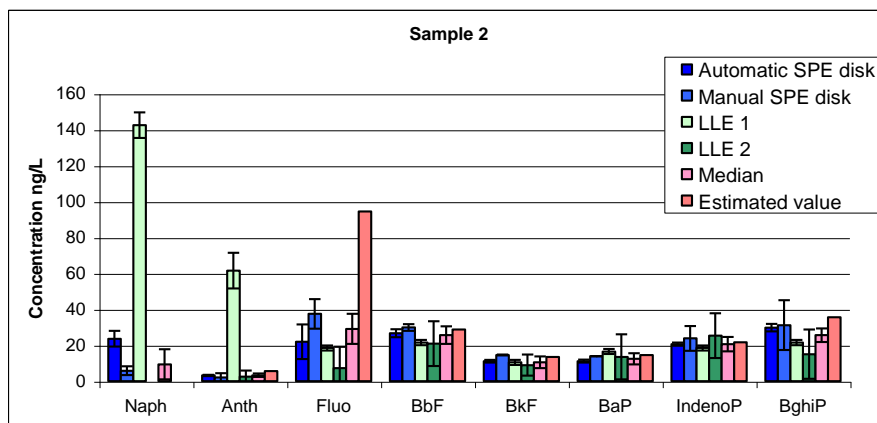


Figure 12: Results of the final ENV08 intercomparison, sample 2 (natural mineral water spiked with SPM (fine dust) contaminated with the target analyte(s)), measurements in duplicate

Contrary to sample 1 SPE disk extraction and LLE gave comparable results for sample 2. Sample 1 and sample 2 contained about the same amount of SPM (40 mg/L and 20 mg/L respectively). The presence of HA in sample 1 could be the reason for the difference between the two extraction methods. However, another reason could be a difference in the type of SPM in both samples which may have led to different interferences in the extraction and separation step.

3.3.5. Conclusion

Objective 1 of the project, a traceable validated reference method capable of quantifying the target priority pollutant at EQS level in whole water samples, was achieved for 6 out of the 8 PAH congeners regulated under the WFD. SPE disk extraction in combination with GC-MS was found to be the most suitable method for the quantification of the 8 WFD PAHs. It was optimised and validated for whole water samples according to the WFD criteria and the validation gave the following results:

- the LOQs required by the WFD were achieved in whole water samples for all PAH congeners except indeno(1,2,3-cd)pyrene and benzo(ghi)perylene. These congeners have the highest retention time of all PAHs studied, they elute at the end of the chromatogram and GC/MS is not sensitive enough for reliable quantification, even if preconcentration steps and large volume injection were applied
- the measurement uncertainties for the 8 WFD PAHs met the criterion of relative expanded uncertainties $\leq 50\%$ at EQS level for all PAH congeners.

The PAH method was used in the final ENV08 intercomparison (see Section 3.5 and objective 4) and disseminated to stakeholders through the project's final workshop (see Section 4.1.4).

The QA/QC Directive states that "In the absence of relevant environmental quality standard for a given parameter, or in the absence of method of analysis meeting the minimum performance criteria set out in, Member States shall ensure that monitoring is carried out using best available techniques not entailing excessive costs." For the analysis of PAHs, and considering the limits of the WFD, the extraction method developed for PAHs by the project can be considered as state of the art (or the best technique available) and therefore it should be considered by Member States for environmental monitoring as per the QA/QC Directive.

3.4. Study of the interaction and partitioning of pollutants (i.e. water pollutants PBDE, TBT) in environmental aquatic compartments by FFF techniques

3.4.1. Introduction

Objective 2 of the project was a systematic study of the interaction and partitioning of pollutants (i.e. water pollutants PBDE, TBT) in environmental aquatic compartments by FFF techniques; in order to provide mass balance data for the size based distribution of pollutants in whole water samples. For this purpose FFF and classical (ultrafiltration and centrifugation) techniques were developed and tested for the separation of environmentally relevant colloids in synthetic and natural whole water samples. The optimised methods were then applied to natural and synthetic whole water samples containing the WFD priority contaminants PBDE and TBT. Research of this type had not been performed prior to this project and the developed new measurement capabilities allowed better elucidation and understanding of the interaction of critical environmental pollutants with natural environmentally present colloids in water. Mass balance data obtained was also important to evaluate the trueness of the analytical methods for whole water samples. Furthermore FFF was used to characterise the size distribution in the SPM materials used for the preparation of whole water samples for method development and reference materials.

3.4.2. Development of an FFF method for PBDEs and bromine

Methodology was developed based on the on-line coupling of asymmetric flow FFF with ICP-MS and UV detection for the partitioning/detection of HA, which is a main component of the organic-rich colloidal fraction in environmental waters. Particular attention was paid to the selection of FFF conditions (e.g. carrier ionic strength, pH, etc) to favour stability and recovery of HA from the FFF membrane. A FFF membrane composed of polyethersulfone (PES 1 kDa), 500 μm channel spacer and 10 mM NH_4NO_3 (pH of 5.7) as FFF carrier were selected for further work. PBDE spiking of a HA standard (Sigma) was performed and the Br signal of the HA-fraction was detected, suggesting possible absorption of PBDEs to the HA colloids. Furthermore, the feasibility of using FFF injection volumes of up to 1000 μl for on-line (on channel) preconcentration of HA colloids without affecting their partitioning was investigated. It was found that with the increase of the injection volume up to 1 mL, the signal intensity also increased and no adverse fractionation effects (e.g. obvious agglomerate/ aggregate formation) were observed. Despite this improvement, the use of larger injection volumes required longer focusing times to be used and this had to be further investigated. The fractionation conditions were further optimised (pH, membrane type, carrier ionic strength, etc) to achieve separation of main water fractions - humic/fulvic acids and inorganic-rich colloids, using on-line ICP-MS/UV detection. Since both the carrier pH and ionic strength affect the particles fractionation their correlative effect should be taken into account. A schematic diagram of the carrier pH/ionic strength optimisation experiments is presented in Figure 13.

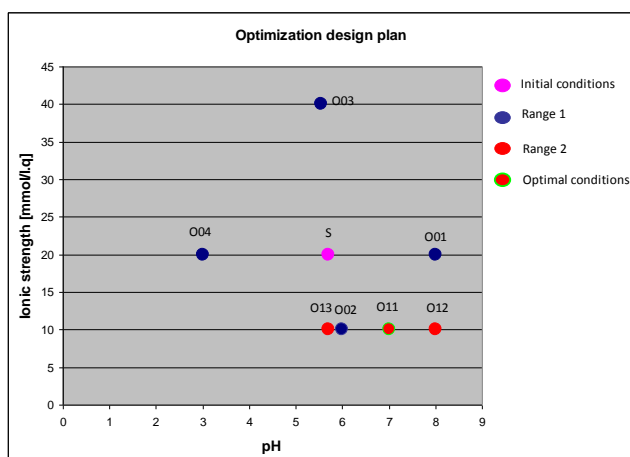


Figure 13: Schematic plan for FFF carrier ionic strength and pH optimisation

Size calibration was investigated using spherical sulfonated polystyrene and NIST gold reference material particles (size/concentration not certified). A Finnish lake water sample rich in inorganic colloids (like aluminosilicates) was analysed under optimal conditions; bromine was present in one of the peaks eluting at

the same time as the humic/fulvic acid fraction, which was a further indication of possibility PBDEs to be bind to HA/FA fraction. The results are presented in Figure 14 and Figure 15. Tin was also detected in the same size fraction as bromine in this sample type (lake water). Similar results were obtained for the fractionation of a river water sample from the UK region (Figure 16).

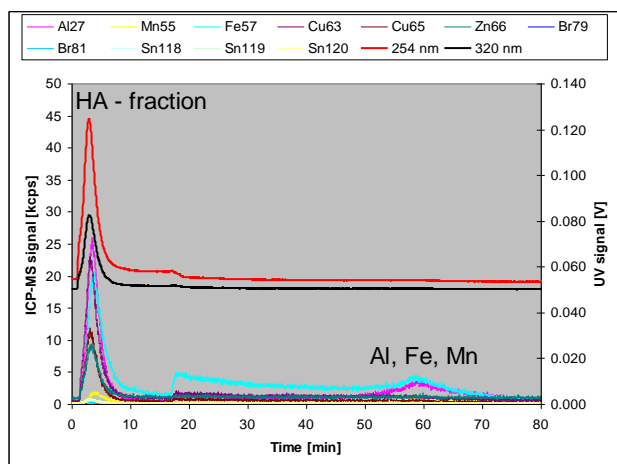


Figure 14: FFF fractionation of Finnish lake water sample rich in organic and inorganic colloids

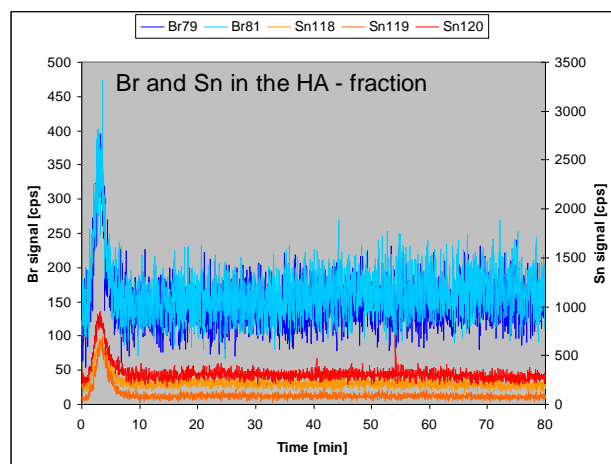


Figure 15: Presence of Br and Sn in Finnish lake water sample fractions. Determination by ICP-MS in He mode.

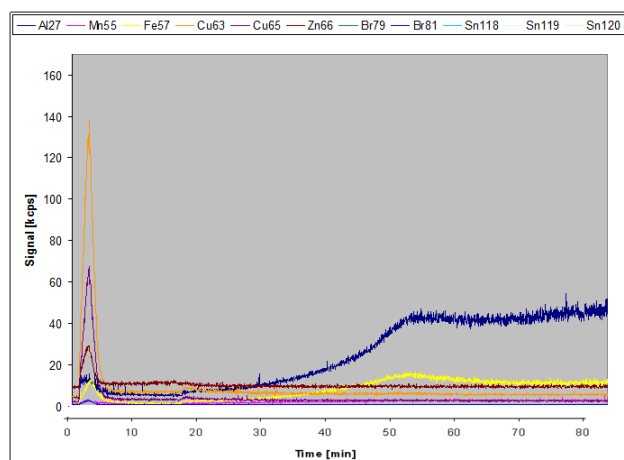


Figure 16: Fractogram of Thames River water (London, UK)

Using the FFF-ICP-MS/UV optimal conditions, the effect of multiple injections on on-channel preconcentration was investigated. The size distribution and elemental composition of the Finnish water were monitored for that purpose, along with freshly prepared synthetic water sample containing HA. Fractograms obtained by injection of 1 mL of a HA standard (20 µg/g powder concentration) were compared with those obtained by 3 times injection of a 1:3 - fold diluted solution of the same HA standard. The results indicated no effect of multiple sample injection on the size distribution of the real water sample suggesting the feasibility of on channel pre-concentration. Also, 1 mL and 3 mL of the same HA standard concentration were injected; results suggested that absorption of HA on the FFF membrane do occur due to high sample load. The recovery of a re-injected HA fraction was then analysed by on-line coupling of FFF to 3 detectors (UV, MALS and ICP-MS) to obtain information about particles' identity, size and elemental distribution. With the developed FFF methodology the recovery was found to be low. To ensure applicability to environmental samples containing priority contaminants, HA material was spiked with PBDE technical mixture and fractionated with FFF. The fractogram is presented on Figure 17. Fractions were collected, off-line pre-concentrated by liquid-liquid extraction, and analysed by GC-ICP-MS. They were found to contain only the spiked PBDEs and therefore the methodology also preserved the composition of the fractions in terms of

PBDE speciation. The results of offline fraction analysis of 2 x 1 ml HA-Na 100 mg/L, 30 ml fraction, 50 µl extract volume are presented on Figure 18.

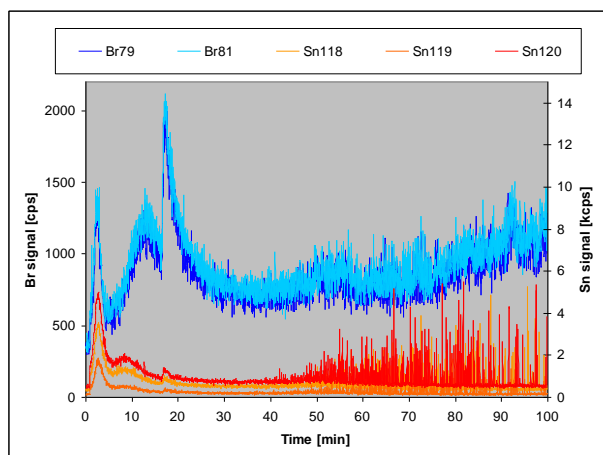


Figure 17: Fractogram of synthetic water sample spiked with PBDE penta-mixture

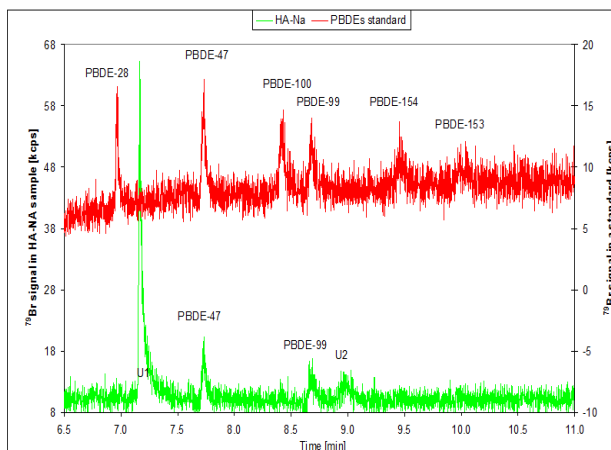


Figure 18: GC-SF-ICP-MS offline analysis of ASF4 water fractions of HA-Na and PBDE spiked water sample

The recoveries of the spiked PBDEs in fraction were still low even with 3 mL sample injections. Furthermore the total bromine concentration in non-filtered natural sample was compared to filtrates through pore sizes of 5000, 450 and 200 nm in an effort to elucidate the reason for low PBDE recoveries. No significant difference in Br content was observed due to the filtration process. Additionally, 3 different nebulisers (micro-mist, slurry and Mira-mist) were compared for achievable transportation efficiency, washing-out times and memory effects. The experiments were conducted with commercially available HA solution and blended elemental standards for some elements frequently found in HA fractions elements (Fe, Cu, Pb). No substantial differences could be proven among the nebulisers regarding the particle-transport efficiencies for HA particles, but the slurry nebuliser showed memory effect and very long washing times. With the selected nebuliser (micro-mist) experiments were performed to estimate the repeatability of the fractionation process and the linearity with increasing particles concentration. Some issues were discovered with the elements frequently found in HA fractions (e.g. Pb, Cu) suggesting possible interaction of these colloids with the membrane of the FFF and/or other fractionation compartments. The total Br content of the whole water and the water fractions, collected from FFF, were determined to estimate the recovery of the FFF fractionation but a significant contribution of endogenous Br-containing compounds hampered this analysis. The study was repeated with an artificial water sample spiked with HA (at expected natural level) and PBDEs at low but environmentally relevant levels and total Br from PBDEs was determined in this sample. Unfortunately, the Br signal from the spiked PBDEs was not detected in the FFF fractions of this sample but was successfully quantified in the whole water sample, suggesting absorption to be the main reason for PBDE losses. In each case the total Br content of whole water and the Br in water fractions was determined by post-FFF injection of the sample using a Br containing standard and/or BCR 611 reference material.

In addition to the FFF fractionation, the applicability of ultrafiltration devices was investigated for pre-concentration of water fraction containing PBDEs that preserves their composition. In an effort to estimate the PBDE distribution between the colloidal and dissolved fraction, conventional 3 kDa ultrafiltration devices were used to investigate the PBDE distribution between the filtrate and retentate in a model water sample containing organic colloids (HA) at relevant environmental levels, non-spiked and spiked with PBDEs (5 ng/g Br). Following LLE and analyses by GC-ICP-MS, PBDEs were found to be above the methodological LOQ only in the retenates with recoveries ranging from about 1% to 15 %. These recoveries were found to be congener dependent and increasing with the BDE bromination rate. PBDEs losses were found to be even larger with FFF fractionation in comparison with ultrafiltration. In similar model samples, PBDE recoveries ranged between 0.1% and 3%. The most probable reason for the low recoveries with both fractionation techniques was believed to be either absorption on the container (FFF channel) walls or on the ultrafiltration membrane. Unfortunately, the significant PBDE absorption losses hampered the efficient off-line pre-concentration for quantitative purposes and only qualitative information was obtained. A last effort was made

to improve the PBDEs recoveries by building specially designed separation channel made of material less absorptive to PBDEs. The channel, injection loops and entire sample pathway, including tubing, were constructed of stainless-steel to minimise PBDEs absorption. However, no improvement was achieved for PBDE recoveries with that channel either, which proved the hypothesis that PBDEs absorb on the fractionation membrane, presented in both FFF and ultrafiltration device separators.

The separation of water fractions using the coupling of asymmetric flow FFF with UV detection (254 nm) was optimised. The model water sample was collected from an artificial pond (fire-fighting water from Germany). Comparison of the fractionation profiles obtained with the optimised method is presented on Figure 19.

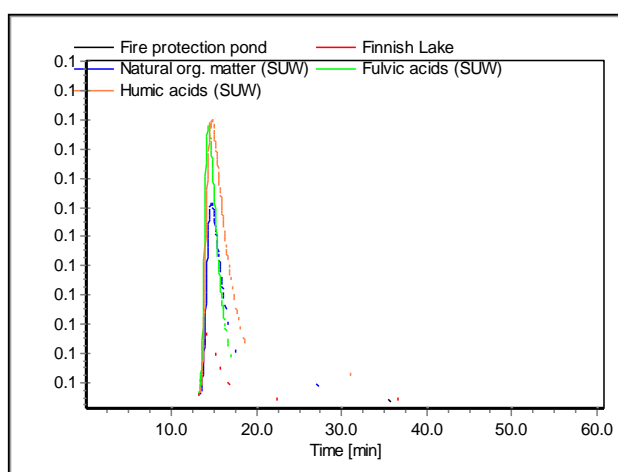


Figure 19: Comparison of the fractionation profiles obtained for natural waters and NOM materials. NOM: natural organic matter

Using FFF, PES membrane of 1 kDa and ultrapure water as a carrier, the influence of operating conditions including cross flow rate, slot outlet flow and spacer height on the size and size distribution of water fractions, were investigated. The increase of spacer height from 250 to 500 μm was found to have a major influence in improving resolution of the detected fractions. Although the water samples were filtered (0.45 μm) before introduction in the FFF system, membrane clogging was experienced, which limited the use of each FFF membrane to up to nine injections (depending on the sample matrix) in order to achieve a good level of repeatability.

A method based on the use of FFF-MALS/UV with NH_4NO_3 carrier and 10 kDa regenerated cellulose membrane with a focus on humic/fulvic acid fractions has also been optimised. Preliminary developments were applied to fractionation of water sample from Finland. Two main fractions (with size 30 nm and 100 nm) were detected in the water sample using UV-vis and light scattering detection. The obtained particles sizes for the HA fraction were out of range in comparison with data reported in the literature (<10 nm). Problems with FFF membrane fouling and clogging were also experienced that could be the reason for the biased results. Therefore, the FFF method development was continued looking at the membrane clogging issue, recovery improvement, investigating influence of other water constituents on particle size distribution. The methodology was applied for the fractionation of natural water samples and laboratory clean water, spiked with natural organic matter. Online-dynamic light scattering (DLS) was used as a detector for these samples but the results were not satisfactory due to the high sample dilution during the fractionation process. Multi-angle light scattering (MALS) detector was then successfully coupled to the FFF and calibrated to allow size determinations of the particulate material. Optimisation of the sample injection volume was performed and the developed method allowed injection of 2.5 ml aqueous sample. Experiments with another carrier (NH_4NO_3) were performed and also proved successful. The carrier concentration was optimised in the range 5 mM to 15 mM solution having the pH 7. Out of the membrane with MWCO 300 Da, 1KDa, 10 KDa and within PES and regenerated cellulose membranes the optimum membrane for analysis was carried out with PES membrane having 1KDa. Spacer widths of 300 and 500 μm were compared and 500 μm spacer was found to provide better separation. The optimal injection volume was found to be 80 μl and was further used for samples fractionation.

PBDE spike recoveries in model water samples made of commercially available mineral water, were determined (PBDE containing SPM (jet-milled sediment) and HA solution). The PBDEs were pre-concentrated on BAKERBOND-Speedisk™ Extraction Disk C18 and the final extract in hexane was

analysed by PTV-LVI and GC/ECNI-MS in SIM mode. Recovery rates ($n=3$) were in the range of 99 to 133 % with standard deviations ranging from 1.7 to 15.9 %.

3.4.3. Development of an FFF method for TBT and tin

As the FFF method was proven applicable for environmental waters fractionation, efforts were focused on the successful fractionation of water samples containing colloids and TBT. After a successful pilot study, fractions of model water containing HA and TBT were collected and distributed for an intercomparison study for total tin and TBT in water fractions.

Different extraction/preconcentration methods using HAc/MeOH (3:1, v/v) and Tetramethylammonium hydroxide (TMAH) extraction assisted by microwave energy, were tested. The total tin results, as determined in the extracts, suggested that the TMAH extraction method provided better extraction efficiency of total Sn than the HAc/MeOH method. A further, two methodologies were developed for TBT extraction from sediments and comparison between ultrasonic and microwave assisted extraction with HAc/MeOH, 3:1 (v/v) was made. Ultrasonic extraction provided better recoveries at a shorter extraction time. Recovery of TBT from environmental water spiked with 1 ng Sn/kg by using ID-GC-ICP-MS was 108% with a relative expanded uncertainty ($k = 2$) of approx. 20%. Experiments were performed to estimate the LOD for total Sn and TBT by ICP-MS and GC-ICP-MS. The achieved LODs were approximately 2 ng/L for total Sn and about 15 ng/L for TBT, which were insufficiently low to allow the quantification of these analytes in the post-FFF fractions. Off-line pre-concentration experiments for TBT, based on solid phase extraction, were then performed and two different cartridges and elution procedures were tested. Experiments were conducted on mineral water matrix and ultrafiltration (UF), freeze-drying (FD) and LLE were tested for applicability as external pre-concentration methods to analyse TBT in the FFF fractions. IDMS and GC-ICP-MS were used for TBT determination in model waters containing about 1 mg kg⁻¹ (as total organic carbon) of HA sodium-salt and doped with about 4 ng(Sn) kg⁻¹ of TBT. UF was a useful approach to separate the particulate/colloidal fraction from the liquid sample, with a recovery of the TBT of 77%. 14% of the recovered TBT mass was found in the concentrate (> 3 kDa), while the 86% was found in the filtrate (< 3 kDa). The LLE method was selected as best pre-concentration method, because it is faster and requires less procedural steps, assuring good recoveries. Results showed that in presence of 1 to 10 mg kg⁻¹ of HA the LLE-IDMS method was able to quantify the expected TBT mass fraction with a recovery of about 115%. Several additional experiments to improve the LLE procedure were also performed. The residence time of TBT and the equilibration time of the isotopic enriched TBT added for the IDMS approach, as well as the extraction procedure, were optimised in waters containing 1 to 10 mg kg⁻¹ of HA. The optimised LLE-IDMS method was used for the evaluation of conventional fractionation techniques and consecutive centrifugation and filtration at different cut-off points were used to fractionate a model water containing 100 mg kg⁻¹ (as total organic carbon) of HA-sodium salt and 50 ng kg⁻¹ of TBT (as tin). Consecutive centrifugations (4°C, 1h 20 min, 8500 rpm) were allowed to separate the whole water in three fractions; the TBT mass in the first fraction was 73.5% of the total TBT mass, in the second fraction 3.1% and in the supernatant 23.4%. The average TBT recovery of the method was 104%. Consecutive filtration was performed using 5 filters with different cut-offs: 0.7 µm, 0.45 µm, 0.22 µm, 0.1 µm and 0.025 µm. 70% of the TBT mass was retained in the fraction > 0.7 µm, while 13.8% of the TBT mass was found in the filtrate, the fraction lower than 0.025 µm. The remaining TBT was distributed between the intermediate fractions. The TBT mass fraction recovery was 65%.

The developed methods were successfully used in the intercomparison to quantify the TBT and total Sn in FFF fractions of model waters containing TBT. The TBT mass balance was established using the results of the TBT quantification by ID in the collected fractions (Figure 20). The TBT was also quantified in the following samples: (1) the model-water injected in the FFF system; (2) the HA solution used to prepare the model-water, that is the matrix-blank; (3) the crossflow, that is the flow passing through the channel membrane and going to the waste, that could transport part of the non-strongly bound TBT. 58% of the injected TBT was found in the collected fractions, while the content in the crossflow was lower than the LOQ. The HA sample contained only 0.1% of TBT. As a consequence, 42% of the injected TBT was lost during the fractionation process.

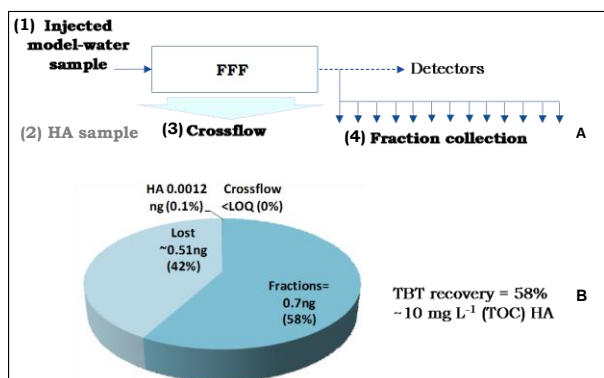


Figure 20: Mass balance of FFF fractionation. A: scheme of the samples used for mass balance calculations. B: TBT distribution in samples coming from the fractionation procedure: collected fractions, crossflow, HA-blank and lost TBT.

A method for quantification of TBT in water fractions was developed. The method was optimised and applied for quantification of TBT in water fractions. A method for quantification of total tin in water fractions was also developed and fractions of model water samples containing HA and TBT were obtained and analysed for total tin.

3.4.4. Conclusion

Objective 2 of the project was a systematic study of the interaction and partitioning of pollutants (i.e. water pollutants PBDE, TBT) in environmental aquatic compartments by FFF techniques; in order to provide mass balance data for the size based distribution of pollutants in whole water samples. To do this, FFF and classical (ultrafiltration and centrifugation) techniques were developed and tested for the separation of environmentally relevant colloids (HA and fulvic acids) in artificial and natural whole water samples. The optimised methods were then applied to natural and synthetic samples containing the priority pollutants PBDE and TBT. Mass balance data for the size distribution of TBT in whole water samples was established.

However, for PBDE low recoveries were observed in the FFF experiments due to adsorption of the target analytes on the membrane and other parts of the equipment. Furthermore, a significant contribution of other endogenous Br-containing compounds hampered the mass balance measurements. These problems could not be fully resolved despite of extensive efforts to optimise the FFF conditions. Therefore, objective 2 was only partly achieved for PBDE.

3.5. Development of preparation methods for aqueous whole water reference materials with proven homogeneity, and short and long time stability, as well as an intercomparison to disseminate the primary reference methods and reference materials developed in the project

3.5.1. Introduction

Objective 3 of the project was the development of preparation methods for aqueous whole water reference materials with proven homogeneity, and short and long time stability, which are as close as possible to real water samples, i.e. including SPM and colloidal matter. Using the methods developed “test materials” were prepared, simulating different phases (dissolved phase and SPM separately) and “whole water” test materials (with both dissolved phase and SPM), with known concentrations of the target compounds. These samples were studied for their short term stability (STS), long term stability (LTS) and homogeneity. Further to this, a systematic study of the interactions of the target analytes PAH, PBDE and TBT with different container materials was conducted in order to select the most suitable containers.

The most suitable whole water test materials were then used in a final ENV08 intercomparison organised for project partners and external laboratories (objective 4). The final ENV08 intercomparison also used the reference methods from objective 1 for TBT, PBDE and PAH in whole water samples.

In addition to this, due to a request from CEN, Mandate 424/TC230, samples were prepared by the project for an second intercomparison for the validation of new or updated CEN methods for TBT, PBDE and PAH in whole water samples.

3.5.2. Selection of suitable containers for the preparation of test materials

Aluminium, amber glass and fluorinated polyethylene (FPE) with a capacity of 0.5 L, 1 L and 2 L were selected and tested (Figure 21). A common spiking and testing protocol was agreed and the results obtained from different partners were comparable. Each partner then received a set of cleaned bottles made of different materials and volumes (cleaned, packed and shipped). As expected the highest adsorption of all compounds under study was observed onto the walls of FPE bottles, so this container material was discarded. For PAHs and PBDEs glass and aluminium were equally suited. For TBT, aluminium bottles were problematic since the required extra cleaning step with strong acid damaged the bottles. No significant differences in the target analyte recoveries were observed between the studied capacities (considering surface to volume ratios) and 1 L amber glass bottles were selected as common container for the next stages in the work. Results of the container study were published in the peer-reviewed literature [7].



Figure 21: Type of bottles tested in the container experiment

3.5.3 Development of concepts for the preparation of test materials

The second step was to develop concepts to prepare “test materials” for PAHs, PBDEs and TBT in water. Three different types of water samples were investigated for the test materials preparation:

1. Materials simulating the dissolved phase in the water, containing dissolved HA and a spike with target compounds in a water-miscible solvent
2. Materials simulating the particulate phase in the water, containing only SPM with the target compounds strongly bound to the particles
3. Materials mimicking “whole water”, including both the dissolved phase and the SPM-phase.

Mineral water: was selected as a matrix for the test material preparations. Soft mineral water offered a good compromise between a natural matrix and availability. More than 500 L of SPA Reine mineral water was purchased, poured into a PFA-lined tank (perfluoroalkoxy polymer) and continuously mixed using an inert bellow-pump. The bottles were thereafter filled using a custom-made system based on displacement of the water by an inert gas from a buffertank. In total (1005 ± 3) mL was filled per bottle (Figure 22).



Figure 22: PFA-lined tank containing SPA mineral water (left) under constant recirculation while automatically filling bottles (right) by accurately recording the mass of water filled.

Test materials without SPM: the preparation of test materials without SPM included the addition of HA in solution to act as a natural ligand for the target compounds. For conservation of the HA-solution, it was filled in amber glass ampoules (25 mL) and then gamma irradiated (Figure 23). The ampoules were then stored at 4 °C until use. Bottles filled with mineral water (SPA) were first spiked with a solution of HA and then with a spike of the compounds of interest in a water miscible organic solvent. It was decided to acidify the mineral water in order to avoid the precipitation of HA (which readily took place at pH 7).



Figure 23: Preparation (left) and ampouling (right) of the HA solution.

Test materials with SPM: for the preparation of a model SPMs (Figure 24); the model SPMs were dispersed in water to form a slurry. After careful and continuous mixing, a specific volume of the slurry was precisely pipetted into bottles filled with mineral water (SPA). Three soil/sediments containing TBT, PBDEs and PAHs were then milled to obtain particle sizes of about 10 μm . Several tests were carried out before preparation of “test materials” in water, such as checking the suitability of the milled soils/sediments as model SPMs. The origin of the TBT-SPM was the CRM BCR-546, (fresh water sediment). For PBDE a candidate CRM was sieved and jet-milled (fresh water sediment). For PAHs a soil PT-material was used.



Figure 24: Model SPMs for PAHs (grey), TBT (black) and PBDEs (red) after jet-milling and filling in vials

Slurry preparation and subsequent pipetting proved to be a highly repeatable method to add model-SPMs to water samples. The SPM loading to the samples was controlled by adding aliquots of the slurry to pre-weighed Petri dishes during the preparation process (two at the beginning, two in the middle of the process and two at the end), air-drying them and calculating the mass of SPM. This control was performed for all sample batches prepared. Relative standard deviations of the added mass of SPM varied from 0.18 % to 1.1 % for the prepared slurries loading model SPMs from 7.5 to 200 mg very precisely (Figure 25).

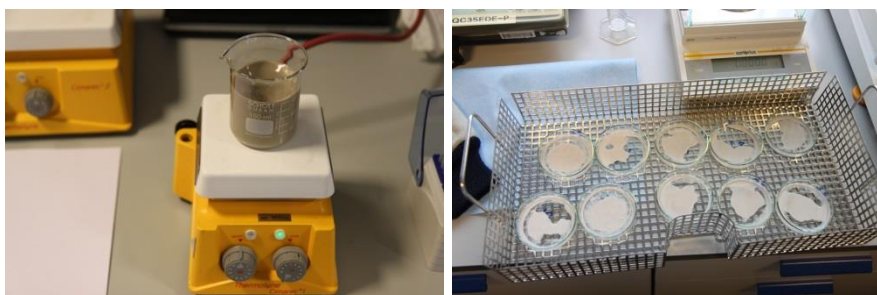


Figure 25: Slurry addition tests. Slurry preparation can be seen to the left and the model SPM in petri dishes after drying to the right.

Test materials with both HAs and SPM: the most complex materials, mimicking “whole water” contained both dissolved HA (5 mg DOC/L) to simulate natural colloids and SPM. In both test materials containing SPM no extra spike of target analytes had to be added to the samples since PAHs, PBDEs and TBT were already present and strongly bound to the particles in the model SPMs. The preparation and characterisation of the different types of test materials is shown in Figure 26.

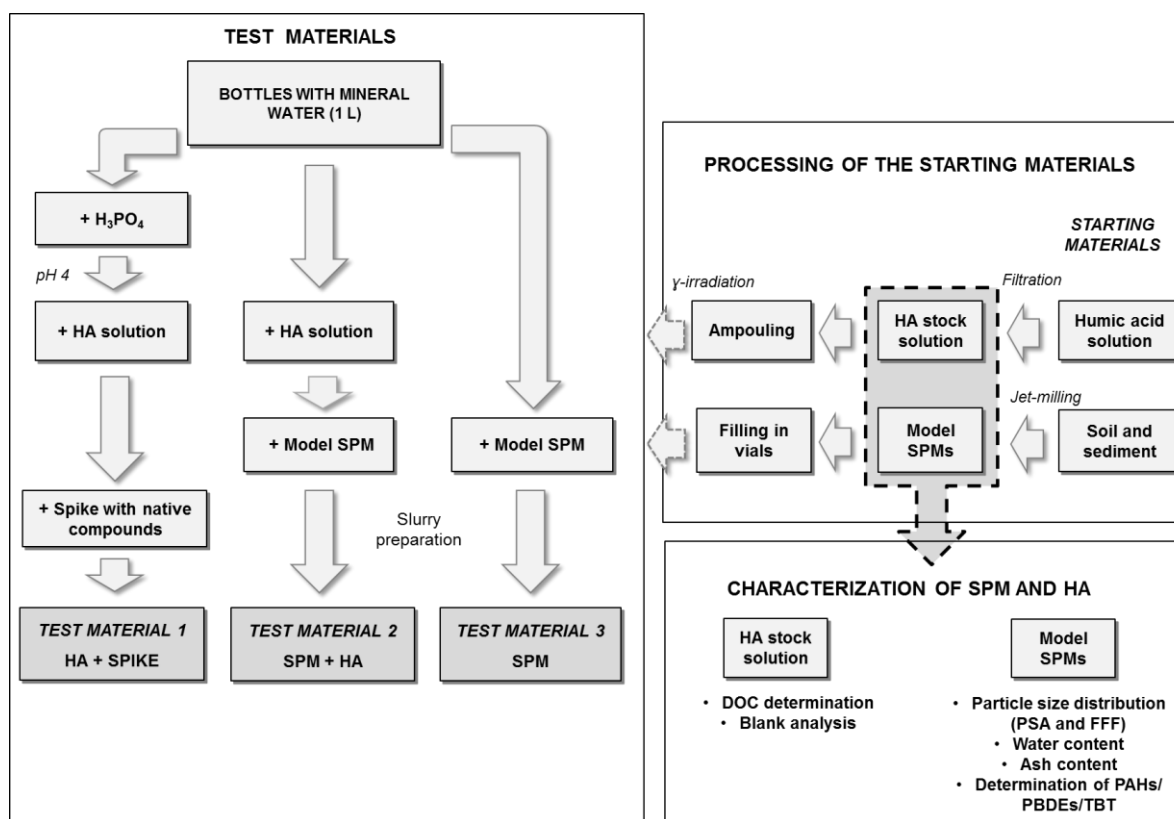


Figure 26: General scheme for the preparation and characterisation of the test materials [8]

3.5.4. Stability and homogeneity studies

Between July 2013 and April 2014 the required test materials with and without SPM were prepared for the short and long term stability and homogeneity studies.

Irradiation of the samples: in order to test the suitability of γ-irradiation for the conservation of the test materials, 5 different test materials (six replicates each) corresponding to three types in the TBT (HA +spike, SPM, and SPM+HA) and two test material in the case of the PAHs (SPM and SPM+HA) were irradiated. Afterwards transport conditions were tested by placing the samples at 4°C, 18°C and 60°C for 7 weeks.

Initial expectations, that a relatively low dose of γ -irradiation would stabilise the samples and the analytes were not confirmed. The irradiation affected TBT and PAHs both in the dissolved phase and in the SPM phase. While TBT disappeared completely in the dissolved phase (below detection limit), quantifiable amounts of TBT and PAHs remained intact in the model SPM. In addition, a clear degradation of the compounds was observed in irradiated samples kept at 60 °C, and TBT and PAHs were nearly completely depleted after 1 week.

Therefore, it was decided not to use irradiation for the future sample preparations. A new set of TBT and PAHs samples were prepared to test their stability at 4°C and 18 °C, since 60 °C was clearly unsuitable for the prepared materials.

Stability studies: samples were checked with respect to stability at +18 and +4 °C, respectively for a period of 1 month (TBT and PAHs) and 7 weeks (PBDEs). The obtained data was collected and evaluated. No significant degradation was observed for the studied compounds. Nevertheless the high uncertainty for the relatively short shelf life studied suggests that some of the analytical methods used were not repeatable enough for some of the analytes. In general, samples kept at 4 °C displayed better recoveries and stability during the studied period than samples kept at 18 °C. The conclusion was that all samples should be shipped for the intercomparison with over-night courier and stored at +4 °C until used for analysis. Under these conditions, samples were considered stable for the lifetime of the intercomparison (3 to 6 weeks).

Homogeneity studies: five identical samples of the different test materials (8 in total) were prepared and sent to partners and collaborators for analysis. All types of TBT materials displayed a relative standard uncertainty (between-bottle heterogeneity, ubb) of below 4 % at ng/L level. Results for PBDEs depended on the type of water testing material and the laboratory analysing them. While ubb <9 % were reported for PBDE materials containing SPM alone for pg/L levels, up to 16% of ubb was found in water samples prepared with HA and a spike. Similar observations were made for PAH materials, for example, two different laboratories tested the homogeneity of PAHs materials containing SPM alone and whilst one of the labs obtained ubb <5% the other laboratory reported values up to 19% ubb. This is problematic since poor analytical performance could be interpreted as inherent flaws in the method for preparation of these water samples. Fortunately better data confirmed that it is possible to prepare water samples with a very high degree of repeatability. Therefore the slurry pipetting approach was chosen for the sample preparation for the intercomparison exercise. In addition, it should be noted that the data sets with the highest precision for the homogeneity studies were performed on the lowest masses of SPM-loadings i.e. from 7.5 mg for TBT to 20 mg for PAH and PBDE, respectively.

Further improvements of some of the existing methods and fully validate methods for PAHs, PBDEs and TBT are required in order to properly characterise and assess prepared test materials (or possibly candidate CRMs). The data with the highest precision obtained with respect to between-bottle-heterogeneity and stability provided sufficient confidence about the quality of the test materials in order to proceed with the final ENV08 intercomparison samples.

3.5.5. Preparation of test materials for the final ENV08 intercomparison

As a final part of the project an intercomparison was organised between project partners and other external laboratories (see section 4.1.4 and objective 4). In total 14 external testing laboratories from 8 European countries participated. The aim of the final ENV08 intercomparison was used to test the new reference methods developed by the project as part of objective 1 (for PAHs, PBDEs and TBT) on realistic samples and to compare the results to other laboratories. In addition, water samples were prepared for a CEN M424/TC230 intercomparison that was validating new and updated CEN standard methods for TBT, PBDE and PAH in whole water.

In the final ENV08 intercomparison, two different types of samples were prepared for all compounds (Table 5). For each compound one of the sample types contained only SPM and the other type contained both SPM and HA. In all cases the PAHs, PBDEs and TBT were present on the SPM so no spiking of analytes took place using standard solutions. The HA solution (final concentration about 5 mg DOC/L) was added to represent natural colloids in the dissolved phase of the water.

Each type of sample was made in triplicate except for PAH samples type 2 (ERM-CZ100) which was prepared in duplicate. Therefore each laboratory analysing PAHs received 5 bottles while laboratories analysing PBDEs and/or TBT received 6 bottles per analyte. The samples without HA were prepared in the same way for the final ENV08 and the CEN M424/TC230 intercomparisons but at different occasions. As the loading of SPM was highly repeatable it was possible to provide almost identical samples to both intercomparisons in the case of one sample type per analyte.

Sample Type	Replicates	Composition	Description/Comments
PAHs			
PAH-1	A,B,C	SPM+HA	40 mg SPM/L + 5 mg DOC / L
PAH-2	A,B	SPM	20 mg ERM-CZ100 / L
PBDEs			
PBDE-1	A,B,C	SPM+HA	200 mg SPM / L + 5 mg DOC / L
PBDE-2	A,B,C	SPM	25 mg SPM / L
TBT			
TBT-1	A,B,C	SPM+HA	7.5 mg SPM / L + 5 mg DOC / L
TBT-2	A,B,C	SPM	7.5 mg SPM / L + 12.5 mg blank SPM / L

Table 5: Sample types in the final ENV08 intercomparison

For TBT and PBDEs only the model SPMs developed in the project were used to prepare the testing materials. In the case of PAHs one type of material (PAH-1) was prepared with the model SPM and the other

one (PAH-2) with the CRM ERM-CZ100 (PAHs in dust). The CRM has a comparable particle size distribution to the model SPMs (90% vol of the particles are below 20 µm).

For the final ENV08 intercomparison 231 bottles in total were prepared using concepts developed in the project. Out of these bottles, 75 were supplied for PAHs (14 participants + 1 spare set), 84 for PBDEs (13 participants + 1 spare set) and 72 for TBT (11 participants + 1 spare set). For the CEN M424/TC230 intercomparison 304 test samples were provided.

3.5.6. Conclusion

Objective 3 of the project, the development of preparation methods for aqueous whole water reference materials with proven homogeneity, and short and long time stability was achieved. The approach of loading a model-SPM and HA to water samples in a controlled manner is a new approach for producing whole water reference materials and the analytical data obtained for PBDE, PAH and TBT at ng/L levels confirmed the production of sufficiently homogeneous and stable reference materials in whole water (containing both SPM and HA).

These reference materials were then used to achieve objective 4 of the project. In the final ENV08 intercomparison, the reference materials for PBDE, PAH and TBT in whole water and the three new reference methods developed as part of objective 1 were validated. Fourteen external laboratories from eight European countries participated in the final ENV08 intercomparison together with 11 project partners. Six different types of water samples were distributed in triplicate, two types each for TBT, PBDE and PAH. The samples contained organic colloids (HA) and/or SPM and therefore met with the WFD's requirement for whole water samples. The results of the final ENV08 intercomparison were evaluated according to ISO 5725-5:2002 "Accuracy (trueness and precision) of measurement methods and results" and performance parameters such as recovery rates, reproducibility and repeatability standard deviations have indicated that the reference materials prepared by the project are sufficiently homogeneous and stable for proficiency testing. It should be highlighted that these results constitute a significant achievement as no such intercomparison has been previously performed on whole water samples with analyte concentrations well below ng/L for some parameters. The concepts for the preparation of the whole water reference materials were published in two open access peer-reviewed papers [7, 8] one more paper is under preparation. Concepts can be freely utilised by stakeholders such as PT-providers and reference materials producers. The results of the final ENV08 intercomparison were also published [9].

In addition, test samples were provided by the project for the CEN M424/TC230 intercomparison for the validation of new or updated CEN methods for TBT, PBDE and PAH in whole water samples (see section 4.3.1). The CEN standard method for TBT was released in 2015, the methods for PBDE and PAH are under approval and expected to be released by the end of 2015. Performance data from the intercomparison are included as part of the standards and a reference to the ENV08 project is also given in the standards.

4 Actual and potential impact

4.1. Dissemination Activities

4.1.1. Metrology achievements

Regarding objective 1; the development of primary reference methods for the analysis of selected priority water pollutants (specified in the WFD) in whole water samples at EQS levels, the following reference methods were developed and then validated (as part of objective 4):

1. TBT cation
2. PBDE (BDE 28, BDE 47, BDE 99, BDE 100, BDE 153, BDE 154)
3. PAH (Naphthalene, Anthracene, Fluoranthene, Benzo(b)fluoranthene, Benzo(k)fluoranthene, Benzo(a)pyrene, Indeno(123,cd)pyrene, Benzo(ghi)perylene)

All reference methods are traceable to the SI and use isotopically labeled standards for calibration. Calibration standards for TBT and PBDE were also assessed for chemical purity by the project to achieve traceability. For PAH, commercially available calibration standards with certified purity from NIST and IRMM were used to trace the analytical results back to the SI.

For the majority of analytes (i.e. TBT, PBDE and PAH) the reference methods met the minimum performance criteria specified in the WFD. Their LOQs are equal or lower than the EQS values and the relative expanded uncertainties are equal or lower than 50 % at EQS level. The exceptions were Indeno(123,cd)pyrene and Benzo(ghi)perylene, as the required LOQ could not be achieved for these two pollutants.

Further to this, a method for the collection of coastal water samples (for PBDE analysis) in the field was developed by a Researcher Excellence Grant as part of the project.

In terms of objective 2, a method for the partitioning of PBDE and TBT in natural and synthetic whole waters by FFF was developed by the project. This method met the objective by determining the interaction and partitioning of pollutants (i.e. water pollutants PBDE, TBT) in environmental aquatic compartments by FFF techniques; in order to provide mass balance data for the size based distribution of pollutants in whole water samples

As part of objective 3, preparation methods for aqueous whole water reference materials with proven homogeneity, and short and long time stability, which are as close as possible to real water samples, were developed by the project. Using these methods the following reference materials were then produced and characterised:

1. model SPM material for TBT, PBDE and PAH
2. natural mineral water with organic colloids for TBT, PBDE and PAH
3. natural mineral water with SPM for TBT, PBDE and PAH
4. natural mineral water with organic colloids and SPM for TBT, PBDE and PAH

Contaminants (i.e. priority water pollutants) in the aqueous whole water reference materials were in the ng/L concentration range relevant for measurements under the WFD and the aqueous reference whole water materials were assessed for homogeneity, and short and long term stability. The between-bottle heterogeneity was sufficiently small and sufficient proof of stability was gathered for all samples kept at 4 °C in the dark for at least 4 weeks [8]. The materials are therefore suitable for proficiency testing and quality assurance. Furthermore, a systematic study of the adsorption of the target analytes on different container materials was conducted in order to select the most suitable container material for TBT, PBDE and PAH. The most suitable containers were amber glass bottles of 1 L capacity [7].

The reference methods from objectives 1 & 2 and the methods for the reference materials in objective 3 are published or will be published in scientific papers (see list in section 6) which allow the user community, field laboratories, PT providers, to have access to them as matrix matched materials are essential to achieve traceable and comparable results.

The project also completed a final ENV08 intercomparison in which the reference methods from objective 1 and the reference materials from objective 3 were validated and compared. The results were then disseminated to stakeholders as part of a final project workshop (for further information see section 4.1.4).

4.1.2. Scientific publications

The project has generated 11 high impact peer reviewed publications in key journals and 3 papers are in preparation. One non-peer reviewed paper [10] has been published in a practitioners journal. A master thesis work on the quantification of TBT in whole water samples [11] was finished within the project and published at Humboldt University, Berlin. These papers incorporate the significant scientific outputs of the project. A list is provided in section 6.

4.1.3. Conferences and workshops

The work carried out in the project has been disseminated to both the wider scientific community in general conferences such as the *International Congress of Metrology* (Paris 2011 and 2013), the *European Conference on Analytical Chemistry (EuroAnalysis)* (Warsaw 2013) or the *38th International Symposium on Environmental Analytical Chemistry* (Lausanne 2014) as well as targeted audiences in specialised conferences such as the *Winter Conference on Plasma Spectrochemistry* (Amelia Island, Florida 2014), the *7th International Franco-Spanish Workshop on Bioinorganic Analytical Chemistry* (Gijon 2012), the *Dioxin* (Madrid 2014) or the *Workshop on Life Sciences and Nanoparticles* (Daejeon, Korea 2013).

In total 18 oral presentations and 20 posters have been presented at conferences and workshops during the lifetime of the project, amongst them was an invited lecture "GC-ICP-MS reference methodology to quantify polybrominated flame retardants in environmental waters relevant to the European Water Frame Directive" given by LGC at the Workshop of the Royal Society of Chemistry "Recent Advances in the Analysis of Complex Environmental Matrices" (London 2013).

4.1.4. Final ENV08 intercomparison and final workshop

As part of objective 4, a final ENV08 intercomparison for consortium members and stakeholders, including European NMIs and expert laboratories was organized by the project. The final ENV08 intercomparison aimed to test and compare the analytical methods developed in the project as well as the concepts developed for reference materials. For the testing laboratories the final ENV08 intercomparison also provided them with an opportunity to test their in-house procedures on the complex whole water reference materials produced by the project.

Thirteen external laboratories from eight European countries participated in the final ENV08 intercomparison together with 11 project partners. Six different types of water samples were distributed in triplicate, two types each for TBT, PBDE and PAH. In total about 250 1-L sample units were prepared and shipped. The samples contained organic colloids (HA) and/or SPM and therefore met with the WFD's requirement for whole water samples. The results of the final ENV08 intercomparison were evaluated according to ISO 5725-5:2002 "Accuracy (trueness and precision) of measurement methods and results" and all participants received a preliminary summary report. The results of performance parameters from the final ENV08 intercomparison such as recovery rates, reproducibility and repeatability standard deviations indicate that the test materials prepared by the project were indeed suitable, i.e. sufficiently homogeneous and stable for proficiency testing. To our knowledge the final ENV08 intercomparison is the first interlaboratory exercise using whole water samples containing defined amounts of SPM and colloidal matter in the aqueous phase. This is a step forward to the production of realistic whole water test and reference materials as required by the European WFD. A paper about the final results has been accepted for publication in Accreditation and Quality Assurance [9].

The final ENV08 intercomparison results were discussed at a project final workshop held in September 2014 in Berlin. Approximately 20 participants attended the workshop, amongst them representatives from CEN, from academic institutions and from field laboratories. The final workshop marked the end of the project and the project's main outcomes and conclusions were presented to stakeholders as well as future collaborations with EMRP JRP ENV51 MeTra.

Through the final ENV08 intercomparison and final workshop objective 4 for the project was achieved (i.e. an intercomparison and workshop for stakeholders to disseminate the primary reference methods and reference materials developed in the project).

4.2 Training and cooperation between partners

The EMRP is aimed at facilitating closer integration of national research programmes and ensuring collaboration between National Metrology Institutes (NMIs) and Designated Institutes (DIs). This project has been a good example of this, by gathering 11 NMIs/DIs and three academic partners (two Research Excellent Grant (REG) researchers and one unfunded partner) from ten European Countries and the EC. NMIs from countries developing their metrological capacity were also included in the project, for example BIM, the Bulgarian NMI, was included as part of a Researcher Mobility Grant (RMG).

The project partners have benefitted from training courses organised within the project. For example, BAM received training in double isotope dilution measurements from LNE and in GC-MS/MS applications from the REG at the University of Oviedo. The REG at Helmholtz Zentrum Geesthacht was also trained in methods for uncertainty estimation by PTB. In total 10 courses were held and approximately 25 consortium researchers were trained.

Further to this, a two day external training course was organised by LGC for the Public Analyst's Laboratory, Dublin, Ireland. The method for quantification of nanoparticles (environmental colloids) developed in the ENV08 project was implemented in the Public Analyst's Laboratory and will be applied for an inter-laboratory performance study: "Detection/quantification of silver nanoparticles in an aqueous matrix" organised by JRC (of the European Commission) to support forthcoming EC regulations.

ISPRA, the Italian Istituto Superiore per la Protezione e la Ricerca Ambientale is responsible for the implementation of the WFD in Italy. This institute was involved as an unfunded partner in the ENV08 project and predominantly contributed to the development of methods for PAH quantification in SPM. It is intended that the outcomes of the project will support ISPRA's efforts in the implementation of the WFD in Italy.

Furthermore, the project supported BIM through an EMRP RMG to further develop capabilities in metrology and analytical chemistry. A researcher from BIM worked at LNE for 6 months and developed analytical methods for the quantification of TBT in Bulgarian coastal lye and sea water. Results of the RMG were presented at the *38th International Symposium on Environmental Analytical Chemistry* in June 2014 in Lausanne, Switzerland and a joint publication between BIM, LNE and BAM is in preparation.

4.3 Intermediate impact

4.3.1. Standardisation committees and stakeholder engagement

One of the main objectives of the project was the development of validated primary methods for the analysis of selected priority water pollutants (specified in the WFD) that are traceable to the SI. These primary methods are not applicable as standard "routine" methods and therefore not directly transferable to standardisation bodies. However, they are a fundamental tool to ensure the metrological traceability of routine measurements and will allow method validation and quality assurance for testing labs via, for example, participation in PT schemes or usage of reference materials.

At the European level, the project established a close cooperation with CEN TC 230 "Water Analysis" and the related Mandate 424. ENV08 project partners attended Mandate 424 meetings and vice versa to exchange information. The ENV08 consortium also provided comments and proposed amendments to the CEN draft standards and technical specifications

- prEN 16691 Water quality - Determination of polycyclic aromatic hydrocarbons (PAH) in whole water samples using liquid solid extraction combined with gas chromatography mass spectrometry (GC-MS)
- prEN 16693 Water quality - Determination of organochlorine pesticides (OCP) in whole water samples using solid phase extraction (SPE) with SPE-disks combined with gas chromatography mass spectrometry (GC-MS)

- prEN 16694 Water quality — Determination of polybromodiphenyl ether (PBDE) in whole water samples using solid phase extraction (SPE) with SPE-disks combined with gas chromatography - mass spectrometry (GC-MS) and
- FprCEN/TS 16692 Water quality - Determination of tributyl tin (TBT) in whole water samples using solid phase extraction (SPE) and gas chromatography with triple quadrupole mass spectrometry.

Four types of model SPM (for TBT, PBDE, PAH and a blank material) prepared in the project were also provided to Mandate 424 for the development and validation of routine standardised methods. Furthermore, water test materials containing different levels of SPM were supplied for the final Mandate 424 validation intercomparison in June 2014. In total more than 250 sample units were prepared and distributed by partner JRC for CEN TC 230. Additionally five ENV08 partners participated in the Mandate 424 intercomparison and provided validation data. FprCEN/TS 16692 has been published in May 2015. The other three standards are under approval and expected to be published later in 2015.

Further to this, a presentation on the preparation of WFD relevant whole water test materials was given by partner JRC at a training workshop organised by CEN. The workshop was attended by about 50 representatives of European field laboratories. Project partners have attended the final Mandate 424 meeting in October 2014 where the results of the validation intercomparisons were discussed.

The Director General of CEN Elena Santiago was also informed about the cooperation between CEN TC 230 and the ENV08 project during a visit to JRC in July 2014. She expressed her appreciation of and support for the direct interactions between CEN and the ENV08 project, as well as the EMRP in general. Furthermore, the support of the ENV08 project in validating new CEN standard methods for water analysis, has been acknowledged by CEN and the newly created STAIR-EMPIR platform. **ST**andardization, **I**nnovation and **R**esearch (STAIR) aims to strengthen ties between fundamental metrological research performed in EMRP projects and necessary developments in the field of CEN standards.

Presentations of the outcomes of the project have also been given at a number of metrological committees, in particular at EURAMET (Teddington 2014) and BIPM-CCQM (Paris 2012) working groups' meetings and the annual meeting of the European Reference Materials (ERM) consortium (Teddington 2014).

At the national level, results of the project have been discussed in several national standardisation bodies such as DIN (German Institute for Standardisation), Standard Committee for water issues, organic compounds, AFNOR (French standardisation body), Committee T91M "Micropollutants in Water" and LAWA (German Working Group on water issues of the Federal States and the Federal Government).

4.3.2. User uptake

Reference values were provided for a French PT scheme for organotin analysis using the project's reference method for the quantification of TBT. The PT scheme was organised by Institut national de l'environnement industriel et des risques (INERIS) and approximately 15 field laboratories participated. Using the reference method and its reference values the field laboratories were able to benchmark the performance of their in-house procedures.

IPROMA (Investigacion y Proyectos de Medioambiente S.L), a Spanish environmental testing company has implemented the project's method for the measurement of the isotopic composition of TBT by Multiple Reaction Monitoring using GC-MS/MS. This method was developed by the Researcher Excellence Grant at the University of Oviedo and allows water surveillance more to be performed more accurately with a lower level of uncertainty of the analytical results.(i.e. the blank value obtained was 0.0116 ± 0.0043 ng/L and the recovery at the EQS level for TBT was 104.9 ± 4 %). To our knowledge, these are the best values achieved for the quantification of TBT by GC-MS/MS to date. The method is suitable for routine analysis and further advantages include the need for less expensive instrumentation (compared with GC-ICP-MS) and the fact that it is a less time consuming method in comparison to other procedures (using external calibration graphs, internal standards or more complicated chromatographic separation techniques).

4.4. Longer-term impact

The project consortium plans to continue disseminating its findings at conferences, through publications and standardisation activities (where possible). Currently, the results have been presented at four international conferences (following the end of the project), e.g. at the Colloquium Analytical Atomic Spectroscopy in

Leipzig, Germany in March 2015 and the Analytical Conference in Graz, Austria in March 2015. As a follow-on of the project a PhD thesis on organotin quantification in whole water samples should also be completed at Humboldt University Berlin in 2016. The PhD thesis will investigate further the interaction of organotin compounds with SPM materials and includes additional SPM materials (i.e. additional to those tested in the ENV08 project).

The primary reference methods as well as the methods for the production of whole water reference materials developed in the project have been published or will be published, a list of papers is provided in section 6. NMIs, reference materials producers and PT-providers have full access to these publications. The reference materials, developed in the project fulfil the ISO Guide 30 definition, but they were not certified within the duration of the project. However, certification has been initiated as JRC-IRMM is currently running a certification campaign for a PAH in water + HA material and BAM is contributing to this campaign. The new Certified reference material (CRM) is expected to be available by the end of 2015. CRMs are performance driven which means that any method, *new* or previously developed, able to reproduce the certified values can be used for analysis i.e. the CRM is used to confirm the performance of a method. Therefore the future certification of the reference materials would support the field of analytical measurements and their uptake by end-users.

The reference materials can also be used for proficiency testing schemes and for the assessment and in-house validation of analytical methods in monitoring laboratories. This will help the latter to maintain an appropriate QA/QC system.

The project provided input to four CEN standard methods as listed in section 4.3.1. These standards have been or will be released in 2015 and will enable field laboratories to do water monitoring more efficiently and economically. The interaction with CEN TC 230 is continuing beyond the end of the project. Project partners also gave two presentations at the CEN TC 230 WG1 meeting in October 2014 in Berlin. Further to this, the project coordinator has also been nominated as expert in CEN TC 230 WG 1 "Physical and Biochemical Methods".

Altogether it is anticipated that the outcomes of the project i.e. the reference materials and improved analytical methods will enhance the quality of water monitoring data and help end-users to achieve the main goal of the WFD, namely good ecological status of surface, ground and coastal waters.

5 Website address and contact details

A public website has been open which informs about the general outline of the project, main achievements, meetings and events: <http://www.emrp-waterframeworkdirective.bam.de/en/home/index.htm>

The contact person for general questions about the project, is Dr Rosemarie Philipp, BAM (rosemarie.philipp@bam.de).

The contact person for the development of reference methods for TBT is Dr Ina Fettig, BAM (ina.fettig@bam.de).

The contact person for the development of reference methods for PBDE is Dr Claudia Swart, PTB (Claudia.swart@ptb.de).

The contact person for the development of reference methods for PAH is Dr Julie Cabillic, LNE (julie.cabillic@lne.de).

The contact person for the study of partitioning of pollutants is Dr Panayot Petrov, LGC (panayot.petrov@lgcgroup.com)

The contact person for the development of concepts for reference materials is Dr Håkan Emteborg, JRC-IRMM (hakan.emteborg@ec.europa.eu)

6 List of publications

- [1] Enrica Alasonati, Barbara Fabbri, Ina Fettig, Catherine Yardin, Maria Estela Del Castillo, Janine Richter, Rosemarie Philipp and Paola Fisicaro, *Experimental design for TBT quantification by isotope dilution SPE-GC-ICP-MS under the European Water Framework Directive*, Talanta **134** (2015) 576-586, DOI: 10.1016/j.talanta.2014.11.064.
- [2] Andrés Rodríguez-Cea, Pablo Rodríguez-González and J. Ignacio García Alonso, *Study of the degradation of butyltin compounds in surface water samples under different storage conditions using multiple isotope tracers and GC-MS/MS*, Environmental Science and Pollution Research, submitted.
- [3] Andrés Rodríguez-Cea, Pablo Rodríguez-González and J. Ignacio García Alonso, *Determination of ultratrace levels of Tributyltin in waters by Isotope Dilution and Gas Chromatography coupled to tandem Mass Spectrometry*, Analytica Chimica Acta, submitted.
- [4] Claudia Swart, Fanny Gantois, Panayot Petrov, John Entwisle, Marjaana Nousiainen, Mine Bilsel, Burcu Biniçi, George Sawal and Adriana Gonzalez-Gago, *Comparison of various measurement procedures as potential primary measurement procedures for PBDE in surface water*, Environmental Science: Processes & Impact, submitted.
- [5] Adriana Gonzalez Gago, Daniel Proefrock and Andreas Prange, *Optimizing GC-ICP-MS for ultra-trace quantification of PBDEs in natural water samples using species specific isotope dilution*, Journal of Analytical Atomic Spectrometry, **30** (2015) 180-190, DOI: 10.1039/C4JA00112E.
- [6] Adriana Gonzalez Gago, Daniel Proefrock and Andreas Prange, *Comparison of GC-(NCI)MS, GC-(ICP)MS and GC-(EI)MS/MS for the determination of PBDEs in water samples according to the Water Framework Directive (WFD)*, Analytical and Bioanalytical Chemistry, submitted.
- [7] Saioa Elordui-Zapatarietxe, Ina Fettig, Janine Richter, Rosemarie Philipp, Fanny Gantois, Claudia Swart and Håkan Emteborg, *Investigation of interaction of 15 different EU-WFD priority substances with glass-, aluminium- and fluorinated polyethylene bottles at ng L⁻¹ levels for the containment of water reference materials*, Accreditation and Quality Assurance, online first, DOI: 10.1007/s00769-015-1150-3.
- [8] Saioa Elordui-Zapatarietxe, Ina Fettig, Rosemarie Philipp, Fanny Gantois, Beatrice Lalere, Claudia Swart, Panayot Petrov, Heidi Goenaga-Infante, Guido Vanermen, Gerard Boom and Håkan Emteborg, *Novel concepts for preparation of reference materials as whole water samples for priority substances at ng L⁻¹ level using model suspended particulate matter and humic acids*, Analytical and Bioanalytical Chemistry **407** (2015) 3055-3067, DOI: 10.1007/s00216-014-8349-8.
- [9] Janine Richter, Saioa Elordui-Zapatarietxe, Håkan Emteborg, Ina Fettig, Julie Cabillic, Enrica Alasonati, Fanny Gantois, Claudia Swart, Taner Gokcen, Murat Tunc, Burcu Binici, Andres Rodriguez-Cea, Tea Zuliani, Adriana Gonzalez Gago, Daniel Proefrock, Marjaana Nousiainen, George Sawal, Mirella Buzoianu and Rosemarie Philipp, *Results of an interlaboratory exercise for the analysis of 15 organic priority substances at ng/L levels in whole water samples*, Accreditation and Quality Assurance, accepted for publication.
- [10] Janine Richter, Ina Fettig, Christian Piechotta und Norbert Jakubowski, *Tributylzinn in Gesamtwasserproben - Entwicklung eines Referenzverfahrens für die EU-Wasserrahmenrichtlinie*, GIT-Laborfachzeitschrift, **9** (2013) 2-4.
- [11] Christiane Retsch, *Quantifizierung von Tributylzinn in Gesamtwasserproben mittels IDMS innerhalb der Wasserrahmenrichtlinie (Quantification of Tributyltin in whole water samples using IDMS for the WFD)*, Master Thesis, Humboldt-Universität, Berlin, 2015.
- [12] Janine Richter, Ina Fettig, Rosemarie Philipp and Norbert Jakubowski, *Tributyltin-Critical pollutant in whole water samples, Development of traceable measurement methods for monitoring under the European Water Framework Directive (WFD) 2000/60/EC*, Environmental Science and Pollution Research, online first, DOI: 10.1007/s11356-015-4614-4.

[13] Julie Cabillic, Sebastian Hein, Petra Lehnik-Habrink, Elisa Calabretta, Monica Potalivo, Ahmed Ceyhan Goren, Mine Bilsel, Ileana Nicolescu, Mugurel Georgescu, Beatrice Lalere and Rosemarie Philipp, *Joint Research Project ENV08 "Traceable measurements for monitoring critical pollutants under the European Water Framework Directive (WFD) 2000/60/EC"*, Proceedings of the 16th International Congress of Metrology, Paris, 2013, DOI: 10.1051/metrology/201310001.