ICES MCWG REPORT 2009

ICES MARINE HABITATCOMMITTEE

ICES CM 2009/MHC:03

REF. SCICOM, ACOM

Report of the Marine Chemistry Working Group (MCWG)

16-20 March 2009

Wageningen, IMARES, Ijmuiden, The Netherlands

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CIEM Conseil International pour l'Exploration de la Mer

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Recommended format for purposes of citation:

ICES. 2009. Report of the Marine Chemistry Working Group (MCWG), 16-20 March 2009, Wageningen, IMARES, Ijmuiden, The Netherlands. ICES CM 2009/MHC:03. 145 pp.

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Executive summary

The Marine Chemistry Working Group [MCWG] (Chair Evin McGovern, Ireland) met at Wageningen IMARES, IJmuiden, Netherlands, 16–20 March, 2009. The meeting was attended by 26 participants, representing 11 countries.

Highlights:

- Much of MCWG2009 was taken up with **delivering products to support coordinated monitoring activities, in response to OSPAR work requests to ICES**. ICES were requested to prepare/revise a number of *technical annexes* for analysis of certain contaminants for inclusion in the *JAMP guidelines for monitoring contaminants in marine biota and sediments*. A feature of MCWG 2009 was increased participation of chemical oceanographers/nutrient experts and the establishment of a chemical oceanography subgroup (COSG) enabled revision of OSPAR eutrophication monitoring guidelines. Other tasks were addressed in *ad hoc* subgroups and in plenary. The status of these annexes is outlined in the table below. Many of the technical annexes produced at MCWG 2007 – 2009 meetings are being prepared for publication in the ICES TIMES series.

Technical annexes for analysis of contaminants	Status of task
Perfluorinated compounds (PFCs) in seawater	Completed. MCWG 2009 Annex 11
Perfluorinated compounds (PFCs) in sediments	Completed. MCWG 2009 Annex 10
Dioxins/furans in marine biota	Completed MCWG 2009. MCWG 2009 Annex 9
Dioxins/furans in marine sediments	Commenced MCWG/WGMS 2009. This will be completed in 2010 in conjunction with WGMS
Co-planar PCBs in biota (Revised organic contaminants in biota technical annex)	Completed revision of annex: Organic contaminants in biota completed. MCWG 2009 Annex 7
Co-planar PCBs in sediment (Revised PCB in sediment technical annex)	Completed revision of annex: PCBs in Sediment completed. MCWG 2009 Annex 8
OSPAR Eutrophication monitoring guidelines Revised guidelines for monitoring nutrients	Completed revision of annex: MCWG 2009 Annex 13
OSPAR Eutrophication monitoring guidelines Revised guidelines for monitoring dissolved oxygen	Completed revision of annex: MCWG 2009 Annex 14

In response to OSPAR requests to define Background Concentrations (BCs) for dioxins in biota, MCWG collated a dataset of dioxin/furan concentrations in fish and shellfish. MCWG suggests indicative low concentrations for dioxins/furans in fish and shellfish that OSPAR could consider on a trial basis to support derivation of background assessment concentrations. However, it should be noted very little data from so-called "pristine" / "remote" areas were available to MCWG and this data was of uncertain quality and generally expressed as Toxic Equivalents. This should be regarded as a preliminary

assessment, although MCWG does not anticipate any notable improvement in data availability in the near future.

- An area of ongoing interest to MCWG is that of **emerging substances**. MCWG reported on developments in analysis of Short Chain Chlorinated Paraffins (SCCPs), and new information on analysis, occurrence in various compartments and effects of polybromodiphenylethers (PBDEs) and poly- and perfluorinated compounds (PFCs). A Canadian study highlighted that large reservoirs of BDE-209 in the environment are likely to present a source of "lighter", more bioaccumulative and toxic BDEs. MCWG also identified nanoparticles as substances for which more data on occurrence and impacts in the marine environment were needed.
- Building on the experience of the MCWG/WGMS passive sampler trial survey (2007), MCWG produced technical guidance to assist new users of silicone **passive samplers**. The guidance covers deployment, analysis and interpretation of results. (ANNEX 15). MCWG also has produced a table summarising the demonstrated or achievable performance of various passive sampling techniques for monitoring a wide range of priority substances (ANNEX 16)

Other issues:

Quality Assurance: MCWG continues to provide feedback and information exchange with the QUASIMEME (laboratory proficiency-testing scheme). This is a key service underpinning marine chemistry monitoring and research activities.

Nutrients and chemical oceanography: A plenary presentation highlighted trend monitoring of pH and carbonate parameters in Icelandic waters. A time series commencing in 1983 indicates a reduction of surface pH by 0.0024 units $year^{-1}$ in the Iceland Sea. MCWG also reviewed ongoing developments with the International Nutrients Scale Systems (INSS), progress towards establishing an Absolute Salinity scale (MCWG 4.3) and highlighted issues with sensor measurement of dissolved oxygen in deep water.

Water Framework Directive (WFD – Dir. 2000/60/EC)/ Marine Strategy Framework Directive (MSFD – Dir. 2008/56/EC): MCWG reviewed developments with respect to chemical monitoring for the WFD and new activities under the MSFD.

Integrated chemical and biological effects: MCWG considered how it contribute to ongoing ICES activities in this field

Update on relevant projects: MCWG members provided information on projects of general interest to the group, including information on a global survey of PAH and organochlorines (Galathea survey), chemical weapons in the Baltic Sea (MERCW project), bioavailability of sediment-bound chemicals and the MODELKEY project.

1 Opening of the meeting

The Marine Chemistry Working Group [MCWG] (Chair Evin McGovern, Ireland) met at Wageningen IMARES, IJmuiden, Netherlands, 16–20 March, 2009. The director, Dr. Martin Scholten, opened the meeting. The meeting was attended by 26 participants, representing 11 countries. There were a number of apologies from members unable to attend. The participants introduced themselves and their affiliations and described their specific interests within the field of marine chemistry. The list of participants is given in annex 1.

2 Adoption of the agenda

The agenda was adopted with no amendments.

3 Report of the 96th ICES statutory meeting

The Chair reported on the Marine Habitats Committee meeting at the ICES 96th statutory meeting in Halifax, Nova Scotia in September 2008. MCWG can see a clear role for itself within the new ICES science plan adopted, especially in relation to the topic "Population and community level impacts of contaminants, eutrophication, and habitat changes in the coastal zone". MCWG acknowledged that the ICES Science implementing structure is still under development. MCWG 2009 also noted the technical minutes of RGChem 2008 (Annex 12 MCWG 2008 report)

4 Reports on related activities

4.1 OSPAR and Helcom

MCWG noted that OSPAR 2008 adopted four technical annexes, produced by MCWG 2007, for analysis of certain brominated flame retardants in biota and sediment. OSPAR 2008 also adopted low concentrations for metals and PAH in molluscs as recommended by MCWG 2008. Various other technical annexes produced by MCWG 2008 are pending.

MCWG also noted a draft HELCOM report, supplied by ICES for information, on Hazardous Substances in the Baltic Sea.

4.2 Laboratory Performance Study QUASIMEME

Michiel Kotterman represented the QUASIMEME Project Office, Wageninen UR at MCWG2009 and presented an update on latest QUASIMEME activities.

There have been changes in staff at the QUASIMEME secretariat over the past year, but there is now a full complement of staff with the additions of Anne-Marie Ryan and Michiel Kotterman. Additionally QUASIMEME are cooperating with WEPAL (Wageningen Evaluating Programmes for Analytical Laboratories), an accredited and international organiser of proficiency testing schemes based within Wageninen UR. QUASIMEME is adopting the quality procedures of WEPAL, which will assist QUASIMEME gaining accreditation as anticipated for October 2009.

The work engine of the QUASIMEME website (http://www.quasimeme.org) has been updated, with publicly accessible general information, and detailed information for participants available by sharepoint. The newsletters are also made available online. The QUEST programme used for data submission will be phased out and replaced by

an internet based application, to be further developed into an interactive platform (e.g. search engines for data). This will be tested by a group of German laboratories during the next round.

No workshops were organised in 2008, but two workshops are planned for 2009, one on perfluorinated compounds (PFCs), to be held in Amsterdam in October 2009, and one on PAHs and alkylated PAHs, to be held in Aberdeen in December 2009. A workshop on chlorinated alkanes and PCBs is planned for 2010.

In terms of new materials, there will be a PFCs development exercise (DE-15) and a Paralytic Shellfish Poisoning (PSP) toxin development exercise (DE-14) during 2009. New sediment and biota (mussel) materials with relatively high levels of organotins were also prepared. However, due to a lack of participants, the toxaphene, alkyl phenols and passive samplers exercises are cancelled. It is suggested that participants will be reimbursed through credit towards future rounds.

The update also addressed issues raised by MCWG2008. The request for 'high' and 'low' concentrations in test materials to better tailor materials to the needs of laboratories was taken into consideration. However, a pitfall to this approach would be the low participation of laboratories potentially resulting into reference values not being assigned to materials due to a limited dataset. To the suggestion that QUASIMEME should reassess constant errors for Water Framework Directive (WFD) relevant substances in the context of the requirements on environmental quality standards (EQS) (i.e. Limit of Quantification be equal to or less than 0.3 times the EQS and the uncertainty be equal or less than 5%), QUASIMEME have taken the MCWG2008 suggestion on board and the reassessment of constant errors has taken place. With regards to multiple contaminants materials (e.g. one material for PAHs, PBDEs, PCBs) these are difficult to source with appropriate concentrations, but will be used if available.

The QUASIMEME project office stressed that it takes all complaints seriously, but these need to be formulated with specific details so that issues can be investigated properly, findings archived and discussed at the QUASIMEME Scientific Advisory Group (SAG).

Several requests from the QUASIMEME project office were also highlighted. First of all, participants to the next round of BT-4 material were asked to analyse alkyl-PAHs of their choice. The information will be used in the 2009 workshop on alkyl-PAHs as a pilot, the aim being to set-up alkyl-PAHs in the QUASIMEME schedule. Also, in the next round of AQ1&2, the determination of Dissolved Organic Carbon (DOC) is optional and will be added at a small additional fee in the following rounds. The AQ1&2 round is still considered to be at a development stage. The analysis of suspended solids will be included in the 2009 programme if there is enough interest. MCWG 2009 participants were asked to show their interest to the QUASIMEME project office. Finally, QUASIMEME is looking for new snail sources (other then Netherlands and UK) for use in imposex studies.

In discussion, several issues were raised by MCWG 2009. Although improvements have been noted, communication between the QUASIMEME project office and participants was still less than optimal and need further improvement. The suitability of some materials was questioned. For example, concentrations of PBDEs in sediments were too low and could not be detected by most participating laboratories and this should be avoided in future. It was remarked that participants pay to get an assessment of their methods and this is often key to their accreditation process. This was discussed at the QUASIMEME Advisory Board and will not happen again. One

member enquired about the presence of additional peaks in PAH materials when analysed by HPLC-fluorescence.

More information on sample type and the provision of a range of concentrations would be useful when the annual programme is produced so that participating laboratories can decide whether the material is suitable for their purpose. It would be helpful for participants to know the origin of the samples (e.g. sediment from harbour or offshore), and it would be useful to have an indication of levels of lipids in biota samples. It was commented that it would be interesting to analyse the changes in participant results over the years in order to evaluate the effects of changes in analytical methodologies used by participants but MCWG also recognized that this would be a very substantial task.

The 'Inconsistent' classification was questioned in the case where a result is reported as being below the method Limit of Quantification (LOQ), but the assigned value is close to the LOQ and therefore the LOQ is within a z-score of ±2. MCWG 2009 recommended that the terminology should be reviewed by QUASIMEME.

MCWG reiterated that it is valuable to maintain a strong link with QUASIMEME. Some of the comments made at MCWG2008 have been dealt with, and most have been considered. MCWG strongly welcomes QUASIMEME's plan to achieve accreditation. Three members of the QUASIMEME Advisory Board were participants at MCWG 2009 and will bring MCWG's comments to QUASIMEME and provide feedback to MCWG2010.

4.3 Other Activities

MCWG reviewed two ongoing activities in the field of chemical oceanography which should be of interest to ICES.

- The **International Nutrient Scale System**, (INSS), is an initiative to harmonize nutrients measurements in the world oceans. This will be facilitated by use of certified reference materials for nutrients and by writing a manual for best practice in the measurement of nutrients in seawater ("Go-Ship manual"). CRMs for nutrients currently are being produced by the Meteorological Research Institute in Japan. Seawater of Pacific origin is being used for those but samples of Atlantic origin will also be produced. An international intercalibration exercise was undertaken with about 60 participants to test this material. First results were recently introduced at a workshop, co-sponsored by ICES. MCWG intends to follow progress of this work next year.
- **Information about the process to establish Absolute Salinity scale**
  The SCOR/IAPSO Working Group 127 has been working to replace the conductivity-based Practical Salinity with Absolute Salinity. The definition of Absolute Salinity is necessary for a more accurate description of the thermodynamic properties of seawater (e.g. density, entropy, enthalpy, specific heat capacity, etc.). Whilst Practical Salinity is described by a function of conductivity, temperature and pressure, Absolute Salinity is defined as the mass of dissolved material per unit mass of seawater. The unit of Absolute Salinity is g kg^{-1}, which follows the SI unit system.

  Initial work has established an algorithm for 'Standard Seawater' (Atlantic surface water) to relate Practical Salinity to Absolute Salinity. The next steps are the development of an algorithm that accounts for the variation

of seawater composition from the standard composition. For this extended algorithm additional data will be needed which may either be measured parameters (e.g. total alkalinity, silicate and nitrate, etc.) or more simply the spatial locations longitude, latitude and pressure. It is expected that the Working Group 127 will produce more detailed information during 2009. The MCWG should wait for the outcome and discuss this item again next year.

5 Plenary Presentations

5.1 Quantification method of the analysis of polychlorinated paraffins: Analyses in marine species

Tatsiana Rusina (MARES Netherlands)

Chlorinated paraffins (CPs) are ubiquitous, persistent and bioaccumulative environmental pollutants. Ongoing production and regulation requires their environmental monitoring and therefore reliable quality assured analytical methods are a prerequisite. Gas chromatography electron capture negative ionization mass spectrometry (GC-ECNI-MS) is one of the most frequently used techniques for the determination of CPs, because it provides sufficient sensitivity and enables distinction of chain length and chlorination degree. However, the main drawback of the technique is the dependence of the ECNI response factor on the number of chlorine atoms in the molecule. Furthermore, congener groups with less than five chlorine atoms are discriminated by the technique. The most critical point in the analysis using GC-ECNI-MS is quantification, since composition, and in particular chlorine content of the CPs in the samples and in the external standards used for quantification, can be very different. Four quantification methods for analysis of short-chain chlorinated paraffins (SCCP) in marine species were presented. The average concentrations found in tested fish species including hake, cod, salmon and eel were 27-250 ng g^{-1} wet weight (ww) or 160-500 ng g^{-1} lipid weight (lw). Methods based on visual comparison of the SCCP patterns of the external standard and the sample generally have higher quantification errors. The methods relating relative total response factors (RTRFs) of SCCP mixtures and their total degree of chlorination in the standards and in the fish samples resulted in reduced quantification error. Individual standards of polychlorinated alkanes (PCA) were also applied for quantification purpose. But because not all the necessary standards were commercially available the quantification errors were higher. Nevertheless, the clear advantage of the method is that the standards are defined, making quantification results comparable between different laboratories. Improvement of the method could be expected if more individual PCA standards were to become commercially available.

5.2 Time series of inorganic carbon in Icelandic waters

Sólveig Ólafsdóttir

An overview of research on inorganic carbon by the Marine Research Institute in Reykjavik was given. Time series are maintained at two stations south-west and north-east of Iceland in two different hydrographic regimes, the Irminger and Iceland Seas respectively. The measurements for surface waters go back to 1983 and from 1991 a full profile has been sampled. The data show that the air to sea flux of CO_2 is an order of magnitude higher north of Iceland than south. Uptake of CO_2 leads to reduction of pH and surface pH is lowered by 0.0024 units $year^{-1}$ in the Iceland Sea. Changes in pH and aragonite saturation state have also occurred in the deep waters of the Iceland Sea where the $\Omega_{ARAGONITE}$=1 saturation horizon rises about 2 m $year^{-1}$.

This equates to approximately 400 km² of seabed becoming under-saturated with respect to aragonite annually.

5.3 New threats for the Marine Environment *Jacob de Boer*

Jacob de Boer gave a presentation in which he focused on emerging contaminants. The growing world population is causing higher and higher demands for all sorts of materials. Although safer materials are being developed, sooner or later some of the alternatives will also reach the marine environment. Methods for detection of contaminants have become ca. 100 million times more sensitive over the last thirty years. Therefore, not all toxic compounds that are being detected pose a problem to environmental and human health at the occurring concentrations. Obviously, many natural compounds also have toxic properties but nevertheless may not cause environmental problems. As marine chemists we have to assess possible risks of anthropogenic compounds. This can be done by target analysis, as has mainly been done until now, e.g. PCB, DDT, mercury analyses, etc. A newer approach is the so-called Effect-Directed Analysis (EDA). In that approach extracts that have shown a certain effect in bioassays are fractionated, after which they are again tested in a bioassay. Then they are fractionated again, tested again, etc. This iterative process of narrowing down the fractions continues until the effect-causing agent is identified by chemical analysis. In this way the result will always be relevant, as there is always a relationship with a specific effect.

A large number of emerging compounds were mentioned. Most of these have been already found in the marine environment. Examples are:

(i) organosilicon compounds: linear organosilicons are being used as anti-foulants (as alternatives for tributyltin), but accumulate as a coating of sediments in shipping lanes; cyclic silicones have been reported in fish from the Oslofjord;

(ii) organophosphorus flame retardants (PFRs): these have been reported in sediment and water samples from the UK and Flanders (Belgium);

(iii) a variety of brominated flame retardants and related compounds has been identified in fish samples, e.g. pentabromotoluene, decabromodiphenylethane, 1,2-bis(2,4,6-bribromphenoxy)ethane, and dibromo-anilines, -phenols and –anisols; UV screens (ethylhexylmethoxycinnamate and bezophenone-3) have been found in German rivers and lakes, and the Oslofjord;

(iv) a large suite of nanoparticles is being used in many applications (paints, washing machines, personal care products, etc.). They are expected to reach the marine environment. Their effects should be assessed and compared with possible effects of naturally occurring nanoparticles. Environmental nanoparticle research may require different instrumentation such as electronic microscopes, ICP-MS, X-ray instruments, etc.

Summarizing, there is a need for a more holistic approach in the risk identification of the large suite of contaminants that are entering the marine environment. Effect-directed analysis is one option to identify some of these risks.

6 Main agenda

MCWG tackled the tasks below in breakout groups and in plenary. A chemical oceanography subgroup (COSG) consisting of Elisabeth Sahlsten, Klaus Nagel,

Sólveig Ólafsdóttir, Carmen Rodriguez, Naomi Greenwood, with some input from the MCWG chair, was established for tasks 6.c, 6.e and 6.j.

6.1 Together with WGMS prepare the following Technical Annexes for inclusion in the JAMP Guidelines for Monitoring Contaminants in Sediments (OSPAR agreement 2002-16) and JAMP Guidelines for monitoring Contaminants in Biota (OSPAR agreement 1992-2) according to the structure of the existing technical annexes as follows:

6.1.1 Technical annexes for dioxins/furans (polychlorinated dibenzo-p-dioxins & polychlorinated dibenzofurans - PCDD/Fs) and coplanar chlorinated biphenyls (CBs)

Three documents were introduced to the group: a draft update of the technical annex on the analysis of CBs in sediment and a draft technical annex on the analysis of dioxins/furans in sediment, prepared by Patrick Roose and Francesca Pelizatto at WGMS, and, a draft technical annex on the analysis of dioxins/furans in biota prepared by Katrin Vorkamp and Philippe Bersuder in advance of MCWG.

WGMS opted to update the CB guideline by including the dioxin-like CBs (DL-CBs) rather than including the latter in the dioxin guideline, as proposed by MCWG 2008 as they are often analysed together. After a brief plenary discussion, the group decided that there was merit in including the DL-CBs both in the CB guideline and the dioxin guideline.

A small subgroup consisting of Philippe Bersuder, Patrick Roose, Katrin Vorkamp, Peter Lepom, Jacob de Boer and Lynda Webster, developed and updated these documents, (dioxins/furans and dioxin-like CBs in biota, CBs in biota and CBs in sediment). Work on the dioxin in sediment annex will continue intersessionally with aim of finalisation at MCWG 2010/WGMS 2010. MCWG recommends that OSPAR adopt the following annexes to the JAMP guidelines.

a) MCWG 2009 Annex 7: Draft revised technical annex on organic contaminants in biota

b) MCWG 2009 Annex 8: Draft revised technical Annex on analysis of PCBs in sediments (with WGMS 2009)

c) MCWG 2009 Annex 9: Draft technical Annex on PCDD/Fs in biota and dioxin-like PCBs in biota

MCWG2009 recommends that to achieve wider dissemination of this work that the technical annexes for dioxins in sediment and biota are combined and edited for publication within the ICES TIMES series.

MCWG also suggests that the existing OSPAR guidelines for monitoring contaminants in biota would benefit from some restructuring. Specifically, technical annex 1 on organic contaminants in biota could be split into a part that deals with sampling and sample handling common to all types of organic contaminant analysis, and the analysis of CBs could become a separate technical annex (e.g. 1a) linked to the first. Other organic contaminant groups could be added as annexes 1b, 1c, etc.

6.1.2 Technical annexes for monitoring of PFOS in sediments and water

Technical annexes for the analysis of perfluorinated compounds (PFCs) in sediments and seawater were prepared by Lutz Ahrens, Christiaan Kwadijk, Ralf Ebinghaus, Philippe Bersuder, Peter Lepom and Evin McGovern and are attached as Annexes 10 and 11 respectively. The technical annex for the PFC analysis in seawater samples is

based on the new ISO25101 guideline but is adapted for water samples containing suspended particle matter (SPM) and broadened to cover the analysis of other PFCs besides perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA).

The technical annex prepared for the analysis of PFCs in sediments is based on the technical annex for the analysis of PFCs in biota from the MCWG2008 since much of the analytical information needed was already included. Changes to the MCWG2008 technical annex are specific to sample handling, extraction and clean-up.

MCWG recommends that OSPAR adopts the proposed technical annexes for PFCs in sediment and seawater as part of the JAMP guidelines for monitoring contaminants. MCWG recommends to achieve wider dissemination of this work that the technical annexes for PFCs in sediment, biota and seawater are combined and edited for publication within the ICES TIMES series

6.2 Develop background concentrations for dioxins (OSPAR request 4, 2008 ICES work programme);

MCWG 2009 recalled the practical approach taken by MCWG 2008 for developing background concentrations for contaminants in biota was to consider a percentile of contaminant data from areas that could be considered "remote" or "pristine" as a basis for recommending *low concentrations.* MCWG 2008 recognized that these were not necessarily representative of "background" concentrations, uninfluenced by Man's activities, as it was not possible to account for regional variation and the influence of long range transport of anthropogenic pollutants.

Michael Haarich and Stepan Boitsov collated data on PCDD/Fs in marine biota supplied by members and augmented this with data available in the scientific literature. Very little data were available in remote locations for the preferred species/matrix combinations so a more inclusive approach was taken.

- Data for different species/locations (~96 datasets) were extracted from twenty different studies (published literature, reports) from areas were not representative of polluted areas in so far as the group could determine,
- From these data sets, again the minimum values for the **sum of PCDD/Fs** (expressed as pg WHO-TEQ PCDD/F g^{-1} wet weight) were selected,
- 20 values were rejected due to outstanding high values obviously not representing "pristine"/un- or low affected areas (Figure 1).

Nearly all of the samples are from coastal areas due to the fact that most recent studies have been performed for seafood consumer protection purposes on fish muscle, bivalve and crustacean species (sea food). The results are summarized in table 1. The entire data selected for this review are in Annex 12. It should be noted that the data included do not necessarily represent "remote" or "pristine areas" and, that remote areas may not be pristine due to long range transport of PCDD/Fs. Furthermore, it was not practical to collate data as the preferred fresh or lipid weight concentrations, as opposed to toxic equivalents. There was little information available on the concentrations of PCDD/Fs in fish liver, which is the recommended matrix for most finfish species included in the OSPAR guidelines. Due to the wide variety of species all fish were summarized as well as bivalves and crustaceans to get a sufficient number of data to be able to perform basic statistic calculations. The mean/median values for bivalves are lower than for fish muscle, whereas the crustaceans are similar to fish.

Table 1: Minimum values for sum of PCDD/Fs from selection of lowest contaminated locations of reviewed data sets (pg WHO-TEQ PCDD/F g^{-1} wet weight)

	Fish muscle	Bivalves	Crustaceans
Mean	0,17	0,09	0,17
SD	0,13	0,09	0,08
RSD,%	75	98	50
Median	0.150	0.059	0.149
1.quartile (25%)	0.062	0.022	0.100
10-percentile	0.016	0.012	0.089
Minimum	0.010	0.001	0.060
Max of selected data (cut off)	0.5	0.323	0,3
n	49	17	10
*Mean PCDD/F limit of quamtification**		*0.024 ± 0.022 pg g^{-1} ww ; sample intake ca. 24 g*	

***Source Greenland mussels**

OSPAR's MON Working Group could consider the medians of minimum values as indicative low concentrations; 0.15 pg WHO-TEQ PCDD/F g^{-1} wet weight for marine fish muscle, and 0.06 pg WHO-TEQ PCDD/F g^{-1} wet weight for bivalve molluscs. MCWG stresses that these are crude estimates (e.g. species differences are not accounted for) based on extremely limited data but suggest that MON could use these values on a trial basis or, alternatively consider a pragmatic limit of quantification (e.g. estimated from data within the ICES database), as a starting point for calculating Background Assessment Concentrations (BACs). This could be reexamined at a future stage, but MCWG 2009 does not foresee any substantial additional information being available in the near future.

6.3 Review and update of JAMP Eutrophication monitoring guidelines for nutrients and dissolved oxygen (OSPAR request 6, 2009 ICES work programme) and specifically:

The COSG dealt with this agenda point. Furthermore, the OSPAR request to ICES included a request for updating chlorophyll guidelines. While not on MCWG 2009 agenda COSG indicated that, if not completed by another working group during the 2009 meeting cycle, MCWG 2010 would be willing to update this.

6.3.1 Guidelines on inorganic/organic nitrogen

including

i) a more detailed explanation of contamination risks during sampling and analysis and appropriate temperatures and duration of preservation

ii) standards and protocols for moored instrumentation

The COSG reviewed the JAMP eutrophication monitoring guidelines for nutrients and updated the text to include the fluorometric determination of ammonia. Further information was included regarding possible sources of contamination (in particular ammonia) and a more detailed discussion of preservation issues. Greater detail has been included regarding the quality assurance which should be undertaken. There are no generally agreed procedures for preserving nutrient samples and therefore the

guidelines now stipulate that laboratories need to validate their own preservation procedures.

Developments in *in situ* nutrient analysers were noted. Information has been included on the way in which they may be used, the issues which arise with their use and the importance of quality assurance of the data. MCWG did not have statistical expertise to review section 3 (Quantitative Objectives) of the guidelines (see 6.3.3). The revised guideline is appended as Annex 13 of this report.

6.3.2 Guidelines on oxygen

including

i) a more detailed explanation of contamination risks during sampling and analysis and appropriate temperatures and duration of preservation

ii) advantages of developing sampling methodology and analysis

The COSG went through the existing JAMP guideline on oxygen and stated that there are no generally new methods, but several technical developments improving oxygen measurements or allowing high frequency sampling. Due to specific requirements of different sampling sites, no general recommendation for oxygen determination can be made.

In response to OSPAR's request for more detailed explanation of contamination risks and consideration of new techniques COSG added some remarks to the guideline concerning the use of different modifications of the basic methods, analytical errors which may be caused by improper handling of samples and the use and limitations of oxygen sensors. Furthermore, it is highlighted that a validated and documented quality assurance system is essential, including all steps of oxygen determination and the maintenance and handling of the equipment. MCWG did not have statistical expertise to review section 3 (Quantitative Objectives) of the guidelines (see 6.3.3). The revised guideline is appended as Annex 14 of this report.

6.3.3 Discuss how MCWG might contribute to providing ICES advice to OSPAR as follows (OSPAR request no. 6. ICES work programme 2009):

- advice on the period and frequency of sampling to have an accurate idea on winter nutrient concentrations
- recommendations on accurate analysis of trends (decreased concentration, increased frequency of low O2 concentration, increased consumption rate)

The chemical oceanography subgroup discussed this and acknowledged that this has been a long-standing task within OSPAR which has not been fully resolved to date. A report on *Temporal and spatial monitoring of eutrophication variables in CEMP* prepared by SMHI (Axe *et al*., 2008) was considered by the subgroup. This concluded *inter alia* that the optimum sampling programme to observe rapid events is likely to be a combination of ferrybox systems, which appear to be reliable and give both good spatial and temporal coverage, combined with buoy observations. To ensure data of sufficient quality, these must be controlled against conventional research vessel observations. Research vessels still have a role in seasonal mapping, and in providing data of sufficient quality for trend analysis from a large area.

In addition, a paper from work conducted by CEFAS (UK) (Heffernan *et al*., 2009) was considered. This used a statistical tool to test the performance of different monitoring designs and assessment methods. The authors concluded that even at background concentrations of TOxN, daily data from a platform such as SmartBuoy or

multi annual discrete sampling would be required to give sufficient power in the assessment.

MCWG made the following observations:

- An improved definition of the monitoring goals would facilitate work on this topic. The group noted discussions at OSPAR's joint ETG/MON *ad hoc* working group in 2004, which suggested examples for a statistical approach to definition of thresholds against which monitoring data could be assessed. This had not been taken forward by OSPAR;
- Apart from the Swedish report and the UK paper, MCWG is unclear as to what work has already been carried out within OSPAR contracting parties to address this, but recognise there may be more information available;
- Guidance on the period, frequency and spatial coverage would need to account for differences between areas/water bodies. In particular salinity variability in inshore coastal waters can complicate assessments, although mixing diagrams can be used to normalise where conservative mixing occurs;
- High frequency monitoring using SmartBuoy or ferrybox systems could support traditional sampling approaches by enabling estimates of temporal variability and short-term trends during winter for specific areas. Data produced by these methods should be quality assured and 'fit for purpose';
- Both statistical expertise and a good knowledge of the water bodies/areas and processes underlying the data are required to address this issue. It may be best addressed by a group consisting of statisticians and eutophication/nutrient experts working together. One approach might be for such a group to work on case studies of specific areas utilising data available, for example, in the ICES database.

While these comments were considered in relation to winter nutrients monitoring, in general they are also valid for dissolved oxygen.

Axe, P. 2008 Temporal and spatial monitoring of eutrophication variables in CEMP SMHI report RO 38.

Heffernan, J., Barry, J., Devlin, M., and Fryer, R. 2009 A simulation tool for designing nutrient monitoring programmes for eutrophication assessments. In press

6.4 Report on new information regarding emerging contaminants in the marine environment; *inter alia* perfluorinated compounds, tris(4-chlorophenyl)methanol (TCPM) and tris(4-chlorophenyl)methane (TCPMe) in flatfish, and brominated flame retardants;

MCWG considers information on emerging contaminants as a rolling agenda point. In 2009 there were updates on perfluorinated compounds (PFCs) and brominated flame retardants (BFRs) and in particular Decabromodiphenylether (BDE -209).

6.4.1 Perfluorinated compounds

Three presentations were given, covering PFCs in the water and biota of the North Sea and the Baltic Sea (Lutz Ahrens), spatial and temporal distribution of PFCs in the aquatic environment of the Netherlands (Christiaan Kwadijk) and atmospheric PFCs in the Baltic Sea environment (Ralf Ebinghaus).

Concern about PFCs has increased in the last few years due to their global distribution, ubiquitous detection in the environment, bioaccumulation in e.g. marine mammals and potential toxic effects. As a result, the 3M Company, the major producer of perfluorooctyl sulfonyl fluoride (POSF), voluntarily discontinued the production in 2000. In 2006, the U.S. Environmental Protection Agency (U.S. EPA) launched a voluntary stewardship program to reduce emissions of perfluorooctanoic acid (PFOA) and related chemicals from facilities and products. In the European Union (EU) the general use of perfluorooctane sulfonate (PFOS) and their derivates have been prohibited since June 2008.

- **Polyfluorinated Compounds in the marine environment (Lutz Ahrens)**

The first part of the presentation focused on the concentration levels of PFCs in waste water treatment plant (WWTP) effluents and in surface water of the river Rhine and the North Sea. In the water phase, the highest concentrations were for PFOA, perfluorobutanesulfonate (PFBS) and perfluorobutanoic acid (PFBA). ∑PFCs concentrations in the North Sea ranged between 0.1 ng L^{-1} offshore and 15 ng L^{-1} in the coastal area, with a strong concentration gradient from the coastline. In the Baltic Sea, ΣPFC ranged between 0.8 and 6.0 ng L^{-1}. The river Elbe and Rhine were identified as local input sources for PFCs to the North Sea. Along the river Rhine a strong increase in the concentrations of PFBA and PFBS was observed between Leverkusen and Dusseldorf, Germany, from 2.3 to 75.1 ng/L and 3.8 to 36.7 ng L^{-1}, respectively. As a result, these compounds are the dominant PFCs in the river Rhine. Industrial WWTP effluents might be the source of this contamination.

Additionally, PFCs were analysed in liver samples of harbour seals *(Phoca vitulina)* collected from the German Bight to examine the temporal trend between 1999 and 2008. Concentrations of various PFCs, including C_4-C_{10} perfluorinated sulfonates (PFSAs), perfluorooctane sulfinate (PFOSi), perfluorooctane sulfonamide (FOSA) and C_8-C_{15} perfluorinated carboxylic acids (PFCAs) were quantified. The main compound was PFOS, with on average over 90% of ΣPFC. Regression analysis of logarithmic-transformed PFC mean concentrations indicated significantly decreasing concentrations for C_5-C_7 PFSAs, PFOSi, FOSA and PFOA between 1999 and 2008. Furthermore, PFOS decreased by 49% between 1999 and 2008, which agrees with decreasing concentration levels of its metabolic precursors PFOSi and FOSA of 83% and 95% in the same time period. The reason for the decline during the past 10 years could be an effect of the replacement of these PFCs by shorter chained and less bioaccumulative compounds. But the observations of increasing perfluorodecane sulfonate (PFDS) levels, the high concentrations of PFOS and constant levels of C_9-C_{13} PFCAs indicates that further work on the reduction of environmental emissions of PFCs is necessary.

- **Spatial and Temporal Distribution of Perfluorinated Compounds in the Aquatic Environment (Christiaan Kwadijk)**

Results were presented of a study carried out in 2007 on PFCs in water, sediment and eel *(Anguila anguila)* from 21 different sites in the Netherlands, covering several rivers, lakes and canals. Water samples were extracted using solid phase extraction (SPE), while sediment and biota samples were extracted using sonication and shaking with acetonitrile. A clean-up step on ENVI-carb was applied to all extracts prior to analysis by LC-MS. Perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid(PFOA) were detected in all water samples. The highest concentrations, however, were found for perfluorobutanesulfonate (PFBS), which peaked in the river Rhine at the Dutch-German border, at a concentration of 270 ng L^{-1}. In sediment samples, PFOS and PFOA were also detected in all samples, in addition to perfluorononanoic acid

(PFNA). The highest concentration was again PFBS in the sediment sample from the Rhine at the Dutch-German border, while PFBS concentrations were significantly lower in the other sediment samples.

PFOS was the main PFC compound in fish samples. The PFOS concentrations in eel varied between 7 and 58 ng g^{-1} wet weight. Bioaccumulation factors (BAF), sediment-water distribution coefficients (Kd) and biota-sediment ratios were calculated for the different compounds, and for both the perfluorinated carboxylic acids and the perfluorinated sulfonates a correlation was found between log BAF and the number of perfluorinated carbons in the compounds. Despite a high variation between the log Kd values, on average an increase of log Kd was observed for each perfluorinated carbon It is expected that the characterization of the sediments will decrease this variation. This also applies to the variation in the data for the eel-sediment ratio. Temporal data was presented for eel samples collected at Hollands Diep between 1979 and 2005. The concentrations in the 22 samples varied between 5 and 42 ng g^{-1} wet weight, but no significant trend could be observed.

- **Poly- and perfluorinated compounds in the atmosphere of the Baltic Sea (Ralf Ebinghaus)**

New PFC data were presented of a measurement campaign onboard Maria S. Merian during the cruise MSM 08/03 from Rostock/Warnemünde via Tallinn and Helsinki to Kiel between 16 June to 19 July 2008. The following groups of PFCs were analysed: fluortelomeralcohols (FTOH), perfluorsulfonamide (FOSA), fluortelomeracrylate (FTA), perfluorsulfonamidoethanole (FOSE). Gaseous PFCs were collected on PUF/XAD2/PUF cartridges followed by "cold" extraction with MTBE/Acetone (1:1) and GC-MS analysis, with positive chemical Ionisation (PCI). Particulate-phase PFCs were collected on glass fibre filters followed by Fluidized Bed Extraction (FBE) with MTBE/Acetone (1:1) and HPLC-MS/MS analysis with negative ionization.

Volatile and semi-volatile PFCs were ubiquitously distributed in the Baltic atmosphere with concentrations ranging between 20 and 160 pg m^3. FTOH were the dominating compounds, while FTA and FASA/FASE were 10-20 times lower than average FTOH concentrations. The particulate fraction of PFCs accounted for <20% of the ∑PFC. Ionic PFC concentrations on particles had even lower concentrations. Sulfonamidoethanole were the dominant particulate-phase PFCs, whereas FTOH could hardly be detected. The data obtained during this cruise are comparable with those obtained during a ship cruise between Sweden-Alaska (Shoeib *et al*., 2006). However, higher peak concentrations (factor 7) were measured in the Baltic atmosphere. Baltic marine background concentrations reported here are comparable to North Sea data, but are higher than concentrations reported for the North Atlantic (Jahnke *et al*., 2007; Dreyer *et al*., 2009).

Dreyer, A., and Ebinghaus, R. 2009. Polyfluorinated Compounds in Ambient Air from ship- and land-based measurements in northern Germany, Atmospheric Environment, 43, 1527–1535.

Jahnke, A., Berger, U., Ebinghaus, R., and Temme, C. 2007. Latitudinal gradient of airborne polyfluorinated alkyl substances in the marine atmosphere between Germany and South Africa (53° N-33° S), Environ. Sci. Technol, Vol 41: 9, 3055–3061.

Shoeib, M., Harner, T., and Vlahos, P. 2006. Perfluorinated chemicals in the Arctic Atmosphere, ES&T, 40 (24): 7577–7583.

6.4.2 Brominated flame retardants (BFRs), with focus on BDE-209

Katrin Vorkamp summarised the following two publications, which had been provided by Michel Lebeuf, with focus on new information on decabromodiphenylether (BDE-209):

- Ross, P.S., Couillard, C.M., Ikonomou, M., Johannessen, S., Lebeuf, M., Macdonald, R., Tomy, G. (2008): Polybrominated diphenylethers (PBDEs) in the Canadian marine environment: An emerging health risk for fish, marine mammals and their habitat. Canadian Science Advisory Secretariat (CSAS) 2008/036;
- Ross, P.S., Couillard, C.M., Ikonomou, M.G., Johannessen, S.C., Lebeuf, M., Macdonald, R.W., Tomy, G.T. (2009). Large and growing environmental reservoirs of deca-BDE present an emerging health risk for fish and marine mammals. Mar. Pollut. Bull. 58 (1), 7–10.

The article in Mar. Poll. Bull. is an extract of the longer and more detailed report to the CSAS, which reviews current knowledge of the occurrence and fate of PBDEs in the Canadian environment and presents up-to-date information on the toxicity of PBDEs. The main conclusion of this review is that large reservoirs of BDE-209 are present in the environment and are likely to present a source of "lighter", more bioaccumulative and toxic BDE congeners.

Regarding the toxicity of BDE-209, three studies are cited which have demonstrated altered steroid hormone concentrations and changes in liver weight of rainbow trout and Atlantic tomcod. For non-209 BDEs, a long list of toxicological studies is given, with the following general thoughts and observations:

- Evidence of endocrine disruption.
- Risks associated with impurities in the PBDE formulations.
- Risks associated with breakdown products.
- PBDEs add to an already complex mixture of chemicals.
- Co-occurrence of structurally related compounds, e.g. PCBs.

PBDEs and PCBs appear to bind to the same cellular receptors in biota. Interactions seem to be more than additive.

Regarding the occurrence in the abiotic environment, BDE-209 was found to be the main congener in surface water, rain and sediments, at least for the particulate fraction. A mass-balance showed that 30% of BDE-209 in the Strait of Georgia (British Columbia) originated from wastewater, which also was recognised as an important source of non-209 BDEs. The introduction of PBDEs into the Strait of Georgia has surpassed that of PCBs (53 kg y^{-1} vs. 19 kg y^{-1}). Decreasing and increasing trends were also found for PCBs and PBDEs, respectively, in sediment cores from the Strait of Georgia. Several other examples are given of continuously increasing PBDE concentrations in the Canadian environment, in contrast to results from Japan (fur seals) and Europe (human milk), where the concentrations have decreased since the middle of the 1990s.

Regarding the occurrence in biota, BDE-209 is only found at low concentrations at the top of the food chain, i.e. not subject to biomagnification. The following possible reasons are discussed:

- Short half-life in the body, metabolic breakdown.

- Rapid partitioning to the particle-bound phase in the environment, low bioavailability.
- Limited uptake across the gastrointestinal tract (high K_{OW}).
- No steady state in the environment, as indicated by more frequent detection of BDE-209 in recent years.

The authors rule out the limited gastrointestinal uptake as a reasonable explanation, based on a study on grizzly bears, which showed higher concentrations for bears feeding in the terrestrial system than for those feeding on salmon. Apparently, the different concentrations are a consequence of differences in exposure, rather than compound uptake. In general, there seems to be a higher exposure of top predators in the terrestrial than in the marine environment.

However, there are conflicting findings for the bioaccumulation and biomagnification of BDE-209: While increasing concentrations of BDE-209 were found with increasing trophic level in the freshwater food web of Lake Winnipeg, the authors also present the opposite result, i.e. decreasing concentrations of BDE-209 with increasing trophic level in an Eastern Arctic marine food web. These opposite findings were discussed in the light of the above-mentioned possible explanations. The authors emphasise the likelihood of more bioavailable, persistent and toxic BDE congeners being formed from BDE-209. An interesting example was the metabolisation of > 90% of BDE-209 in grizzly bears during hibernation, with no concurrent excretion, i.e. the metabolites were retained.

Katrin Vorkamp also informed about current activities at NERI including brominated flame retardants in the marine environment:

- Review article on BFRs in the Arctic, as part of the International AMAP Assessment, to be published in a special issue of Sci. Total Environ. (de Wit, C.A.; Herzke, D.; Vorkamp. K.: Brominated Flame Retardants in the Arctic environment – trends and new candidates)
- Monitoring of PBDEs, incl. BDE-209, in ringed seals from East and West Greenland (AMAP).
- Retrospective time trend study of HBCD in ringed seals from East Greenland, 1982–2008 (AMAP).
- HBCD in hake from the Mediterranean Sea (Collaboration project with IFREMER).

Philippe Bersuder added information on the joint project on monitoring of DecaBDE (funded by the Bromine Science and Environmental Forum, BSEF) which includes analytical campaigns every other year. The project is ongoing and new data are expected for next year.

New results on HBCD in marine mammals have become available from CEFAS, indicating decreasing concentrations compared with a study conducted in 2003: Law, R.J.; Bersuder, P.; Barry, J.; Wilford, B.H.; Allchin, C.R.; Jepson, P.D. (2008): A significant downturn in levels of hexabromocyclododecane in the blubber of harbour porpoises (*Phocoena phocoena*) stranded or bycaught in the UK: An update to 2006. Environ. Sci. Technol. 42, 9104–9109.

Jacob de Boer informed about the legal status of BDE-209 which is now banned from use in electric and electronic devices in the EU. The proficiency testing exercises indicate progress in the accuracy and precision of the analysis of BDE-209. However, blanks are still an issue. In addition to the DecaBDE project mentioned above, moni-

toring of BDE-209 in human blood has been started, first results of which will be presented at SETAC 2009.

6.4.3 Tris(4-chlorophenyl)methanol (TCPM) and Tris(4-chlorophenyl)-methane (TCPMe) in fish

These compounds have been analysed in the period 2004–2008 in 20–30 biota samples each year. Samples consisted of "seafood" available in the Netherlands; bivalves, shrimps and fish (including flatfish), originating from Dutch fresh water, aquaculture and catch in the North Sea as well as some tropical seas.

Levels of TCPM were very low, only a few samples were above the detection limit (around 0.5 to 0.01 μg kg^{-1} fresh weight, depending on sample type), TCPMe values were even lower. Highest value observed was 1.6 μg kg^{1} TCPM in farmed eel, second highest was 1 μg kg^{-1} in seabass from a harbour. These data will be publicly available later this year.

An anticipated peer-reviewed publication outlining results of an MCWG collaborative study was not available for review at MCWG 2009.

6.4.4 Nanoparticles

MCWG briefly discussed the relevance of nanoparticles (NP) for the marine environment after a short introduction given by Ralf Ebinghaus. Nanoparticles had also been mentioned in Jacob de Boer's presentation "New threats for the marine environment" (see 5.3.)

There are presently three groups of NPs under discussion:

- carbon based NP, such as Carbon Nano Tubes and fullerenes;
- metallic compounds, such as TiO_2 and ZnO; and,
- zero valent metals, such as elemental Fe, Ag and Au.

In aqueous solutions NPs are subject to aggregation and precipitation, which limits their application in aqueous and biomedical applications. Sediments and benthic organisms are expected to be the main sinks and receptors in surface waters since higher ionic strengths lead to increased aggregation and sedimentation and an increased toxicity. This can be especially critical for benthic organisms in estuarine environments.

A recent review by Klaine *et al*. (2008) has identified the following key question related to NP in the environment.

1) Do they retain their nominal nano-scale size and original structure and reactivity in aquatic and soil/sedimentary systems?

2) Does an association exist with other colloidal and particulate constitutents?

3) What are the effects of solution and physical (e.g. flow) conditions?

4) Is their effect on aquatic and sedimentary biota different from that of larger particles of the same materials?

5) Does biota, such as biofilms and invertebrates modify the behaviour of NPs?

NPs are an emerging substance group where little is known about their environmental fate or risk as substances in their own right or as vectors for other substances. Some MCWG members expressed concern at the high quantities of NPs produced

containing toxic substances such as cadmium. .MCWG recognised that this could be area of future interest to the group.

Klaine, S., Alvarez, P. J. J., Batley, G. E., Fernandes, T. F., Handy, R. D., Lyon, D. Y., Mahendra, S., McLaughlin, M. J., and Lead, J. R. 2008. Nanomaterials in the environment: Behavior, fate, bioavailability, and effects. Environmental Toxicology and Chemistry, 27 (9): 1825–1851.

6.5 Review available information regarding the role of nutrients and organically-bound nutrient species as potential drivers for processes which can influence the uptake and distribution of contaminants in the environment and ecosystems;

COSG highlights the function of organically-bound nutrient species in marine ecosystems as a significant source of matter for the recycling of inorganic nutrient species within the system and as a source to fuel microbial activity. Despite this role, there is little information about formation and degradation processes and about stability of organically-bound nutrient species. Furthermore, the relationship between changes in environmental conditions and (elemental) composition of organically - bound nutrients are poorly understood. Within the framework of environmental management it is a fundamental requirement to gather more information about the role and behaviour of organically-bound nutrients.

In addition to the turnover of matter, organically-bound nutrient species can also be involved in complexing trace metals or organic contaminants and by this influence their effects in marine ecosystems.

COSG recommend as a first step, information should be gathered and reviewed for the MCWG meeting next year. The review should include a description of the role and elemental composition of organically – bound nutrient species in relation to recycling of inorganic nutrients. It will not consider the role of organic nutrients in the uptake and distribution of contaminants in the environment and ecosystems.

6.6 Develop (in collaboration with WGMS) draft guidelines for the preparation, use and analysis of passive samplers, review new information on use of passive samplers and consider how they could be incorporated in marine monitoring programme;

6.6.1 Presentation on the outcome of the outcome of the ICES collaborative passive trial survey (Foppe Smedes)

In 2006 a joint Coordinating Group of the ICES WGs on Marine Sediments and Marine Chemistry organized a collaborative trial of the use silicone rubber passive samplers in water and sediment. Target analytes were polycyclic aromatic hydrocarbons (PAHs) and chlorobiphenyls (CBs). Passive sampling of the water phase and sediment pore waters was carried out between October and December 2006 by thirteen laboratories at up to 30 locations through the PSTS project. In addition participants analysed local or deployed mussels in parallel to the passive sampling. The European locations covered estuarine and coastal environments from Norway to Portugal and west to Ireland and the Faeroe Islands. Also, two locations in Brisbane, Australia were sampled. This design permitted intercomparison of participants' performance through comparison with the results obtained by the more experienced central laboratory. An example was shown how sampling rates determined from participants' data agreed with those of the central laboratory

The free dissolved or available concentrations of hydrophobic organic contaminants in the aqueous phases (water column and pore water) were estimated from the uptake by passive samplers.

Notably high concentrations were found in Norwegian areas close to aluminum smelters. A remarkable finding was that in the outer parts of the Scheldt, concentrations of lighter PAHs increased seawards, while heavier PAHs showed a decrease. Possibly this is related to atmospheric input that remains more in the water phase in open sea, whereas in estuarine water suspended particulate matter may bind the deposited PAHs. Concentrations in sediment pore waters reflected known contamination of the Seine and the Scheldt with CBs, and the Elbe with hexachlorobenzene. Low concentrations were found in Norway and Scotland; these areas are likely to be remote from inputs of CBs. Comparison of concentrations in surface and pore water could be made showing agreement in dynamic areas and differences in quiet sedimentation areas. Bioconcentration factors for Mussels water obtained showing a good relation with hydrophobicity (K_{OW}). Outlying results for CB170 and CB180 showed lower uptake for mussels in data from France, Portugal and the Netherlands. This is in agreement with finding in a 6 year monitoring program.

Sediment exposures allowed study of partitioning between sediment and pore water. LogKoc values could be determined for added compounds as well as natively present compounds. For PCBs logKoc values of native and added compounds agreed well, while for PAHs the logKoc for natively present PAHs was, on average, one log unit higher than added compounds. This demonstrates the reduced availability of these compounds.

Smedes, F., Davies, I. M., Tronczynski, J. 2007. ICES Passive sampling trial survey for water and sediment (PSTS) 2006–2007. Part 1: Objectives, Design and Realization, ICES CM 2007/J:02. http://www.ices.dk/products/CMdocs/CM-2007/J/J0207.pdf

Smedes, F., van der Zande, T., Tixier, C., and Davies, I. M. 2007. ICES Passive sampling trial survey for water and sediment (PSTS) 2006-2007. Part 2: Laboratory intercomparison, analytical issues and lessons learned. ICES CM 2007/J:03 http://www.ices.dk/products/CMdocs/CM-2007/J/J0307.pdf

Smedes, F., van der Zande, T., and Davies, I. M. 2007. ICES Passive sampling trial survey for water and sediment (PSTS) 2006-2007. Part 3: Preliminary interpretation of field data, ICES CM 2007/J:04, http://www.ices.dk/products/CMdocs/CM-2007/J/J0407.pdf

6.6.2 Guidelines for the preparation, use and analysis of passive samplers in seawater

During MCWG 2008 it was agreed there was a requirement for general guidelines on passive sampling as well as a need for specific guidance on particular techniques. The first should assist users with the choice of approach depending on the monitoring requirement. The other should be specifically for the use of silicone sheets (PDMS) being aware that such guideline reflects the present status and future developments may lead to improvements to be implemented later.

- **Guidelines for Silicone rubber**

Intersessionally and during the meeting, Foppe Smedes and Kees Booij, worked on the "Guideline for use of silicone membranes for Passive Sampling of Seawater". A final draft of this guideline was discussed and adapted on the basis of comments received (Annex 15). The group felt that although the author(s) pointed out that procedures may be subjected to changes/improvements in future it was the right moment to publish them as a TIMES document to make sure that they are widely available.

Therefore, MCWG 2009 recommends the publication of this guideline in the ICES TIMES series

- **General guidance on Passive Sampling**

MCWG 2008 identified the following considerations that would help to select particular devices:

1) Are the required detection limits achievable by the device?
2) Are the calibration data available for estimating the sampling rates necessary to calculate the aqueous concentrations?
3) Is the technique mature, i.e. has it been thoroughly tested by multiple laboratories working in multiple geographical areas?
4) Can a sufficiently large number of sampling sites be found or established to cover the necessary geographical range (availability of buoys, jetties, and secure mooring sites)?
5) What is the potential of these devices to detect temporal and spatial trends?

To study the last item would require time series over a number of years, which are scarce. One monitoring series from the Netherlands has been reported covering 5 years with sampling only in autumn and winter.

Points 1–3 are related to the capabilities of passive samplers. MCWG summarised the demonstrated or achievable performance of passive sampling techniques for monitoring priority substances listed within WFD, OSPAR, HELCOM, AMAP, BSC, UNEP POP. The performance of semipermeable membrane devices (SPMDs) low-density polyethylene strip sampler, silicone strip samplers, Chemcatcher and polar organic chemical integrated samplers (POCIS) is summarised in appendix 16. Many of the listed nonpolar priority substances have been measured in the past with these samplers, and low detection limits (often in the pg L^{-1}) range are achievable. The field of polar chemical sampling is less well developed, but some polar chemicals have been successfully monitored with the POCIS, and this sampler shows potential for monitoring many other polar chemicals at a detection limit of 0.5 ng L^{-1} for 28 day exposures. For some compounds (particularly the polar chemicals, but also some of the hydrophobic ones) additional calibration data (sampling rates, sampler-water partition coefficients) are needed.

6.7 Report on the developments in Water Framework Directive (WFD) monitoring programmes for physico-chemical parameters (priority substances, other pollutants, nutrient status) in transitional and coastal waters;

6.7.1 Update on WFD activities

Peter Lepom presented information on recent developments with implementation of WFD.

- **DIRECTIVE 2008/105/EC on environmental quality standards**

The Directive has been published on 16 December 2008 and contains relevant provisions for the use of biota and sediment as matrices for chemical monitoring under the WFD.

For the majority of substances the establishment of EQS values at Community level is, at this stage, limited to surface water only. However, as it is not possible to ensure

protection against indirect effects and secondary poisoning for hexachlorobenzene, hexachlorobutadiene and mercury, by setting EQS for surface water alone, EQS have been established at Community level for those three substances in biota. In order to allow Member States flexibility depending on their monitoring strategy, Member States should be able either to monitor and apply those EQS for biota, or to establish stricter EQS for surface water providing the same level of protection.

Member States may establish EQS for sediment and/or biota at national level and apply them instead of the EQS for water set out in the Directive. Such EQS should be established through a transparent procedure involving notifications to the Commission and other Member States so as to ensure a level of protection equivalent to the EQS for water set up at Community level.

Sediment and biota are considered important matrices for the monitoring of certain substances with significant accumulation potential. Member States shall arrange for the long-term trend analysis of concentrations of those priority substances that tend to accumulate in sediment and/or biota, giving particular consideration to anthracene, PBDE, cadmium, C10-13 chloroalkanes, DEHP, fluoranthene, hexachlorobenzene, hexachlorobutadiene, hexachlorocyclohexane, lead, mercury, pentachlorobenzene, certain 5- and 6-ring PAHs and TBT.

- **COMMISSION DIRECTIVE laying down technical specifications for chemical analysis and monitoring of water status**

The Directive has not yet been published but has been adopted by European Water Directors in November 2008. The Directive allows the use of any available analytical method for monitoring provided it is properly validated and meets certain performance criteria as regards LOQ and uncertainty of measurement.

Member States shall ensure that laboratories or parties contracted by laboratories apply quality management system practices in accordance with EN ISO/IEC 17025. Furthermore, laboratories need to demonstrate their competence by participation in proficiency testing programmes and by analysing reference materials that are representative of the samples in the monitoring programmes under the WFD. The proficiency testing programmes shall be organised by accredited organisations or internationally or nationally recognised organizations, which meet the requirements of ISO/IEC guide 43-1. The results obtained in those programmes shall be evaluated on the basis of the scoring systems set out in ISO/IEC guide 43-1 or ISO-13528 or in other equivalent standards accepted at international level.

- **Chemical Monitoring Activity**

CMA-1: The surface water monitoring guidance has been published in February 2009 and is now available via internet (http://circa.europa.eu/Public/irc/env/wfd/library)

A guidance on monitoring of biota is under development, which will be presented to the CMA plenary at its next meeting to be held in Ghent on 5/6 May 2009. A first working draft has been reviewed by MCWG (see 6.7.2).

CMA-2: A common strategy on QA/QC for WFD related chemical monitoring has been developed within the EU-project EAQC-WISE (European Analytical Quality Control in support of WISE) that was finalised in November 2009. Following adaptation to practical needs and discussion with Member States, it is planned to publish this strategy as a CIS-Guidance document.

CMA-3: Under the mandate given to CEN to develop methods for analysis of OCPs, PAHs, PBDEs, TBT compounds, and short-chain chlorinated paraffins in unfiltered

water samples, a consortium has been established and project proposal elaborated, which will be forwarded to DG ENTR via CEN in this month.

6.7.2 Comments on the document - Guidance on Chemical Monitoring of Sediment and Biota under the Water Framework Directive, draft 1

A breakout group consisting of Koen Parmentier (Belgium), Victoria Besada (Spain) and Harri Kankaanpää (Finland) Michiel Kotterman (the Netherlands) plus Peter Lepom (Germany) considered the draft document. MCWG very much welcomed this document as for many substances sediment and biota offer a practical alternative matrix to water. Clearly the current draft owes much to the OSPAR Joint Assessment and Monitoring Programme and it is encouraging that the document draws on this experience. It is acknowledged by MCWG that the document is an early draft and not necessarily at an appropriate stage for comment. However, as MCWG members have long experience of monitoring contaminants in sediment and biota and as the current schedule anticipates the document being finalised in advance of MCWG 2010, it seemed opportune for MCWG 2009 to offer observations which could assist the authors with this task.

General comments

The document would benefit from a clearer structure. Background information should appear first and the document should then continue by presenting subsequent practical information in logical order. Reorganisation and consolidation is recommended.

At the outset, the authors should stress the need to focus on the essential. What aspects are really crucial for the success of a monitoring program – e.g. what compounds are abundant (causing local problems) and which biota species are locally available. A (check-list-type) flow diagram in the beginning of the document could help the orientate reader. The block elements of the flow diagram could include references to the chapters concerned in the document. However, this presentation should be kept as simple as possible.

The document would benefit from more consistent and up-to-date references. Example analytical methods rather than every method could be highlighted. Field variability and sampling error could be highlighted (selection of stations/natural heterogeneity of biota, etc).

Selection of species is critical. The authors could examine the philosophy of selecting an appropriate species that has relevance to the local ecosystem with respect to abundance, representativity of the area and their contaminant concentration levels. Also, MCWG would like to point out that in the HELCOM area there are other important contaminants, such as phycotoxins, that affect the local ecological status. These should be taken into account in the local implementation of the WFD.

The ICES Working Group on Marine Sediments (WGMS) has already discussed the issue on normalization of contaminant concentrations in sediments, and thus this point was not discussed in detail. However, it is advised that the authors need to pay attention to clear presentation of key elements involved in normalization to support spatial and/or temporal comparison of concentrations in sediment. The authors should make proper references to the sediment working group documents. There are considerable differences between sediments from different regions in OSPAR and HELCOM areas and therefore the need for normalization may vary.

MCWG encourages the authors to continue developing this document and take account the various comments provided by the MCWG. These comments consist of what is presented here plus an additional document containing a list of more specific comments which will be informally transmitted to EC working group.

6.7.3 Update on the Marine Strategy Framework Directive 2008/56/EC (MSFD):

MCWG were updated on the new task groups (TGs) established to provide scientific recommendations for criteria and methodological standards to assess progress in achieving Good Environmental Status (GES) for 11 descriptors under the MSFD. Of potential interest to MCWG are TG5 on eutrophication (lead JRC), TG 8 on contaminants and pollution effects (JRC lead), and TG9 on contaminants in fish/seafood (DG Sanco lead)

6.8 Provide expert knowledge and guidance to the ICES Data Centre (via subgroup) on a continuous basis

No requests from ICES were received by MCWG 2009.

6.9 Review information on integrated chemical and biological effects monitoring and assessment including information on effect directed chemical analysis

6.9.1 Models for Assessing and Forecasting the Impact of Environmental Key Pollutants on Marine and Freshwater Ecosystems and Biodiversity (ModelKeY)

Harri Kankaanpää provided an overview of the EU 6th framework ModelKey project, involving 26 countries (Coordinator Werner Brack, UFZ Centre, Leipzig), and running from 2005–2010. This program aims at assessing and predicting impacts of environmental key pollutants on marine and freshwater ecosystems and biodiversity. There are six subprojects:

- Keytox: identification of contaminants and toxins
- Basin: Database establishment & evaluation
- Expo: basin scale exposure monitoring
- Effect: effect-related models for diagnosis, prediction & mechanism
- Site: verification of exposure and effects predicted by EXPO & effect
- Decis: Generation of risk indices and decision support systems: tools for prioritisation and mitigation

The project also consists of a distinct training component. Case studies are conducted in the rivers Elbe, Scheldt and Llobregat. The WFD strives towards a good ecological status for the environment in 2015. Currently biodiversity is reduced in both freshwater and marine environment and this program must bridge knowledge gaps, and help in diagnosis of toxic impacts, efficient mitigation of the stressors and identification of the stressors

Moreover, the program intends:

- "to assess, forecast, and mitigate the **risks of traditional and recently evolving pollutants** on fresh water and marine ecosystems and their biodiversity at a river basin and adjacent marine environment scale,
- to provide **early warning strategies** on the basis of sub-lethal effects *in vitro* and *in vivo*,

- to provide a better understanding of **cause-effect-relationships** between changes in biodiversity and the ecological status, as addressed by the Water Framework Directive (WFD), and the impact of environmental pollution as causative factor, "
- to provide **methods for state-of-the-art risk assessment** and decision support systems for the selection of the most efficient management options to prevent effects on biodiversity and to **prioritize contamination sources and contaminated sites**,
- to **strengthen the scientific knowledge** on an European level in the field of impact assessment of environmental pollution on aquatic ecosystems and their biodiversity by extensive training activities and knowledge dissemination to stakeholders and the scientific community.

Keytox looks for effect-directed identification of toxicants, with feed-back between biological and chemical analysis via a confirmation loop

Some recent project outputs:

- *in situ* assessment of toxicity using biofilm translocation (Llobregat)
- phytotoxicant-induced shifts in algal community structures (Elbe)
- analysis of estrogenic compounds using sensitive mud snails
- relationships between emergent toxicants and biological structure (Llobregat)

Project website: www.modelkey.org

See also the BONUS BEAST project on Baltic Sea ecosystem health through use of biomarkers: http://www.bonusportal.org/research_projects/research_projects/beast/

6.9.2 Integrated chemical and biological effects monitoring of contaminants

Integrated chemical and biological effects monitoring of contaminants is likely to play a role in assessing good environmental status under the MSFD. Biological effects measurements are necessary to establish the environmental relevance of contaminant concentrations and combined effects of complex environmental mixtures. However, various MCWG members commented on the complexities often faced in assessing biological effects data and the difficulty in drawing clear conclusions. For example, biological responses may be induced by other factors, such as environmental conditions, or stress in fish due to catching. Harmonizing such techniques within international regulatory monitoring programmes thus presents a substantial challenge. MCWG is willing to contribute to ICES activities in this area, for example by providing analytical guidelines or relevant data, but wishes to avoid replicating work of WGBEC or study group on integrated monitoring of contaminants (SGIMC). MCWG noted section 4 of the SGIMC 2009 report mapping future work for this WG and recognize that the role of chemical measurement within integrated monitoring still needs further elaboration. SGIMC 2009 primarily focused on an assessment of EROD data. MCWG noted that as no contaminant data are related to the EROD activity an integrated assessment was not possible in this case.

6.10 Consider data quality issues arising when using dissolved oxygen sensors in deep waters (>2000m)

Measurements of dissolved oxygen concentration in coastal waters and open ocean are made regularly in a variety of scientific research and management activities. In the last decade the incorporation of oxygen sensors into CTD rosette packages has increased considerably, and these are now being used regularly in oceanographic cruises. Whilst oxygen sensors may be less precise than the Winkler method for discrete samples, and require frequent calibration to obtain accurate data, they provide a continuous record of the water column. Pressure hysteresis in electrochemical sensors has limited the usefulness of this technology at high pressure, invalidating sensor data if the calibration procedure does not take this problem into account.

Specific manufacturers have developed a new calibration algorithm using coefficients derived from Winkler titrations of discrete samples collected with each cast. These improved algorithms must be tested to demonstrate an improvement in the quality of oxygen sensor measurements in deep waters.

6.11 MCWG members to report information on projects of relevance to MCWG activities

A number of members presented some information to the group on a variety of ongoing projects

- **Sediments and bioavailability – Bart Koelmans (Netherlands IMARES/Wageningen University)**

Bioavailability of sediment-bound chemicals is important for retrospective as well as prospective risk assessments. For retrospective assessments or monitoring purposes, measurement of aqueous (pore water) concentrations with, for instance, passive samplers is adequate. In prospective assessments typically models are used. New work on calibration of such models shows that the *in situ* chemical availability of compounds like pesticides, PAH, PCB, PCDDs and PCDFs in harbour sediments can be modelled using three domain sorption models accounting for sorption to amorphous organic matter, oil and black carbon. Monte Carlo simulations, however, show that uncertainty in modelled values is considerable due to the accumulating error in underlying variable - and parameter values.

- **Chemical Weapons in the Baltic Sea - MERCW project (Harri Kankaanpää)**

Harri Kankaanpää (Finland) presented information from the MERCW project in chemical weapons in the Baltic Sea. This EU 6th framework programme (2005-2010), examines the threats related to the post World War II chemical weapons dumped in the Baltic Sea. The programme is divided into eight work packages that cover geophysical, hydrographic and biological investigations plus modelling of checmical warfare agents (CWA) and their breakdown product migration. Additionally bioaccumulation of the CWAs in the area is being examined.

The main CWAs dumped and analysed in this programme by VERIFIN (Helsinki, Finland) include mustard gas (sulphur mustard), As-containing adamsite, chlorobenzene, chloroacetophenone, clark I and tabun. Analytes include also the hydrolysis products of sulfur mustard (thiodiglycol and thiodiglycol sulphoxide). The MERCW has been focusing on studies on CWAs dumped around the Bornholm deep (southern Baltic Sea).

The mobilization of CWAs is a possible scenario, for example due to seabed-related activities such as dredging and trawling. During the MERCW several wrecks and objects have been pin-pointed. Partly based on this information the trans-Baltic Sea NordStream gas pipeline has now been rerouted to avoid the Bornholm deep dumpsite(s).

Chemical analyses (GC-MS and LC-MS/MS) by VERIFIN of recent surface sediment, pore water and near-bottom water samples revealed no parental CWAs. However, degradation products were detected in most sediment samples and a few pore water samples. It remains unknown how long ago these CWAs have been released from munitions/containers. Elevated As concentrations have been present at the dumpsite apparently for decades. The CWAs, e.g. adamsite, have a patchy distribution in the soft sediments and concentrations of degradation products of adamsite vary from non detectable to ca. 40,000 μg/kg. When released into water, parental sulphur mustard quickly hydrolyses and polymerises. Indeed, no sulphur mustard has been detected, but its degradation product was present in one sediment sample. So far there is no information about CWA bioaccumulation into local biota.

Bacteria tolerant to sulphur mustard have been isolated from deep waters surrounding the dumpsite, and cultured. These bacteria are not only tolerant to mustard gas but are also capable of degrading sulphur mustard even in a time frame of weeks.

The MERCW project will continue until 2010 and more information pertaining to the CWAs in the local environment are expected. At this point the data obtained suggests that actual ecological risks due to CWAs remain low and localised.

- **Galathea Survey: Marine pollution – organochlorines and PAHs in a global perspective. (Katrin Vorkamp)**

As part of the Danish Galathea 3 expedition, NERI has conducted the project "Analytical-chemical assessment of environmental pollutants and metals and molecular genetic characterisation of xenobiotic metabolising genes/enzymes in the marine environment in the vicinity of international shipping routes", in collaboration with the universities of Roskilde and Copenhagen. The expedition followed the traditions of Galathea 1 and Galathea 2, launched in 1845 and 1950, respectively, and circumnavigating the globe in the name of scientific research. Bivalve samples were collected at the Faroe Islands, Greenland, Ghana, South Africa, Australia, the Salomon Islands, New Zealand, Chile, the US Virgin Islands (St. Croix), Boston, Newfoundland and the Shetland Islands. The PCB concentrations in the bivalve samples varied with three orders of magnitude between the highest and lowest (detectable) concentrations. The highest PCB and DDT concentrations were found in Boston harbour (approximately 350 ng g^{-1} dw for ΣPCB and 80 ng g^{-1} dw forΣDDT) and the Sydney estuary (ΣPCB:150-280 ng g^{-1} dw, ΣDDT: 140 ng g^{-1} dw). Clearly higher PCB/DDT ratios were found for Nuuk, compared with the remaining Greenland samples, possibly indicating local impacts in Nuuk.

The lowest PAH concentrations (< 200 ng g^{-1} dw for ΣPAH) were found for Hobart and Chile, the highest concentration was again found for Boston harbour (about 6000 ng g^{-1} dw). The second highest concentration was found for Nuuk (4000 ng g^{-1} dw), with indications of an influence from petroleum sources. Different pollution sources were studied by Principal Component Analyses and it was possible to group the stations according to their pyrogenic/petrogenic influence. For most of the PAHs (phenanthrene, fluoranthene, pyrene, benz(a)pyrene, indeno(1.2.3-cd)pyrene, benzo(g,h,i)perylene, C1- and C2-phenanthrenes and C1- and C2-dibenzothiophenes), the samples of this study had higher concentrations than the

ICES "low concentrations" for PAHs in mussels. The lowest concentrations were found in samples from Greenland, but still above the ICES "low concentrations". For some other compounds (Benz(a)anthracene, Chrysene/Triphenylene, Benz(b,j,k)fluoranthene, Benz(e)pyrene and C3-Phenanthrenes), 20–100% of the Galathea reference samples were below the "low concentrations", but most of them < LOQ at the same time. This comparison with a global set of samples, including numerous reference sites, shows that the ICES "low concentrations" indeed describe very low PAH levels.

Additional data were presented for mercury and TBT in the same samples and compared with OSPAR Background/Reference Concentrations (B/RCs)[1] and Ecotoxicological Assessment Concentrations (EACs). For TBT, the samples spanned a range for three orders of magnitude, with the highest concentrations in Copenhagen harbour and St. Croix. About half of the samples exceeded the EAC. For Hg, only 4 of the 64 samples were within the B/RC range, none of them below. The median Hg concentration was 5 times above the upper B/RC. This tendency was also found for Cd, Pb, Cu and Zn. However, calculations of a Pollution Load Index indicated a "fair" health of the oceans, with regard to metals and TBT.

6.12 Complete guidelines for publication in ICES TIMES series: Determination of HBCD in sediment and biota; determination of PBDEs in marine biota and sediment; determination of PAH in marine biota and sediment and determination of organotins in marine biota

The TIMES papers combined the sediment and biota technical annexes, prepared by MCWG in 2007 and 2008, with the aim of increasing the accessibility of these guidelines. The report on PAHs includes examples of chromatograms and integrations for the alkylated PAHs, and was completed prior to the meeting, no further changes were made. The HBCD, PBDE and organotin TIMES reports were reviewed and finalised during the meeting.

7 Plenary discussion of draft report

Plenary discussion of the draft report took place on Friday 20 March.

8 Any other business

Following the "Smedes" protocol, Katrin Vorkamp was elected by acclaim as incoming co-chair subject to approval by ICES. Evin McGovern agreed to remain as co-chair for one further year to facilitate the transition.

9 Recommendations and action list

Recommendations, Resolutions for TIMES reports and Actions are listed in Annexes 4, 5 and 6, respectively.

1 This assessment is not based on revised OSPAR assessment criteria proposed for adoption in 2009 and applied during 2008 MON assessment. Consequently, this does not apply *low concentration* for mercury in mussels proposed by MCWG 2008 in lieu of background concentrations.

10 Date and venue of the next meeting

MCWG received and welcomed an invitation from Patrick Roose (MUMM) to host MCWG 2010 in [Ghent/Ostende}, Belgium [tbc]. The proposed dates for MCWG 2010 are [tbc].

11 Closure of the meeting

The meeting was closed at 13:00 on Friday 20 March.

Annex 1: List of participants

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Annex 2: MCWG Agenda: 31st meeting

IMARES, IJmuiden, Netherlands, 16–20 March, 2009

1 OPENING OF THE MEETING

The meeting will begin at 10.00 am on the first day, and 09.00 am thereafter.

The Meeting will be opened by Dr. Martin Scholten, Wageningen IMARES

2 ADOPTION OF THE AGENDA

3 REPORT OF THE 96th ICES STATUTORY MEETING

4 REPORTS ON RELATED ACTIVITIES

4.1 OSPARCOM AND HELCOM

Any official requests from OSPARCOM or HELCOM which arose prior to the production of the agenda have been included.

4.2 Laboratory Performance Study QUASIMEME

An update on the QUASIMEME scheme will be given.

4.3 Other Activities

All members who wish to make a presentation under this item should prepare a note for MCWG.

5 PLENARY PRESENTATIONS

5.1 *Tatsiana Rusina* Quantification method of the analysis of polychlorinated paraffins: analyses in marine species

5.2 *Sólveig Ólafsdóttir* Time series of inorganic carbon in Icelandic waters

5.3 *Jacob de Boer* New threats for the Marine Environment

6 MAIN AGENDA

a. together with WGMS prepare the following Technical Annexes for inclusion in the JAMP Guidelines for Monitoring Contaminants in Sediments (OSPAR agreement 2002-16) and JAMP Guidelines for monitoring Contaminants in Biota (OSPAR agreement 1992-2) according to the structure of the existing technical annexes as follows:

i.) prepare technical annexes for monitoring of dioxins in biota and sediments[2] (OSPAR request 4, 2008 ICES work programme);

ii.) prepare technical annexes for monitoring of PFOS in sediments and water (OSPAR request 4, 2008 ICES work programme);

iii.) review the existing technical annexes on monitoring of chlorinated biphenyls in biota and sediment and propose revisions so that they are adequate for monitor-

2 taking into account advice from SIME 2007 that monitoring of dioxins in sediments should only be carried out in specific areas (such as sedimentation areas or estuaries) because of time lag (10–12 years) in deposition of quantities required for sampling

ing of planar CBs in these compartments.[3] (OSPAR Request 4, 2008 ICES work programme);

b. develop background concentrations for dioxins (OSPAR request 4, 2008 ICES work programme);

c. Review and update of JAMP Eutrophication monitoring guidelines for nutrients and dissolved oxygen (OSPAR request 6, 2009 ICES work programme) and specifically:

i.) provide further clarification in the guidelines on inorganic/organic nitrogen including

- a more detailed explanation of contamination risks during sampling and analysis and appropriate temperatures and duration of preservation
- standards and protocols for moored instrumentation

ii.) provide further clarification in the guidelines on oxygen including

- a more detailed explanation of contamination risks during sampling and analysis and appropriate temperatures and duration of preservation
- advantages of developing sampling methodology and analysis

iii.) discuss how MCWG might contribute to providing ICES advice to OSPAR as follows (OSPAR request no. 6. ICES work programme 2009):

- advice on the period and frequency of sampling to have an accurate idea on winter nutrient concentrations
- recommendations on accurate analysis of trends (decreased concentration, increased frequency of low O2 concentration, increased consumption rate)

d. report on new information regarding emerging contaminants in the marine environment; *inter alia* perfluorinated compounds, tris(4-chlorophenyl)methanol (TCPM) and tris(4-chlorophenyl)methane(TCPMe) in flatfish, and brominated flame retardants;

e. review available information regarding the role of nutrients and organically-bound nutrient species as potential drivers for processes which can influence the uptake and distribution of contaminants in the environment and ecosystems;

f. develop (in collaboration with WGMS) draft guidelines for the preparation, use and analysis of passive samplers, review new information on use of passive samplers and consider how they could be incorporated in marine monitoring programme;

g. report on the developments in Water Framework Directive monitoring programmes for physico-chemical parameters (priority substances, other pollutants, nutrient status) in transitional and coastal waters;

h. provide expert knowledge and guidance to the ICES Data Centre (via sub-group) on a continuous basis;

i. review information on integrated chemical and biological effects monitoring and assessment including information on effect directed chemical analysis (related to OSPAR request 8);

j. Consider data quality issues arising when using dissolved oxygen sensors in deep waters (>2000m)

k. MCWG members to report information on projects of relevance to MCWG activities;

l. Complete guidelines for publication in ICES TIMES series: Determination of HBCD in sediment and biota; determination of PBDEs in marine biota and

3 taking into account advice from SIME that monitoring in sediments should be undertaken only if levels of marker PCBs are e.g. 100 times higher than the BACs and that for biota monitoring of concentrations in seabird eggs could provide an alternative matrix

sediment; determination of PAH in marine biota and sediment and determination of TBT in marine biota.

7 PLENARY DISCUSSION OF DRAFT REPORT

8 ANY OTHER BUSINESS

9 RECOMMENDATIONS AND ACTION LIST

10 DATE AND VENUE OF THE NEXT MEETING

11 CLOSURE OF THE MEETING

Annex 3: MCWG Terms of Reference for the next meeting

2009/2/SCICOM00 The **Marine Chemistry Working Group** [MCWG] (Co-Chairs: E. McGovern, Ireland, Katrin Vorkamp, Denmark) will meet in [Ostende/Ghent], Belgium from [dates to be confirmed] 2010 to:

a) Report on developments with regard to quality assurance of marine chemistry, in particular with respect to QUASIMEME

b) Report on developments in relation to ongoing chemical oceanography issues, including the International Nutrient Scale System and SCOR/IAPSO progress on developing algorithms for reporting absolute salinity of seawater;

c) Finalize technical annex for monitoring PCDD/Fs and "dioxin like" PCBs in sediments (with WGMS).

d) report on new information regarding emerging contaminants in the marine environment

e) review available information regarding the role and the elemental composition of organically-bound nutrient species in relation to recycling of inorganic nutrients

f) report on the developments in Water Framework Directive monitoring programmes for physico-chemical parameters (priority substances, other pollutants, nutrient status) in transitional and coastal waters; and developments in defining MSFD GES descriptors (Task groups 5 - eutrophication, TG8 - contaminants and their effects in the marine environment, TG 9 contaminants in seafood)

g) MCWG to contribute, as may be required, to ICES activities on integrated chemical and biological effects monitoring and review new information on effect directed chemical analysis

h) Complete guidelines for publication in ICES TIMES series: Determination of PFCs in marine sediment, biota and seawater; Guidelines on use of silicone rubber passive samplers

i) MCWG members to report information on projects of relevance to MCWG activities;

j) provide expert knowledge and guidance to the ICES Data Centre (via subgroup) on a continuous basis;

MCWG will report by 30 April 2009 for the attention of SCICOM and ACOM.

Supporting information

Priority:	This Group maintains an overview of key issues in relation to marine chemistry, both with regard to chemical oceanography and contaminants. The activities are considered to have a high priority. MCWG provides input across the field of marine chemistry, which underpins the advice given by ICES, and also supports the work of national and international collaborative monitoring programmes, e.g., within OSPAR.
Scientific Justification and relation to Action Plan:	a) MCWG has a particular interest in quality assurance and

	maintains strong links with QUASIMEME with a view to supporting quality assurance activities in this field. b) This activity is initiated by MCWG to reinforce its chemical oceanography activities and to track key international developments in this field that are of potential interest ICES; c) Preparation of technical annexes for JAMP guidelines is in response to OSPAR request 4, 2008 ICES work programme. d) This was initiated among MCWG members on the basis of concerns regarding emerging contaminants in the marine environment and is an ongoing area of interest to the group e) This activity is initiated by MCWG to reinforce its nutrient activities and to create a better link between contaminant dynamics and ecosystems drivers; f) This work was initiated by MCWG and will be of interest to OSPAR/EC/ HELCOM; g) This item was initiated by MCWG members and will be of interest to OSPAR in relation to OSPAR request no. 8, 2008; h) This work was initiated by MCWG with a view to - achieving wider dissemination of PFC guidelines initially prepared in response to an OSPAR request for technical annexes - meeting a need perceived by MCWG for guidelines to assist new users of passive samplers in the marine environment i) MCWG are interested in receiving reports on relevant projects and activities from its members; j) This is in direct response to a request by the ICES Data Centre;
Resource Requirements:	The resource required to undertake activities within the framework of this group is negligible.
Participants:	The Group is normally attended by some 20–35 members.
Secretariat Facilities:	None.
Financial:	No financial implications.
Linkages to Advisory Committees:	ACOM
Linkages to other Committees or Groups:	WGMS, WGBEC, SCICOM
Linkages to other Organisations:	The work of this group is closely aligned with work being undertaken within the EU Chemical Monitoring Group on the requirements and implementation of the Water Framework Directive. The group provides the basis for some advice to OSPAR.

Annex 4: Recommendations

RECOMMENDATION	FOR FOLLOW UP BY:
1. MCWG recommends that MCWG and QUASIMEME should retain a close working link and that QUASIMEME should take into account feedback and suggestions as given in MCWG 2009 report (agenda 4.2)	QUASIMEME
2. MCWG recommends that OSPAR adopt the revised annex on organic contaminants in biota and the annex on dioxins and co-planar CBs in biota as technical annexes annexes to the JAMP guidelines for monitoring contaminants in biota.	OSPAR
3. MCWG recommends that the OSPAR Jamp monitoring guidelines for contaminants in biota are restructured to separate general guidelines on sampling and sample handling from techncal annex 1 on organic contaminants in biota	OSPAR
4. MCWG recommends that OSPAR adopt the revised annex on CBs in sediment as a technical annex to the JAMP guidelines for monitoring contaminants in sediment .	OSPAR
5. MCWG recommends that OSPAR adopts the proposed technical annexes for PFCs in seawater and sediment as part of the JAMP guidelines for monitoring contaminants.	OSPAR
7. MCWG recommends that OSPAR adopts the revised Eutrophication monitoring guidelines for nutrients and dissolved oxygen in seawater	OSPAR
8. MCWG recommends that the authors of the CMA document on monitoring sediment and biota take account of the various comments provided by the MCWG.	WFD CMA drafting group on monitoring sediment and biota
9. MCWG recommends that ICES publishes the TIMES series reports on HBCD in sediment and biota, PBDEs in sediment and biota and Organotins in biota as per MCWG 2008 resolutions	ICES
10. MCWG recommends that, given the number of ICES groups involved in assessment of contaminants and their effects in the marine environment, coordination of ICES efforts in the field of integrated monitoring is required	ICES

Annex 5: MCWG Draft Resolutions for TIMES Publications

The following reports are proposed for publication on the ICES Techniques in Marine Environmental Science series (TIMES).

PUBLICATION TITLE	MCWG LEAD	ESTIMATED PAGE NUMBERS
Guidance for monitoring polychlorinated dibenzodioxanes and furans (PCDD/Fs) and planar chlorinated biphenyls (CBs) in marine biota and sediment	Katrin Vorkamp	20
Guidance for monitoring polyfluorinated compounds (PFCs) in marine biota, sediment and seawater	Lutz Ahrens	16
Guidelines for passive sampling of hydrophobic contaminants in water using silicone strip samplers	Foppe Smedes	14

The Marine Chemistry Working Group agrees to submit the final draft of the proposed publication by September 2010.

Supporting Information

Priority:	Monitoring guidelines are required to support monitoring under the regional sea conventions (e.g. OSPAR) and under activities (e.g. EC directives). These analytical guidelines support general monitoring activiti by providing best practice for monitoring these specific substances in marine biota and sediment.
Scientific justification and relation to action plan:	These products are based on technical annexes for monitoring contaminant in biota and in sediment produced by MCWG and WGMS in response to OSPAR requests. For relatively little additonal effort, publication in the TIMES series would make the information more widely available to, for example, scientists engaged with other regional sea conventions and EC directives such as the WFD and MSFD.
Resource requirements:	Cost of production and publication of four (in average) 17-page TIMES. The material in the reports is fairly straightforward, and therefore no specific additional costs are necessary.
Participants:	Approximately one month's work is required by the editor to finalise the drafts.
Secretariat facilities:	Help with document preparation/publication. Final editing.
Financial:	Publication costs.
Linkages to advisory committees:	
Linkages to other committees or groups:	The work will be coordinated with WGMS.
Linkages to other organizations:	These will be based on guidelines produced by MCWG on request for OSPAR. They will also support monitoring activities of other regional convention and the EC's Chemical Monitoring Activity which provides implementation guideance for monitoring under the WFD.

Annex 6: Actions

ACTION	WHO
Provide link between MCWG and QUASIMEME and to bring MCWG 2009 feedback and suggestions to QUASIMEME project office and advisory board	Michiel Kotterman Patrick Roose
Report to MCWG 2010 on developments on the International Nutrient Scale System and	Patrick Roose
Report to MCWG 2010 on progress of SCOR/IAPSO on developing algorithms for reporting absolute salinity of seawater	Klaus Nagel
Develop draft technical annex for dioxins and "dioxin-like" CBs in sediment for finalisation at WGMS/MCWG 2010	Philippe Bersuder, Patrick Roose, Katrin Voorkamp and Lynda Webster and Francesca Pelizatto (WGMS)
Contact ICES secretariat to identify if further work is required on revising the OSPAR eutrophication chlorophyll guidelines at MCWG 2010	Evin McGovern
Report to MCWG 2010 on HBCD time trend in Greenland	Katrin Vorkamp
Report new information on occurrence of benzotriazole in the marine environment at MCWG 2010	Ralf Ebinghaus, Norbert Theobald?
Report new information on occurrence of alkyl- PAH in the marine environment	Michiel Kotterman
Report new information on the occurrence of organophosphorus flame retardants in the marine environment	Philippe Bersuder
Collate available information in advance of MCWG 2010 regarding the role of nutrients and organically-bound nutrient species	Klaus Nagel & COSG
Bring attention of MCWG 2009 comments on draft "Guidance on Chemical Monitoring of Sediment and Biota under the Water Framework Directive" to CMA drafting group	Patrick Roose
Update MCWG 2010 on developments wrt contaminants monitoring under the WFD and progres of task groups defining GES descriptors for contaminants in the marine environment	Peter Lepom
MCWG chair to contact SGIMC chair prior to MCWG 2010 to identify potential input and or collaboration between EGs	Evin McGovern

Annex 7: Revised technical Annex on organic contaminants in biota

Organic contaminants

This annex is intended as a supplement to the general OSPAR JAMP guidelines. The Annex applies to analysis of all types of organic contaminants but are not intended as a complete description or a substitute for detailed instructions. Advice and recommendations given in documents prepared through the QUASIMEME project (Quality Assurance of Information for Marine Environmental Monitoring in Europe) are frequently cited.

1. Species

1.1 Fish and shellfish

1.1.1 Criteria for the selection of species for temporal trend monitoring

Species for temporal trend monitoring can only be selected in the light of information on fish stock composition and history. It is essential that long time series with one species are obtained. Care should be taken that the sample is representative of the population and can be repeated annually. Fish and shellfish species currently used for trend monitoring are listed in Tables 1 and 2 of the main guidelines.

1.1.2 Criteria for the selection of species for spatial distribution monitoring

In order to standardise results the first choice species *Limanda limanda*, *Gadus morhua* and *Mytilus edulis* or *M. galloprovincialis* should be used whenever possible. The second choice species *Merlangius merlangus, Merluccius merluccius, Platichthys flesus* and *Crassostrea gigas* should only be used when none of the first choice species are available.

First choice species

Limanda limanda **(dab)**

Dab is a ground dwelling species confined to the shelf seas. It has replaced the previously recommended plaice and flounder for the following reasons:

its migration is less pronounced, thus it is more likely to represent the area in which it is caught;

it has been used successfully in disease studies, thus complementary information from such studies would be available (in fish disease studies a length range for individual fish of 20-25 cm is used).

The southern distribution limit of dab is the north coast of Spain.

Gadus morhua **(cod)**

Cod normally live near the seabed but may also be pelagic. Cod occur in coastal areas and to 600 m depth. Cod may also be found in the open ocean and so may also be used for monitoring oceanic regions of the Maritime Area. The southern distribution limit of cod is at 45°N. A sampling size range of 30-45 cm is specified because cod of that size and age tend to feed on a fairly uniform diet.

Mytilus sp. **(mussel)**

Mytilus edulis occurs in shallow waters along almost all coasts of the Contracting Parties. It is therefore suitable for monitoring in nearshore waters. No distinction is made between *M. edulis* and *M. galloprovincialis* because the latter, which may occur along Spanish and Portuguese coasts, cannot easily be discerned from M. edulis. A sampling size range of 3-6 cm is specified to ensure availability throughout the whole maritime area.

Second choice species

Platichthys flesus (flounder)

The distribution of flounder extends further south than that of dab and might therefore represent the flatfish of choice for certain Portuguese coastal areas and Spain's northwestern coastal areas. Flounder is not suitable for monitoring in open sea areas due to its migration pattern. A sampling size range of 15-35 cm ensures individuals of the 2-3 year age class.

Merlangius merlangus (whiting)

Whiting can be caught in coastal waters and to 200 m depth. Its distribution is from Portugal to Iceland and Norway, thus covering all the maritime area subject to monitoring by Contracting Parties. It is a suitable substitute for Cod. The sampling size range, 20-35 cm, may need adjustment in the light of future experience.

Merluccius merluccius (hake)

Hake live at 100-300 m along the shelf margins. The sampling size range is 20-35 cm. The sampling size interval suggested is arbitrary and may need adjustment in the light of future experience.

Crassostrea gigas (Pacific oyster)

The Pacific oyster should be sampled in areas where *Mytilus sp.* is not available. The sampling size should be within the length range 9-14 cm to ensure individuals of the 2 year age class.

1.2 Seabirds

Relevant references concerning the use of seabirds in contaminant monitoring programmes include Gilbertson, 1987; Becker, 1989 and 1991; Becker *et al*., 1991 and 1992; Walker, 1992 and Bignert *et al*., 1995.

Sterna hirundo (common tern)

The common tern is widely distributed over the European and North American Atlantic coasts as well as the Baltic Sea, but does not occur in Iceland. It feeds in marine, brackish and fresh waters.

Haematopus ostralegus (oystercatcher)

The oystercatcher is widely distributed along the coasts of the North-west Atlantic, including Iceland, and also occurs in the Baltic Sea. The species is not strictly marine as it also feeds inland. It feeds on benthos. In contrast to other seabirds, nest sites are accessible and the eggs within reach.

Uria aalge (guillemot)

The guillemot feeds in the open sea and nests on the coasts of northern Europe, in the Baltic Sea and on the North American coast.

2. Sampling

Two alternative sampling strategies are described: sampling to minimise natural variability and length-stratified sampling. References of relevance to sampling and statistics include Gilbert, 1987; Bignert *et al.*, 1993 and 1994; Tema Nord, 1995 and Nicholson *et al.*, 1996 and 1997.

2.1 Sampling to minimise natural variability

Gain in precision of the contaminant data can be obtained by minimising variance from the biological covariables. For fish, this can be achieved by sampling and analysing individually at least 12 young fish of the same sex, *e.g.* 2-3 year old female fish. For shellfish, a sample should be collected with the number of individuals large enough to be divided into at least 3 equal pools with each pool consisting of at least 20 animals and enough soft tissue for all analyses. The length of the individuals collected should be constant from year to year at each station, or should at least fall within a very narrow range, *e.g.* within 5 mm. To reflect recent levels of contamination, young individuals should be chosen. In selecting the sample, care should be taken to ensure that it is representative of the population and that it can be obtained annually.

2.2 Length-stratified sampling

Where successfully ongoing, length-stratified time series should be continued.

2.2.1 Fish

Gain in precision of the contaminant data can also be obtained from stratification using biological variables. Although several biological parameters are appropriate, length appears to be the parameter which can most easily be applied onshore and at sea and which has also been shown to be significant in many analyses. Much discussion has been devoted as to whether simple linear or log-linear (multiplicative) models give the better fit. General experience with other fish and other types of data indicate a preference for the log-normal model at least for the present. As the length dependence of the contaminant concentration is not well understood, sampling should keep the length/contaminant relationship under constant surveillance, *i.e.* the entire length range should be covered evenly. Care should be taken that the samples are not unduly clustered within a particular length interval. More length intervals could be used and the test of the hypothesised contaminant/length relationship becomes stronger if the lengths are evenly distributed. It is essential to keep the length stratification identical from one year to the next. The length range should be defined on the basis of practical considerations. For fish, the upper limit should be chosen in such a way that at least 5 fish in the largest length interval can easily be found. The length stratification should be determined in such a way that it can be maintained over many years. The length interval should be at least 2 cm in size. The length range should be split into 5 length intervals, which are of equal size after log transformation. For example, if the length range is 18-36 cm, then the interval boundaries could be (rounded to 0,1 cm) as follows:

18 - 20,720,8-23,8 23,9-27,3 27,4-31,3 31,4-36 cm.

2.2.2 Shellfish

For shellfish, the upper limit should be chosen in such a way that at least 20 mussels in the largest length interval can easily be found. The length stratification should be determined in such a way that it can be maintained over many years. The length interval should be at least 5 mm in size. The length range should be split into at least 3 length intervals (small, medium and large) which are of equal size after log transformation. For example, if the length range is 40-70 mm, then the interval boundaries could be (rounded to 1 mm) as follows:

a) 5 intervals: 40 - 45 46 - 50 51 - 56 57 - 63 64 - 70

b) 3 intervals: 40 - 48 49 - 58 59 - 70

2.3 Seabird eggs

2.3.1 Permission

Permission to collect the eggs must be received from the appropriate national authorities.

2.3.2 Sampling period and frequency

Eggs should be sampled annually at each site in May or June. Only clutches from the first laying cycle within a single year should be selected.

2.3.3 Number of eggs and sampling procedure

Eggs should only be taken from full clutches (*i.e.* common tern 3 eggs, oystercatcher 3-4 eggs). Eggs should not be taken from abandoned clutches. Only one egg should be taken from each clutch. Ten eggs should be selected in total (*i.e.* one egg from 10 separate clutches) and it is important to choose the egg from each clutch randomly. As the eggs must be fresh (*i.e.* between 1-5 days incubation) information about the incubation stage of each egg is required. Two methods are recommended for determining incubation stage:

a) locate 12-15 clutches containing one egg only and mark these by placing a peg about 1 m from the nest. Check the clutches every other day until they are complete. Take one egg randomly from the completed clutch;

b) fill a 1 litre plastic beaker with water and place the egg in the water:

  i) fresh eggs (*i.e.* of 1-2 days incubation) will lie on the bottom with the long axis parallel to the bottom;

  ii) eggs of 3-6 days incubation will rest with the small end on the bottom of the beaker and the long axis forming an angle of 30-45°;

  iii) eggs which float or stand vertically with the small end on the bottom are of more than 7 days incubation and should not be selected.

Each nest from which an egg has been taken should be marked, using a peg or some other type of marker, to ensure that a second egg is not taken. While still in the field the egg selected should be put into a numbered plastic egg box. The number of the box should be written on the shell of the egg in soft pencil. The clutch size from which the egg was taken, the nest number and the sampling date should be recorded.

2.3.4 Materials

For each species, area and year the following are required:

- nest pegs;
- a non-toxic, waterproof marker;
- a 1 litre plastic beaker;
- numbered egg boxes (*e.g.* for oystercatcher: 100 ml, polypropylene polyethylene, ø 55*73 mm, and for common tern: 50 ml, polystyrol/ polyethylene, ø 41*49 mm).

3. Transportation

3.1 Fish and shellfish

Samples should be kept cool and frozen at <-20°C as soon as possible after collection. Length and weight should be determined before freezing. Live mussels should be transported in closed containers at temperatures between 5-15°C, preferably <10°C. Frozen samples should be transported in closed containers at temperatures <-20°C. More rigorous conditions will be necessary for samples for biological effects monitoring, *e.g.* storage in liquid nitrogen.

3.2 Seabird eggs

Eggs should be kept cool and frozen at -18°C as soon as possible after collection.

4. Pre-treatment and storage

4.1 Contamination

Sample contamination may occur during sampling, sample handling, pre-treatment and analysis (Oehlenschläger, 1994), due to the environment, the containers or packing material used, the instruments used during sample preparation or from the chemical reagents used during the analytical procedures. The risk of contamination is particularly high for dioxin-like PCBs which occur at ultra-trace levels in the environment. Controlled conditions are therefore required for all procedures, including the dissection of fish organs on board ship. One way of minimising the risk is to conduct dissection in a clean area, such as within a laminar-flow hood away from the deck areas of the vessel.

4.2 Fish

4.2.1 Dissection and storage

Ungutted fish should be wrapped separately in suitable material (*e.g.* pre-cleaned aluminium foil) and frozen. Plastic materials, except polyethylene or polytetrafluorethylene, must not be used (*cf.* Smedes and de Boer, 1994). The frozen samples should be stored in suitable containers to avoid damage. Sub-samples (*e.g.* of liver) should be stored in pre-cleaned containers made of glass (*e.g.* borosilicate glass), stainless steel or aluminium, or should be wrapped in pre-cleaned aluminium foil and frozen quickly in liquid nitrogen or a blast freezer. The individual samples should be clearly labelled and stored together in a suitable container placed in a deep freeze at <-20°C until analysis. Sub-samples for enzyme tests should be stored in vials suitable for storage in liquid nitrogen, labelled clearly and stored in liquid nitrogen until analysis.

When samples are processed at sea the dissection must be done by trained personnel preferably on a clean bench wearing clean gloves and using clean stainless steel knives. Stainless steel tweezers are recommended for holding tissues during dissec-

tion. After each sample has been prepared, the tools should be cleaned. Washing in acetone or alcohol and high purity water is the procedure recommended.

4.2.2 Sub-sampling

To sample fish muscle, care should be taken to avoid including any epidermis or subcutaneous fatty tissue in the sample. Samples should be taken underneath the red muscle layer. In order to ensure uniformity, the right side dorso-lateral muscle should be sampled. If possible, the entire right dorsal lateral filet should be homogenised and sub-samples taken for replicate dry weight and contaminant determinations. If however the amount of material to be homogenised would be too large, a specific portion of the dorsal musculature should be chosen. It is recommended that the portion of the muscle lying directly under the first dorsal fin be used in this case. As both fat and water content vary significantly in the muscle tissue from the anterior to the caudal muscle of the fish, in order to ensure comparability it is important to obtain the same portion of the muscle tissue for each sample (see Oehlenschläger, 1994).

When dissecting the liver, care should be taken to avoid contamination from other organs. The whole liver should be homogenised or freeze-dried. If however the amount of material homogenised was too large, a specific portion of the liver should be chosen in order to ensure comparability.

Where pooling of tissues is necessary, an equivalent quantity of tissue must be taken from each fish, *e.g.* a whole fillet from every fish. If the total quantity of tissue so yielded would be too large to be handled conveniently, the tissue may be subsampled, but a fixed proportion of each tissue must then be taken, *e.g.* 10% of each whole fillet or 10% of each whole liver or for muscle tissue 10% of the fish.

Personnel must be capable of identifying and removing the desired organs according to the requirements of the investigations.

4.3 Shellfish

4.3.1 Depuration

Mussels should be placed on a polyethylene tray elevated above the bottom of a glass aquarium. The aquarium should be filled with filtered sub-surface sea water collected from the same site as the samples and which has not been subject to contamination from point sources if possible. The aquarium should be aerated and the mussels left for 20-24 hours at water temperatures and salinity close to those from which the samples were removed.

4.3.2 Opening of the shells

Mussels should be shucked live and opened with minimum tissue damage by detaching the adductor muscles from the interior of one valve. The mussels should be inverted and allowed to drain on a clean towel or funnel for at least 5 minutes in order to minimise influence on dry weight determinations.

4.3.3 Dissection and storage

The soft tissues should be removed and deep frozen (<-20°C) as soon as possible in containers appropriate to the intended analysis. The dissection of the soft tissue must be done under clean conditions on a clean bench by trained personnel, wearing clean gloves and using clean stainless steel knives. After each sample has been prepared,

the tools should be cleaned regularly. Washing in acetone or alcohol and high purity water is recommended. When the analysis is eventually undertaken, all fluids that may initially separate on thawing should be included with the materials homogenised. Homogenisation should be performed immediately prior to any sub-dividing of the sample.

4.4 Seabird eggs

Before thawing, each egg should be placed in a previously weighed goblet. The weight of the egg (to the nearest 0.1 g inclusive of shell), the length of the egg between poles and the breadth of the egg at the equator (to the nearest 0.1 mm using callipers) should be recorded. The egg should then be opened (if this has not already happened during thawing) and the content carefully separated from the shell. If the egg contains an embryo, the eye diameter or the "crown-tail" length of the embryo should be measured (to the nearest 0.1 mm using callipers). The content of the egg (*i.e.* the albumen and yolk) should be weighed (to the nearest 0.1 g) and homogenised in the same goblet for each egg (*e.g.* by an Ultra Turrax). Any sub-sampling should be done immediately after homogenisation. The samples can then be analysed or deep frozen for later analysis. The shell (including the shell-skin) should be washed with water and dried in laboratory air for at least a week before weighing (to the nearest 0.01 g). The shell thickness should be measured at three points along the egg equator.

Technical Annex 1a
Determination of chlorobiphenyls in biota – analytical method

1. Introduction

This annex provides advice on (chlorinated biphenyl) CB analysis for all biota samples. The guideline is an update of the an earlier version (OSPAR, 1999) taking into account evolutions in the field of analytical chemistry and also covering the determination of so-called planar or dioxin-like CBs (DL-CBs), i.e. mono-*ortho* (CB105, 114, 118, 123, 156, 157, 167 and 189) and non-*ortho* substituted CBs (CB81, 77, 126 and 169).

The analysis of CBs in biota generally involves extraction with organic solvents, clean-up (removal of lipids and fractionation), and gas chromatographic separation with electron capture or mass-spectrometric detection. All stages of the procedure are susceptible to insufficient recovery and/or contamination. Where possible, quality control procedures are required in order to check the method's performance. These guidelines are intended to encourage and assist analytical chemists to reconsider their methods and to improve their procedures and/or the associated quality control measures where necessary. Due to the low concentrations of, particularly, non-*ortho* substituted CBs in biota compared to those of other CBs, their determination requires an additional separation and concentration step. Therefore, in the relevant sections a distinction will be made between the non-*ortho* CBs and the others.

These guidelines can also be used for several other groups of organochlorine compounds, e.g. DDTs and their metabolites, chlorobenzenes and hexachlorocyclohexanes. Recoveries in the clean-up procedures must be checked carefully. In particular, treatment with H_2SO_4 results in a loss of some compounds (e.g. dieldrin and endosulfanes (de Boer and Wells, 1996)).

These guidelines are not intended as a complete laboratory manual. If necessary, further guidance should be sought from specialised laboratories. Whichever analytical procedure is adopted, the laboratory must demonstrate the validity of the procedure. Analyses must be carried out by trained staff.

2. Analysis

2.1. Precautionary measures

Solvents, chemicals and adsorption materials must be free of CBs or other interfering compounds. If not they should be purified using appropriate methods. Solvents should be checked by concentrating the volume normally used in the procedure to 10% of the final volume if practical and then analysing for the presence of CBs and other interfering compounds. If necessary, the solvents can be purified by redistillation but this practice is not favoured by most analytical laboratories as they generally opt to buy high quality solvents. Chemicals and adsorption materials should be purified by extraction and/or heating. Glass fibre materials (*e.g.* thimbles for Soxhlet extraction) should be pre-extracted. Alternatively, full glass thimbles with a G1 glass filter at the bottom can be used. Generally, paper filters should be avoided in filtration and substituted for by appropriate glass filters. As all pre-cleaned materials are prone to contamination (*e.g.* by the adsorption of CBs and other compounds from laboratory air), materials ready for use should not be stored for long periods. All containers, tools, glassware *etc.* which come into contact with the sample must be made

of appropriate material and must have been thoroughly pre-cleaned. Glassware should be extensively washed with detergents, heated at >250°C and rinsed immediately before use with organic solvents or mixtures such as hexane/acetone. In addition all glassware should preferably be covered with aluminium foil and stored in cupboards to keep out any dust. Old and scratched glassware is more likely to cause blank problems because of the larger surface and therefore greater chance of adsorption. Furthermore, scratched glassware is more difficult to clean.

2.2 Lipid determination

The determination of the lipid content of tissues can be of use in characterising the samples and reporting concentrations in biota on a wet weight or lipid weight basis. The total lipid content of fish or shellfish should be determined using the method of Bligh and Dyer (1959) as modified by Hanson and Olley (1963) or an equivalent method such as Smedes (1999). Extractable lipid may be used, particularly if the sample size is small and lipid content is high. It has been shown that if the lipid content is high (>5%) then this will be comparable to the total lipid. If extraction techniques are applied which destroy or remove lipid materials (e.g., PLE with fat retainers), the lipid content should be determined on a separate subsample of the tissue homogenate. Other relevant information concerning lipid determination are provided by QUASIMEME, 1994 and Roose *et al*., 1996.

2.3. Dry weight determination

Dry weight determinations should be carried out by drying homogenised subsamples of the material to be analysed to constant weight at 105°C.

2.4. Homogenisation and drying

Prior to analysis, the samples should be sufficiently homogenised. Homogenisation is commonly carried out on fresh tissue. Care should be taken that the sample integrity is maintained during the actual homogenisation. When the analysis is undertaken, all fluids that may initially separate on thawing should be included with the materials homogenised. Homogenisation should be performed prior to extraction and clean-up procedures. When homogenising samples after drying, classical techniques using a ball mill can be used. Cryogenic homogenisation of dried or fresh materials at liquid nitrogen temperatures using a PTFE device (*cf.* Iyengar and Kasperek, 1977) or similar techniques is also possible (*cf.* Iyengar, 1976; Klussmann *et al*., 1985).

CBs can be extracted from wet or dried samples, although storage, homogenisation and extraction are easier when the samples are dry. Drying the samples however may alter the concentrations e.g. by the loss of compounds through evaporation or by contamination. Potential losses and contamination should be checked as part of the method validation.

Chemical drying can be performed by grinding with e.g. Na_2SO_4 or $MgSO_4$ until the sample reaches a free-flowing consistency. It is essential that there are at least several hours between grinding and extraction to allow for complete dehydration of the sample; residual water will decrease extraction efficiency.

Freeze-drying is also a popular technique, although its application should be carefully considered. Possible losses or contamination must be checked. Losses through evaporation are diminished by keeping the temperature in the evaporation chamber below 0°C. Contamination during freeze-drying is reduced by putting a lid, with a hole of about 3 mm in diameter, on the sample container.

2.5. Extraction

Recovery standards should be added prior to extraction. When using a Soxhlet a combination of polar and apolar solvents is recommended. Alternatively, saponification may be used. This technique is highly effective, but conditions should be controlled as saponification could result in the decomposition of certain pesticides and under certain conditions of some CB congeners.

Although the use of binary non-polar/polar solvent mixtures and Soxhlet is still the benchmark for CB extraction, there have been numerous attempts to find alternative procedures, which are less time-consuming, use less solvent and/or enable miniaturisation. Amongst these novel approaches are pressurized liquid extraction (PLE) and related subcritical water extraction (SWE), microwave-assisted extraction (MAE), matrix solid-phase dispersion (MSPD), ultrasound extraction (US) and supercritical fluid extraction (SFE).

From among the techniques mentioned, PLE or Accelerated Solvent Extraction (ASE) has – so far – been most successful. Soxhlet methods are easily translated into PLE as the same solvent compositions can be used. The method further allows interesting modifications that include in-cell clean-up of samples by adding fat retainers, such as acid-impregnated silica, florisil or alumina, to the cell. New promising techniques have been described, e.g. the use of a small carbon column in the extraction cell, which selectively adsorbs dioxin-like compounds (subsequently isolated by back-flushing with toluene), but are not established for routine analysis (Sporring *et al.*, 2003). PLE and MAE have the shared advantage over SFE that they are matrix-independent, which facilitates method development and changing-over from the classical Soxhlet extraction. Recent years have also seen an increased use of ultrasound-based techniques for analytes isolation from solid samples. With most applications, extraction efficiency is fully satisfactory, and sonication time often is 30 min or less (Roose and Brinkman, 2005).

All the methods described above are in principle suitable for extracting CBs from biota. However, Soxhlet extraction is still the reference for alternative approaches.

2.6 Clean-up

The extraction procedures above will result in the co-extraction of lipids, which will need to be removed from the extract. Furthermore, tissue extracts will always contain many compounds other than CBs, and a suitable clean up is necessary to remove those compounds which may interfere with the subsequent analysis. Different techniques may be used, either singly or in combination, and the choice will be influenced by the selectivity and sensitivity of the final measurement technique and also by the extraction method employed. Most CBs are stable under acid conditions; therefore treatment with sulphuric acid or acid impregnated silica columns may be used in the clean-up.

The most commonly used clean-up methods involve the use of alumina or silica adsorption chromatography, but gel permeation chromatography (GPC) is also employed. Any water residues in the extract should be removed prior to clean-up, e.g. by Na_2SO_4.

As CBs are apolar, clean-up using normal-phase chromatography is the most appropriate technique for the separation from other compounds. Using an apolar solvent (*e.g.* hexane or isooctane) as an eluent, CBs normally elute very rapidly. All polar solvents used in the extraction should be removed before further clean-up. The last con-

centration step is usually performed by evaporation with a gentle stream of nitrogen. Evaporation to dryness should always be avoided, but for the analysis of DL-CBs, very small final volumes might be necessary to achieve detectable concentrations.

Deactivated Al_2O_3 (5-10% water) is often used as a primary clean-up. Al_2O_3 sometimes gives a sufficiently clean extract for a GC-ECD analysis of the sample. Al_2O_3 removes lipid compounds from the extracts (although samples with a very high lipid content and low CB concentrations may require additional clean-up).

Deactivated silica (1-5% water) does not retain CBs (including non-*ortho* CBs) and only slightly retains polycyclic aromatic hydrocarbons (PAHs) when eluted with hexane or isooctane. When organochlorine pesticides are also to be determined in the same extract, deactivation of the silica with a few percent of water is necessary.

For high activity silica (overnight at 180°C) the retention of CBs is negligible, while PAHs are more strongly retained. The CBs and a few other organochlorine compounds are eluted with apolar solvents. More polar solvents (*e.g.* hexane/acetone) should be avoided as some interfering organochlorine pesticides would be eluted.

For the separation of CBs from lipids or oil components, reversed-phase HPLC can be used. In reversed-phase chromatography CBs elute during a solvent gradient of 80 to 90% methanol together with numerous other compounds of the same polarity. Most of the above mentioned extraction methods and clean-up procedures yield an extract containing an apolar solvent. These cannot be injected directly for reversed-phase chromatography, and so compounds must be transferred between solvents several times *e.g.* before injection and after elution. When using polar solvents for extraction reversed-phase columns could be used directly for clean-up. When eluting an acetonitrile extract from a C_{18} solid phase extraction (SPE) column with acetonitrile, high molecular hydrocarbons are strongly retained while CBs elute in the first few column volumes.

The above mentioned normal-phase chromatographic procedures on silica and Al_2O_3 can be transferred to HPLC having the advantages of higher resolution and better reproducibility.

When using GPC the elution of CBs should be carefully checked. Two serial columns are often used for improved lipid separation. Solvent mixtures such as dichloromethane/hexane or cyclohexane/ethyl acetate can be used as eluents for GPC. However, a second clean-up step is often required to separate the CBs from other organohalogenated compounds and/or remove residual lipids.

One advantage of using PLE extraction is that it is possible to combine the clean up with the extraction, especially where mass spectrometry will be used as the detection method. If Soxhlet extraction is used for biota, then there is a much greater quantity of residual lipid to be removed than in the case of PLE with fat retainers. An additional clean-up stage may therefore be necessary. Methods have been developed for online clean-up and fractionation of dioxins, furans and CBs with PLE for food, feed and environmental samples (Sporring *et al*., 2003), utilising a fat retainer for the online clean-up of fat. Silica impregnated with sulphuric acid, alumina and florisil have all been used as fat retainers. A non-polar extraction solvent such as hexane should be used if fat retainers are used during PLE.

Non-*ortho* CBs require a more specialised clean-up that is generally associated with the analysis of dioxins. Although initial clean-up may very well proceed along the lines described above, the larger sample intake results in even more co-extracts and care has to be taken that the capacity of the adsorption columns is not exceeded

and/or that lipids are adequately removed. Often, more rigorous procedures are applied to remove the excess material by e.g. shaking the sample with concentrated sulphuric acid. A more efficient alternative is to elude the sample over a silica column impregnated with sulphuric acid (40 % w/w).

Non-*ortho* CBs are nearly always separated from the other CBs with advanced separation techniques. A very efficient method is to inject the extracts (after concentrating them) into a HPLC system coupled to PYE (2-(1-pyrenyl) ethyldimethylsilylated silica) column. Column dimensions are typically 4.6 x 150 mm column, but combinations of several columns are sometimes used. PYE columns not only allow the separation of *ortho*, mono-*ortho* and non-*ortho* CBs on the basis of structural polarity from each other but also from dibenzodioxins and –furans. The eluting solvent is an apolar solvent such as *iso*-hexane. Coupled to a fraction collector, the use of a HPLC system allows the automatic clean-up of a considerable number of samples. Alternatively, HPLC systems equipped with porous graphitised carbon can be used. Column sizes are in the order of 50 x 4.7 mm and care has to be taken that the column is not overloaded. Similarly to PYE columns, they will separate non-ortho CBs from the other CBs and from dioxins. Fully automated systems, such as Powerprep™, that combine several steps are routinely used

2.7 Pre-concentration

Evaporation of solvents with a rotary-film evaporator was up until recently the common method. However, evaporation of solvents using this technique should be performed at low temperature (water bath temperature of ≤ 40°C) and under controlled pressure conditions, in order to prevent losses of the more volatile CBs. To reduce the sample to the final volume, solvents can be removed by blowing-down with a gentle stream of nitrogen. Only nitrogen of a controlled high quality should be used.

Turbovap sample concentrators can also be used to reduce solvent volume. This is a rapid technique, but needs to be carefully optimised and monitored to prevent both losses (both of volatiles and solvent aerosols) and cross-contamination. The use of rotary-film evaporators is more time consuming but more controllable. Here also, evaporation to dryness should be avoided at all costs. Syncore™ parallel evaporators (Buchi, Switzerland) can be used with careful optimisation of the evaporation parameters. The Buchi Syncore™ Analyst also uses glass tubes but the system is sealed, avoiding contamination from the laboratory air during evaporation. It does not use a nitrogen stream, thus reducing the loss of volatiles and if the flushback module is fitted the sides of the tubes are rinsed automatically thus reducing the loss of the heavier components. Again water-bath temperatures should be minimised to prevent losses. When reducing the sample to the required final volume, solvents can be removed by a stream of clean nitrogen gas. Suitable solvents for injection into the gas chromatograph (GC) include hexane, heptane, toluene and *iso*-octane.

2.8 Calibration and preparation of calibrant solutions

Internal standards (recovery and quantification standards) should be added in a fixed volume or weight to all standards and samples. The ideal internal standard contains no CBs or negligible (non-detectable) concentrations and does not coelute with other CBs. All CBs with a 2,4,6-substitution (e.g. CB112, CB155, CB 198) are, in principle, suitable. Alternatively, 1,2,3,4-tetrachloronaphthalene or homologues of dichloroalkylbenzylether can be used. For GC analysis with mass selective detection (GC-MS), ^{13}C labelled CBs should be used for each degree of chlorination. This is especially

critical for the non-*ortho* CBs. If possible, the labelled calibrant solutions should correspond to the unlabelled determinants. For the non-*ortho* CBs a labelled standard is available for each congener and use of all of them is recommended. When preparing a calibration solution for a new determinant for the first time, two independent stock solutions of different concentrations should always be prepared simultaneously to allow cross checking. A new calibration solution should also be cross-checked to the old standard solution. Crystalline CBs of known purity can be used for preparing calibration solutions, but for health and safety reasons, the purchase of solutions is recommended for dioxin-like CBs. In recent years, a lot of certified commercial custom made standards have become available and laboratories have been switching to these. If the quality of the standard materials is not guaranteed by the producer or supplier, it should be checked by GC preferably with mass spectrometric detection. Solid standards should be weighed to a precision of 10^{-5} grams. Calibration solutions should preferably be stored in ampoules in a cool and dark place. Commercially available screw-cap vials with a capillary opening (Certan™) combine of advantages of ampoules and vials, and, have proven to be reliable. When stored in containers the weight loss during storage should be recorded.

2.9. Instrumental determination

2.9.1 Injection techniques

The two modes commonly used are splitless and on-column injection. In split injection, strong discrimination effects may occur. The liner should possess sufficient capacity with respect to the injected volume after evaporation, but should not be oversized to avoid poor transfer to the column and losses by adsorption. Liners with light packing of (silylated) glass wool may improve the performance for CBs, but may degrade some organochlorine compounds like DDT, which are often included in national monitoring programmes.

Recently, other techniques such as temperature-programmed or pressure-programmed injection have become more prominent. They offer additional advantages such as an increased injection volume without the negative effects previously associated with that, but should be thoroughly optimised before use. Increasing the injection volume will allow either or both the elimination of an extra evaporation step and lowering the detection limits.

2.9.2 Carrier gas

Hydrogen is the preferred carrier gas and is indispensable for columns with very small inner diameters. Helium is also acceptable and the standard carrier for GC-MS.

2.9.3 Columns

Only capillary columns should be used. The following parameters are recommended:

Minimum Length	50 m (for microcolumns of internal diameter <0,1 mm, shorter columns can be suitable).
Maximum internal diameter	0.25 mm. Note that for diameters <0.15 mm the elevated pressure of the carrier gas needs special instrumental equipment as most of the instruments are limited to 400 kPa.
Film thickness	0.2-0.4 μm.

Columns which do not fulfil these requirements generally do not offer sufficient resolution to separate CB28, CB105 and CB156 from closely eluting CBs. A wide range of stationary phases can be used for CB separation. The chemical composition is differ-

ent for many producers and depends on the maximum temperature at which the column can be operated. Further advice may be found in the producer's catalogues, where compositions, applications and tables to compare products from different manufacturers are included.

In recent years, new chromatographic phases have become available that result in an improved separation of critical CB pairs. A good example is the HT-8 phase (1,7-dicarba-closo-dodecarborane phenylmethyl siloxane) (Larsen *et al*., 1995) that shows a remarkable selectivity for CBs (Table 1). This column is currently recommended for CB analysis.

2.9.4 Detection

The electron capture detector (ECD) is still frequently used for CB analysis. Injection of chlorinated solvents or oxygen-containing solvents should therefore be avoided. When using mass selective detectors (MSD) negative chemical ionisation mode (NCI) is extremely sensitive for pentachlorinated to decachlorinated CBs and is approximately ten fold better than ECD. However, MS systems have improved considerable allowing analysis by electron impact ionisation (EI), whereas before, negative chemical ionisation (NCI) was often necessary in order to detect the low concentrations of, in particular the non-*ortho* CBs. Suggested target and qualifier ions for *ortho* CBs (including non-*ortho* CBs) are shown in Table 1 and in Table 2 for mono-*ortho* CBs.

Next to conventional GC-MS, the use of ion-trap with its MS^2 option – i.e., increased selectivity – is receiving increased attention. GC-ITMS is a less expensive alternative to high-resolution mass spectrometry (HRMS), which is commonly used to determine PCDD/Fs and as such also ideally suited for all CB groups.

Table 1: Example of retention times for selected CB congeners using a 50 m HT8 column (0.25 mm i.d. and 0.25 μm film), along with possible target and qualifier ions. Temperature programme: 80°C, hold for 1 minute, ramp 20°C/minute, to 170 °C, hold 7.5 minutes, ramp 3 °C/minute to 300 °C, hold for 10 minutes.

CB congener	MW	RT	Target Ion	Qualifier Ion	Number of chlorines
13C-CB28	270	28.371	268	270	3
CB31	258	28.071	256	258	3
CB28	258	28.388	256	258	3
13C-CB52	304	30.317	304	302	4
CB52	292	30.336	292	290	4
CB49	292	30.698	292	290	4
CB44	292	32.024	292	290	4
CB74	292	34.881	292	290	4
CB70	292	35.199	292	290	4
13C-CB101	340	36.612	338	340	5
CB101	326	36.630	326	328	5
CB99	326	37.062	326	328	5
CB97	326	38.267	326	328	5
CB110	326	39.277	326	328	5
CB123*	326	41.2	326	328	5
CB118*	326	41.563	326	328	5
CB105*	326	43.443	326	328	5
CB114*	326	42.2	326	328	5

13C-CB153	374	42.567	372	374	6
CB149	362	40.328	360	362	6
CB153	362	42.584	360	362	6
CB132	362	42.236	360	362	6
CB137	362	43.744	360	362	6
13C-CB138	374	44.437	372	374	6
CB138	362	44.487	360	362	6
CB158	362	44.663	360	362	6
CB128	362	46.307	360	362	6
13C-CB156	374	48.406	372	374	6
CB156*	362	48.366	360	362	6
CB167*	362	46.2	360	362	6
CB157*	362	48.698	360	362	6
13C-CB180	408	48.829	406	408	7
CB187	396	44.787	394	396	7
CB183	396	45.264	394	396	7
CB180	396	48.846	394	396	7
CB170	396	50.684	394	396	7
13C-CB189	406	53.182	406	408	7
CB189*	396	53.196	394	396	7
13C - CB194	442	57.504	442	440	8
CB198	430	50.347	430	428	8
CB194	430	57.514	430	428	8
*mono-ortho CBs					

Table 2 Possible target and qualifier ions for non-ortho CBs, including labelled internal standards

CB	Target ion (m/z)	Qualifier (m/z)	Qualifier (m/z)	Qualifier (m/z)
13CB81	304	302	NA	NA
CB81	292	290	220	222
13CB77	304	302	NA	NA
CB77	292	290	220	222
13CB126	338	340	NA	NA
CB126	326	328	254	256
13CB169	372	374	NA	NA
CB169	360	362	218	220

2.9.5 Separation, identification and quantification

When using GC-ECD and to a certain extent GC-MS, two columns with stationary phases of different polarity should be used, as column-specific coelution of the target CBs with other CBs or organochlorine compounds occurs. The temperature programme must be optimised for each column to achieve sufficient separation of the CB congeners to be determined. An isothermal period in the programme around 200-220°C of approximately 30 minutes is recommended. Care should be taken that CBs of interest do not coelute with other CB congeners (for example CB28 and CB31).

When using GC-ECD, compounds are identified by their retention time in relation to the standard solutions under the same conditions. Therefore GC conditions should be constant. Shifts in retention times should be checked for different parts of the spectrum with the help of characteristic, unmistakable peaks (*e.g.* originating from the internal standard or higher concentrated CBs such as CB153 and CB138. Using a GC/MS system, the molecular mass or characteristic mass fragments or the ratio of two ion masses can be used to confirm the identity of separated CBs. Since calibration curves of most CBs normally non-linear using a GC-ECD, but should be linear for GC-MS, a multilevel calibration of at least five concentrations is recommended. The calibration curve must be controlled and the best fit must be applied for the relevant concentration range. Otherwise, one should strive to work in the linear range of the detector. Analysis of the calibration solutions should be carried out in a mode encompassing the concentrations of the sample solutions (or alternatively by injecting matrix-containing sample solutions and matrix-free standard solutions distributed regularly over the series). When the chromatogram is processed with the help of automated integrators the baseline is not always set unambiguously and always needs to be inspected visually. When using GC-ECD, peak height is preferable to peak area for quantification purposes. From the two columns of different polarity the more reliable result should be reported.

Recent years have witnessed the emergence of so-called comprehensive two-dimensional gas chromatography (GCxGC) – a technique that can be used to considerably improve analyte/matrix as well as analyte/analyte separation. Briefly, a non-polar x (semi-)polar column combination is used, with a conventional 25–30 m long first-dimension, and a short, 0.5–1 m long, second-dimension column. The columns are connected via an interface called a modulator. The latter device serves to trap, and focus, each subsequent small effluent fraction from the first-dimension column and, then, to launch it into the second column. The main advantages of the comprehensive approach are that the entire sample (and not one or a few heart-cuts, as in conventional multidimensional GC is subjected to a completely different separation, that the two-dimensional separation does not take any more time than the first-dimension run, and that the re-focusing in the modulator helps to increase analyte detectability. The most interesting additional benefit for CBs is, that structurally related as CB congeners show up as so-called ordered structures in the two-dimensional GCxGC plane. The very rapid second-dimension separation requires the use of detectors with sufficiently high data acquisition rates. Initially, only flame ionisation detectors could meet this requirement. However, today there is also a micro-ECD on the market that is widely used for GCxGC-μECD of halogenated compound classes. Even more importantly, analyte identification can be performed by using a time-of-flight mass spectrometer or – with a modest loss of performance, but at a much lower price – one of the very recently introduced rapid-scanning quadrupole mass spectrometers. So far, the use of GCxGC has been limited to qualitative purposes and still seems inappropriate for routine quantification.

3. Quality assurance

Planners of monitoring programmes must decide on the accuracy, precision, repeatability, and limits of detection and determination which they consider acceptable. References of relevance to QA procedures include HELCOM, 1988; QUASIMEME 1992; Wells *et al.*, 1992; Oehlenschläger, 1994; Smedes *et al.*, 1994 and ICES, 1996.

3.1 System performance

The performance of the GC system should be monitored by regularly checking the resolution of two closely eluting CBs. A decrease in resolution points to deteriorating GC conditions. The signal-to-noise ratio yields information on the condition of the detector. A dirty ECD-detector or MS-source can be recognised by the presence of an elevated background signal together with a reduced signal-to-noise ratio. Chromatograms should be inspected visually by a trained operator.

3.2 Recovery

The recovery should be checked and reported. One method is to add an internal (recovery) standard to each sample immediately before extraction and a second (quantification) standard immediately prior to injection. If smaller losses occur in extraction or clean-up or solutions are concentrated by uncontrolled evaporation of solvents (*e.g.* because vials are not perfectly capped) losses can be compensated for by normalisation. If major losses are recognised and the reasons are unknown, the results should not be reported, as recoveries are likely to be irreproducible. A control for the recovery standard is recommended by adding the calibration solution to a real sample. Recoveries should be between 70 and 120%, if not, samples should be repeated.

3.3 Blanks

A procedural blank should be measured for each sample series and should be prepared simultaneously using the same chemicals and solvents as for the samples. Its purpose is to indicate sample contamination by interfering compounds, which will lead to errors in quantification. Even if an internal standard has been added to the blank at the beginning of the procedure, a quantification of peaks in the blank and subtraction from the values obtained for the determinands must not be performed, as the added internal standard cannot be absorbed by a matrix. An alternative may be using a CB-free oil as a matrix blank.

3.4 Accuracy and precision

A Laboratory Reference Material (LRM) should be included, at least one sample for each series of identically prepared samples. The LRM must be homogeneous, well characterised for the determinands in question and stability tests must have shown that it produces consistent results over time. The LRM should be of the same type of matrix (*e.g.* liver, muscle tissue, fat or lean fish) as the samples, and the determinant concentrations should occur in a comparable range to those of the samples. If the range of determinant concentrations in the sample is large (> factor of 5) it's preferable to include two reference materials in each batch of analyses to cover the lower and upper concentrations. It is good practice to run duplicate analyses of a reference material to check within-batch analytical variability. A quality control chart should be recorded for a selected set of CBs. When introducing a new LRM or when it is suspected from the control chart that there is a systematic error possibly due to an alteration of the material, a relevant Certified Reference Material (CRM) of a similar matrix to the material analysed should be used to check the LRM. Additionally a duplicate of at least one sample should be run with every batch of samples. Each laboratory should participate in interlaboratory comparison studies and proficiency testing schemes on a regular basis, preferably at an international level.

3.5 Data collection and reporting

The calculation of results and the reporting of data can represent major sources of error. Control procedures should be established in order to ensure that data are correct and to obviate transcription errors. Data stored on databases should be checked and validated, and checks are also necessary when data are transferred between databases. If possible data should be reported in accordance with the latest ICES reporting formats.

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Acknowledgements: This technical annex was prepared by the ICES Marine Chemistry Working Group with contributions from Lynda Webster, Patrick Roose, Katrin Vorkamp, Philippe Bersuder, Jacob de Boer, Peter Lepom, Franscesco Pellizato (WGMS).

Annex 8: Revised technical Annex on analysis of PCBs in sediments

Determination of chlorobiphenyls in sediments - analytical method

1. Introduction

This annex provides advice on (chlorinated biphenyl) CB analysis for all sediment fractions and suspended particulate matter (*e.g.* <2mm fraction and <20µm fraction). The guideline is an update of an earlier version (Smedes and de Boer, 1994 and 1997) taking into account evolutions in the field of analytical chemistry and also covering the determination planar or dioxin-like CBs (DL-CBs). Basically, these consist of mono-*ortho* (CB105, 114, 118, 123, 156, 157, 167 and 189) and non-*ortho* substituted CBs (CB81, 77, 126 and 169).

The analysis of CBs in sediments generally involves extraction with organic solvents, clean-up (removal of sulphur and column fractionation), and gas chromatographic separation with electron capture or mass-spectrometric detection. All stages of the procedure are susceptible to insufficient recovery and/or contamination. Where possible, quality control procedures are recommended in order to check the method's performance. These guidelines are intended to encourage and assist analytical chemists to reconsider their methods and to improve their procedures and/or the associated quality control measures where necessary. Due to the low concentrations of, particularly, non-*ortho* substituted CBs in sediments compared to those of other CBs, their determination requires an additional separation and concentration step. Therefore, in the relevant sections a distinction will be made between the non-ortho substituted CBs and the others.

These guidelines can also be used for several other groups of organochlorine compounds, *e.g.* DDTs and their metabolites, chlorobenzenes and hexachlorocyclohexanes. Recoveries in the clean-up procedures must be checked carefully. In particular, treatment with H_2SO_4 results in a loss of some compounds (*e.g.* dieldrin and endosulfanes (de Boer and Wells, 1996). Also, the clean-up procedure with silver ions can result in low recoveries for some pesticides (*e.g.* hexachlorocyclohexanes).

These guidelines are not intended as a complete laboratory manual. If necessary, guidance should be sought from highly specialised research laboratories. Whichever analytical procedure is adopted, each laboratory must demonstrate the validity of each step in the procedure. In addition, the use of a second (and different) method, carried out concurrently with the routine procedure is recommended for validation. Analyses must be carried out by experienced staff.

2. Sampling and storage

Plastic materials (except polyethylene or polytetrafluorethene) must not be used for sampling due to the possible adsorption of contaminants onto the container material. Samples should be stored in solvent washed aluminium cans or glass jars. Aluminium cans are better as glass jars are more susceptible to breakage. Samples should be transported in closed containers; a temperature of 25°C should not be exceeded. If samples are not analysed within 48 h after sampling, they must be stored in the short term at 4°C. Storage over several months should be limited to frozen (<–20°C) and dried samples.

3. Precautionary measures

Solvents, chemicals and adsorption materials must be free of CBs or other interfering compounds. If not they should be purified using appropriate methods. Solvents should be checked by concentrating the volume normally used in the procedure to 10% of the final volume and then analysing for the presence of CBs and other interfering compounds using a GC. If necessary, the solvents can be purified by re-distillation but this practice is not favoured by most analytical laboratories as they generally opt to buy high quality solvents. Chemicals and adsorption materials should be purified by extraction and/or heating. Glass fibre materials (e.g. Soxhlet thimbles and filter papers used in pressurised liquid extraction (PLE)) should be cleaned by solvent extraction or pre-baked at 450°C overnight. Alternatively, full glass thimbles with a G1 glass filter at the bottom can be used. Generally, paper filters should be avoided in filtration and substituted for by appropriate glass filters. As all super cleaned materials are prone to contamination (*e.g.* by the adsorption of CBs and other compounds from laboratory air), materials ready for use should not be stored for long periods. All containers, skills, glassware *etc.*, which come into contact with the sample must be made of appropriate material and must have been thoroughly pre-cleaned. Glassware should be extensively washed with detergents, heated at >250°C and rinsed immediately before use with organic solvents or mixtures such as hexane/acetone. In addition all glassware should preferably be covered with aluminium foil and stored in cupboards to keep out any dust. Old and scratched glassware is more likely to cause blank problems because of the larger surface and therefore greater chance of adsorption. Furthermore, scratched glassware is more difficult to clean. All glassware should be stored in clean cupboards, ensuring dust cannot enter (QUASIMEME, 2007)

4. Pre-treatment

Before taking a subsample for analysis, the samples should be sufficiently homogenised.

CBs can be extracted from wet or dried samples, although storage, homogenisation and extraction are much easier when the samples are dry. Drying the samples however may alter the concentrations *e.g.* by the loss of compounds through evaporation or by contamination (Smedes and de Boer, 1994 and 1997). Losses and contamination must be accounted for.

Chemical drying can be performed by grinding with Na_2SO_4 or $MgSO_4$ until the sample reaches a free-flowing consistency. It is essential that there are at least several hours between grinding and extraction to allow for complete dehydration of the sample; residual water will decrease extraction efficiency.

Freeze-drying is becoming a more popular technique, although its application should be carefully considered. Possible losses or contamination must be checked. Losses through evaporation are diminished by keeping the temperature in the evaporation chamber below 0°C. Contamination during freeze-drying is reduced by putting a lid, with a hole of about 3 mm in diameter, on the sample container.

5. Extraction

The target compounds must be extracted from the sediment with an organic solvent prior to analysis. Extraction methods do not differ for DL-CBs but, because of the low

concentrations, a substantially larger sample intake has to be considered. Generally, at least a 100 g sample of freeze-dried sediments is required.

5.1 Wet sediments

Wet sediments are extracted in a step-wise procedure by mixing them with organic solvents. Extraction is enhanced by shaking, Ultra Turrax mixing, ball mill tumbler or ultrasonic treatment. Water miscible solvents are used (especially in the first step) such as methanol, acetone, acetonitrile, *etc.* The extraction efficiency of the first step is low as there will be a considerable amount of water in the liquid phase. The extraction is continued with a mixture of polar and apolar solvents (*e.g.* acetone/hexane or methanol/dichloromethane). For a sufficient extraction of target compounds, wet sediments must be extracted with organic solvents at least three times. The contact time with the solvent should be sufficient to complete the desorption of the CBs from the sediment.

When using a Soxhlet, extraction of wet sediments should be done in two steps. A polar solvent, such as acetone, is first used to extract the water from the sediment and then the flask is replaced and the extraction continued with a polar/apolar mixture such as acetone/hexane.

In both cases water must be added to the combined extracts and the CBs must be extracted to an apolar solvent such as hexane.

5.2 Dry sediments

For dried sediments Soxhlet extraction is the most frequently used technique. A mixture of a polar and an apolar solvent (*e.g.* acetone/hexane) is recommended for efficient extraction; a good choice is 25% acetone in hexane. A greater proportion of polar solvent increases the extraction efficiency, but the polar solvent must be removed prior to gas chromatographic analysis. Extraction can be carried out with a normal Soxhlet or a hot Soxhlet. A sufficient number of extraction cycles must be performed (~ 8 h for the hot Soxhlet and 12 to 24 h for normal Soxhlet). The extraction efficiency must be checked for different types of sediments by a second extraction step. These extracts should be analysed separately.

Although the use of binary non-polar/polar solvent mixtures and Soxhlet is still the benchmark for CB extraction, there have been numerous attempts to find alternative procedures, which are less time-consuming, use less solvent and/or enable miniaturisation. Amongst these novel approaches are pressurized liquid extraction (PLE) and related subcritical water extraction (SWE), microwave-assisted extraction (MAE), matrix solid-phase dispersion (MSPD), ultrasound extraction (US) and supercritical fluid extraction (SFE).

From among the techniques mentioned, PLE or Accelerated Solvent Extraction (ASE) has – so far – been most successful. Soxhlet methods are easily translated into PLE as the same solvent compositions can be used. The method further allows interesting modifications that include in-cell clean-up of samples by adding fat retainers, such as florisil or alumina, to the cell, and the use of a small carbon column in the extraction cell, which selectively adsorbs dioxin-like compounds (subsequently isolated by back-flushing with toluene) (Sporring *et al*., 2003). PLE and MAE have the shared advantage over SFE that they are matrix-independent, which facilitates method development and changing-over from the classical Soxhlet extraction. Recent years have also seen an increased use of ultrasound-based techniques for analytes isolation from

solid samples. With most applications, extraction efficiency is fully satisfactory, and sonication time often is 30 min or less (Roose and Brinkman, 2005).

All the methods described above are in principle suitable for extracting CBs from sediments. However, Soxhlet extraction is still the reference for alternative approaches.

6. Clean-up

6.1 Removal of sulphur and sulphur-containing compounds

An aqueous saturated Na_2SO_3 solution is added to a hexane extract. In order to allow the transfer of the HSO_3^- ions to the organic phase, tetrabutylammonium salts (TBA) and isopropanol are then added to the mixture. Water is subsequently added to remove the isopropanol. The aqueous phase must then be quantitatively extracted with hexane (Jensen *et al*., 1977). If the extraction was performed by a polar solvent miscible with water, then a Na_2SO_3 solution can be added directly after extraction. If the extraction mixture also contains an apolar solvent, then depending on the ratio of the solvents, the addition of TBA and isopropanol may or may not be necessary. Any excess Na_2SO_3 and reaction products can be removed by the addition of water and thus partitioning between apolar solvent and water.

Japenga *et al*. (1987) developed a column method for the removal of sulphur and sulphur-containing compounds. The column material is made by mixing an aqueous solution of Na_2SO_3 with Al_2O_3. Some NaOH is also added to improve the reaction with sulphur. Subsequently the material is dried under nitrogen until a level of deactivation equivalent to 10 % water is reached. Storage must be under nitrogen because sulphite in this form may easily be oxidised to sulphate. Eluting the extract (hexane) through a column filled with this material results in removal of the sulphur in combination with further clean-up of the sediment extract. The sulphur removal properties are somewhat difficult to control.

Mercury, activated copper powder, wire or gauze (Smedes and de Boer, 1994 and 1997; Wade and Cantillo, 1996) remove the sulphur directly from an organic solvent. Although mercury is appropriate for removing sulphur, it should be avoided for environmental reasons. Copper can be applied during or after Soxhlet extraction. Ultrasonic treatment might improve the removal of sulphur. If sulphur appears to be present in the final extract the amount of copper or mercury used was insufficient and the clean-up procedure must be repeated.

Silver ions strongly bind sulphur and sulphur compounds. Loaded onto silica, $AgNO_3$ is a very efficient sulphur removing agent. It can be prepared by mixing dissolved $AgNO_3$ with silica and subsequently drying under nitrogen. Compounds containing aromatic rings are strongly retained, but for CBs this retention is reduced, probably due to shielding of the rings by the chlorine atoms. Retained compounds can easily be eluted by using cyclohexene, or another solvent with double bonds, as a modifier (Eganhouse, 1986; Japenga *et al*., 1987).

Elemental sulphur is strongly retained on a polystyrene-divinylbenzene copolymer column as generally applied for gel permeation chromatography (GPC). In addition GPC combines sulphur removal with a clean-up.

All these methods have advantages and disadvantages. For different samples the use of multiple methods may sometimes prove necessary. Several methods leave some aromatic sulphur compounds in the extract. These compounds elute from the GC column at the same retention time as the lower CBs. The major part of these com-

pounds can be removed by eluting an apolar extract over a column with silica loaded with concentrated H_2SO_4. Other disturbing compounds (*e.g.* phthalates and fatty acid esters) are removed as well by this procedure.

6.2 Further clean-up

The extraction procedures above will result in the co-extraction of many compounds other than CBs. The extract may be coloured due to chlorophyll-like compounds extracted from sediment, and may also contain sulphur and sulphur-containing compounds, oil, PAHs and many other natural and anthropogenic compounds which will need to be removed from the extract. Different clean-up techniques may be used, either singly or in combination, and the choice will be influenced by the selectivity and sensitivity of the final measurement technique and also by the extraction method employed. Most CBs are stable under acid conditions; therefore treatment with sulphuric acid or acid impregnated silica columns may be used in the clean-up.

The most commonly used clean-up methods involve the use of alumina or silica adsorption chromatography, but gel permeation chromatography (GPC) is also employed.

As CBs are apolar, clean-up using normal-phase chromatography is the most appropriate technique for the separation from other compounds. Using an apolar solvent (*e.g.* hexane or isooctane) as an eluent, CBs normally elute very rapidly. All polar solvents used in the extraction or sulphur removal step should be removed before further clean-up. The last concentration step is usually performed by evaporation with a gentle stream of nitrogen. Evaporation to dryness should always be avoided.

Deactivated Al_2O_3 (5–10% water) is often used as a primary clean-up. Provided that sulphur has been removed, Al_2O_3 sometimes gives a sufficiently clean extract for a GC-ECD analysis of the sample. Al_2O_3 removes lipid compounds from the extracts (although samples with a very high lipid content and low CB concentrations may require additional clean-up).

Deactivated silica (1–5% water) does not retain CBs (including DL-CBs) and only slightly retains polycyclic aromatic hydrocarbons (PAHs) when eluted with hexane or isooctane. When organochlorine pesticides are also to be determined in the same extract, deactivation of the silica with a few percent of water is necessary.

For high activity silica (overnight at 180°C) the retention of CBs is negligible, while PAHs are more strongly retained. The CBs and a few other organochlorine compounds are eluted with apolar solvents. More polar solvents (*e.g.* hexane/acetone) should be avoided as some interfering organochlorine pesticides would be eluted.

For the separation of CBs from lipids or oil components, reversed-phase HPLC can be used. In reversed-phase chromatography CBs elute during a solvent gradient of 80 to 90% methanol together with numerous other compounds of the same polarity. Most of the above mentioned extraction methods and clean-up procedures yield an extract containing an apolar solvent. These cannot be injected directly for reversed-phase chromatography, and so compounds must be transferred between solvents several times *e.g.* before injection and after elution. When using polar solvents for extraction (*e.g.* for wet sediments) reversed-phase columns could be used directly for clean-up. When eluting an acetonitrile extract from a C_{18} solid phase extraction (SPE) column with acetonitrile, high molecular hydrocarbons are strongly retained while CBs elute in the first few column volumes.

The above mentioned normal-phase chromatographic procedures on silica and Al_2O_3 can be transferred to HPLC having the advantages of higher resolution and better reproducibility.

When using GPC the elution of CBs should be carefully checked. When applying GPC, two serial columns are often used for improved lipid separation. Solvent mixtures such as dichloromethane/hexane or cyclohexane/ethyl acetate can be used as eluents for GPC. However, a second clean-up step is often required to separate the CBs from other orgnaohalogenated compounds.

One advantage of using PLE extraction is that it is possible to combine the clean up with the extraction, especially where mass spectrometry will be used as the detection method. If Soxhlet extraction is used for biota, then there is a much greater quantity of residual lipid to be removed than in the case of PLE with fat retainers. An additional clean-up stage may therefore be necessary. Methods have been developed for online clean-up and fractionation of dioxins, furans and CBs with PLE for food, feed and environmental samples (Sporring *et al*., 2003). The first method utilises a fat retainer for the on-line clean-up of fat. Silica impregnated with sulphuric acid, alumina and florisil have all been used as fat retainers. A non-polar extraction solvent such as hexane should be used if fat retainers are used during PLE.

Non-ortho CBs require a more specialised clean-up that is generally associated with the analysis of dioxins. Although initial clean-up may very well proceed along the lines described above, the larger sample intake results in even more co-extracts and care has to be taken that the capacity of the adsorption columns is not exceeded and/or that sulphur is adequately removed. Often, more rigorous procedures are applied to remove the excess material by e.g. shaking the sample with concentrated sulphuric acid. A more efficient and safer alternative is to elute the sample over a silica column impregnated with sulphuric acid (40 % w/w).

Non-ortho CBs are nearly always separated from the other CBs with advanced separation techniques. A very efficient method is to inject the extracts (after concentrating them) into a HPLC system coupled to PYE (2-(1-pyrenyl) ethyldimethylsilylated silica) column. Column dimensions are typically 4.6 x 150 mm column, but combinations of several columns are sometimes used. PYE columns not only allow the separation of *ortho,* mono-*ortho* and non-*ortho* CBs on the basis of structural polarity from each other but also from dibenzodioxins and –furans. The eluting solvent is an apolar solvent such as iso-hexane. Coupled to a fraction collector, the use of a HPLC system allows the automatic clean-up of a considerable number of samples. Alternatively, HPLC systems equipped with porous graphite carbon. Column sizes are in the order of 50x4.7 mm and care has to be taken that the column is not overloaded. Similarly to PYE columns, they will separate non-ortho CBs from the others and from dioxins. Fully automated systems, such as Powerprep, that combine several steps are routinely used (Focant and De Pauw, 2002).

7. Pre-concentration

Evaporation of solvents with a rotary-film evaporator was up until recent the common method. However, evaporation of solvents using this technique should be performed at low temperature (water bath temperature of ≤ 30°C) and under controlled pressure conditions, in order to prevent losses of the more volatile CBs. To reduce the sample to the final volume, solvents can be removed by blowing-down with gently streaming nitrogen. Only nitrogen of a controlled high quality should be used. As a solvent for the final solution to be injected into the GC, iso-octane is recommended.

Turbo-vap sample concentrators can also be used to reduce solvent volume. This is a rapid technique, but needs to be carefully optimised and monitored to prevent both losses (both of volatiles and solvent aerosols) and cross-contamination. The use of rotary-film evaporators is more time consuming but more controllable. Here also, evaporation to dryness should be avoided at all costs. Syncore parallel evaporators (Buchi, Switzerland) can be used with careful optimisation of the evaporation parameters. The Buchi Syncore Analyst also uses glass tubes but the system is sealed, avoiding contamination from the lab air during evaporation. It does not use a nitrogen stream, thus reducing the loss of volatiles and if the flushback module is fitted the sides of the tubes are rinsed automatically thus reducing the loss of the heavier components. Again water-bath temperatures should be minimised to prevent losses. When reducing the sample to the required final volume, solvents can be removed by a stream of clean nitrogen gas. Suitable solvents for injection into the gas chromatograph (GC) include hexane, heptane, toluene and iso-octane.

8. Calibration and preparation of calibrant solutions

Internal standards (recovery and quantification standards) should be added in a fixed volume or weight to all standards and samples. The ideal internal standard contains no CBs or negligible (non-detectable) concentrations and does not coelute with other CBs. All CBs with a 2,4,6-substitution (e.g. CB 115, CB155, CB 198) are, in principle, suitable. Alternatively, 1,2,3,4-tetrachloronaphthalene or homologues of dichloroalkylbenzylether can be used. For GC analysis with mass selective detection (GC-MS), ^{13}C labelled CBs should be used for each degree of chlorination. This is especially critical for the non-*ortho* CBs. If possible, the labelled calibrant solutions should correspond to the unlabelled determinants. For the non-*ortho* CBs a labelled standard is available for each congener and use of all of them is recommended. When preparing a calibration solution for a new determinant for the first time, two independent stock solutions of different concentrations should always be prepared simultaneously to allow cross checking. A new calibration solution should also be cross-checked to the old standard solution. Crystalline CBs of known purity should always be used for preparing calibration solutions. If the quality of the standard materials is not guaranteed (*e.g.* as in the case for a Certified Reference Material) by the producer or supplier, it should be checked by GC preferably with mass spectrometric detection. Solid standards should be weighed to a precision of 10^{-5} grams. In recent years, a lot of certified commercial custom made standards have become available and laboratories have been switching to these. Calibration solutions should preferably be stored in ampoules in a cool and dark place. When stored in containers the weight loss during storage should be recorded.

9. Instrumental determination

9.1 Injection techniques

The two modes commonly used are splitless and on-column injection. In split injection, strong discrimination effects may occur. The liner should possess sufficient capacity with respect to the injected volume after evaporation, but should not be oversized to avoid poor transfer to the column and losses by adsorption. Liners with light packing of (silylated) glass wool may improve the performance for CBs, but may degrade some organochlorine compounds like DDT, which are often included in national monitoring programmes.

Recently, other techniques such as temperature-programmed or pressure-programmed injection have become more prominent. They offer additional ad-

vantages such as an increased injection volume without the negative effects previously associated with that, but should be thoroughly optimised before use. Increasing the injection volume will allow either or both the elimination of an extra evaporation step and lowering the detection limits.

9.2 Carrier gas

Hydrogen is the preferred carrier gas and is indispensable for columns with very small inner diameters. Helium is also acceptable and the standard carrier for GC-MS.

9.3 Columns

Only capillary columns should be used. The following parameters are recommended:

Minimum Length	50 m (for microcolumns of internal diameter <0.1 mm, shorter columns can be suitable).
Maximum internal diameter	0.25 mm. Note that for diameters <0.15 mm the elevated pressure of the carrier gas needs special instrumental equipment as most of the instruments are limited to 400 kPa.
Film thickness	0.2-0.4 μm.

Columns which do not fulfil these requirements generally do not offer sufficient resolution to separate CB28, CB105 and CB156 from closely eluting CBs. A wide range of stationary phases can be used for CB separation. The chemical composition is different for many producers and depends on the maximum temperature at which the column can be operated. Further advice may be found in the producer's catalogues, where compositions, applications and tables to compare products from different manufacturers are included.

In recent years, new chromatographic phases have become available that result in an improved separation of critical CB pairs. A good example is the HT-8 phase (1,7-dicarba-closo-dodecarborane phenylmethyl siloxane) (Larsen *et al.*, 1995) that shows a remarkable selectivity for CBs. This column is currently recommended for CB analysis. An example of the retention times for various CBs are given in Table 1.

9.4 Detection

The electron capture detector (ECD) is still frequently used for CB analysis. Injection of chlorinated solvents or oxygen-containing solvents should therefore be avoided. When using mass selective detectors (MSD) negative chemical ionisation mode (NCI) is extremely sensitive for pentachlorinated to decachlorinated CBs and is approximately ten fold better than ECD. However, MS systems have improved considerable allowing analysis by Electron impact ionisation (EI), whereas before, negative chemical ionisation (NCI) was often necessary in order to detect the low concentrations of, in particular the DL-CBs. Suggested target and qualifier ions for *ortho* CBs (including non-*ortho* CBs) are shown in Table 1 and in Table 2 for non-*ortho* CBs..

Table 1: Example of retention times for selected CB congeners using a 50 m HT8 column (0.25 mm i.d. and 0.25 μm film), along with possible target and qualifier ions. Temperature programme

CB congener	MW	RT	Target Ion	Qualifier Ion	Number of chlorines
13C-CB28	270	28.371	268	270	3
CB31	258	28.071	256	258	3
CB28	258	28.388	256	258	3
13C-CB52	304	30.317	304	302	4

CB52	292	30.336	292	290	4
CB49	292	30.698	292	290	4
CB44	292	32.024	292	290	4
CB74	292	34.881	292	290	4
CB70	292	35.199	292	290	4
13C-CB101	340	36.612	338	340	5
CB101	326	36.630	326	328	5
CB99	326	37.062	326	328	5
CB97	326	38.267	326	328	5
CB110	326	39.277	326	328	5
CB123*	326	41.2	326	328	5
CB118*	326	41.563	326	328	5
CB105*	326	43.443	326	328	5
CB114*	326	42.2	326	328	5
13C-CB153	374	42.567	372	374	6
CB149	362	40.328	360	362	6
CB153	362	42.584	360	362	6
CB132	362	42.236	360	362	6
CB137	362	43.744	360	362	6
13C-CB138	374	44.437	372	374	6
CB138	362	44.487	360	362	6
CB158	362	44.663	360	362	6
CB128	362	46.307	360	362	6
13C-CB156	374	48.406	372	374	6
CB156*	362	48.366	360	362	6
CB167*	362	46.4**	360	362	6
CB157*	362	48.698	360	362	6
13C-CB180	408	48.829	406	408	7
CB187	396	44.787	394	396	7
CB183	396	45.264	394	396	7
CB180	396	48.846	394	396	7
CB170	396	50.684	394	396	7
13C-CB189	406	53.182	406	408	7
CB189*	396	53.196	394	396	7
13C - CB194	442	57.504	442	440	8
CB198	430	50.347	430	428	8
CB194	430	57.514	430	428	8
*mono-ortho CBs, ** to be checked					

Next to conventional GC-MS, the use of ion-trap with its MS^2 option – i.e., increased selectivity – is receiving increased attention. GC-ITMS is a less expensive alternative to high-resolution mass spectrometry (HRMS), which is commonly used to determine PCDD/Fs and as such also ideally suited for all CB groups (Eppe *et al*., 2004).

Table 2: Possible target and qualifier ions for non-ortho CBs, including labelled internal standards.

CB	Target ion (m/z)	Qualifier (m/z)	Qualifier (m/z)	Qualifier (m/z)
13CB81	304	302	NA	NA
CB81	292	290	220	222
13CB77	304	302	NA	NA
CB77	292	290	220	222
13CB126	338	340	NA	NA
CB126	326	328	254	256
13CB169	372	374	NA	NA
CB169	360	362	218	220

9.5 Separation, identification and quantification

When using GC-ECD and to a certain extent GC-MS, two columns with stationary phases of different polarity should be used, as column-specific coelution of the target CBs with other CBs or organochlorine compounds occurs. The temperature programme must be optimised for each column to achieve sufficient separation of the CB congeners to be determined. An isothermal period in the programme around 200-220°C of approximately 30 minutes is recommended. Care should be taken that CBs of interest do not coelute with other CB congeners (for example CB28 and CB31). When using GC-ECD, compounds are identified by their retention time in relation to the standard solutions under the same conditions. Therefore GC conditions should be constant. Shifts in retention times should be checked for different parts of the spectrum with the help of characteristic, unmistakable peaks (*e.g.* originating from the internal standard or higher concentrated CBs such as CB153 and CB138. Using a GC/MS system, the molecular mass or characteristic mass fragments or the ratio of two ion masses can be used to confirm the identity of separated CBs. Since calibration curves of most CBs normally non-linear using a GC-ECD, but should be linear for GC-MS, a multilevel calibration of at least five concentrations is recommended. The calibration curve must be controlled and the best fit must be applied for the relevant concentration range. Otherwise, one should strive to work in the linear range of the detector must. Analysis of the calibration solutions should be carried out in a mode encompassing the concentrations of the sample solutions (or alternatively by injecting matrix-containing sample solutions and matrix-free standard solutions distributed regularly over the series). When the chromatogram is processed with the help of automated integrators the baseline is not always set unambiguously and always needs to be inspected visually. When using GC-ECD, peak height is preferable to peak area for quantification purposes. From the two columns of different polarity the more reliable result should be reported.

Recent years have witnessed the emergence of so-called comprehensive two-dimensional gas chromatography (GCxGC) – a technique that can be used to considerably improve analyte/matrix as well as analyte/analyte separation. Briefly, a non-polar x (semi-)polar column combination is used, with a conventional 25-30 m long first-dimension, and a short, 0.5-1 m long, second-dimension column. The columns are connected via an interface called a modulator. The latter device serves to trap, and focus, each subsequent small effluent fraction from the first-dimension column and, then, to launch it into the second column. The main advantages of the comprehensive approach are that the entire sample (and not one or a few heart-cuts, as in

conventional multidimensional GC (Dallüge *et al*., 2003) is subjected to a completely different separation, that the two-dimensional separation does not take any more time than the first-dimension run, and that the re-focusing in the modulator helps to increase analyte detectability. The most interesting additional benefit for CBs is, that structurally related as CB congeners show up as so-called ordered structures in the two-dimensional GCxGC plane. The very rapid second-dimension separation requires the use of detectors with sufficiently high data acquisition rates. Initially, only flame ionisation detectors could meet this requirement. However, today there is also a micro-ECD on the market that is widely used for GCxGC-μECD of halogenated compound classes. Even more importantly, analyte identification can be performed by using a time-of-flight mass spectrometer [Dallüge *et al*., 2002] or – with a modest loss of performance, but at a much lower price – one of the very recently introduced rapid-scanning quadrupole mass spectrometers [Korytar *et al*., 2005; Adachour *et al*., 2005). So far, the use of GCxGC has been limited to qualitative purposes and still seems inappropriate for routine quantification.

10. Quality assurance

Planners of monitoring programmes must decide on the accuracy, precision, repeatability, and limits of detection and determination which they consider acceptable. The limit of determination should depend on the purpose of the investigation. A limit of at least 0.1 ng/g (dry weight, fraction <2mm) should be reached, but detection limits of 0.01 ng/g are achievable nowadays. The method for calculating the limit of determination should reflect QUASIMEME advice (Topping *et al*., 1992). The limit of determination that can be achieved depends on the blank, on the sample matrix, on concentrations of interfering compounds and on the mass of sediment taken for analysis. References of relevance to QA procedures include HELCOM, 1988; QUASIMEME 1992; Wells *et al*., 1992; Oehlenschläger, 1994; Smedes *et al*., 1994 and ICES, 1996.

10.1 System performance

The performance of the GC system should be monitored by regularly checking the resolution of two closely eluting CBs. A decrease in resolution points to deteriorating GC conditions. The signal-to-noise ratio yields information on the condition of the detector. A dirty ECD-detector or MS-source can be recognised by the presence of an elevated background signal together with a reduced signal-to-noise ratio. Chromatograms should be inspected visually by a trained operator.

10.2 Recovery

The recovery should be checked and reported. One method is to add an internal (recovery) standard to each sample immediately before extraction and a second (quantification) standard immediately prior to injection. If smaller losses occur in extraction or clean-up or solutions are concentrated by uncontrolled evaporation of solvents (*e.g.* because vials are not perfectly capped) losses can be compensated for by normalisation. If major losses are recognised and the reasons are unknown, the results should not be reported, as recoveries are likely to be irreproducible. A control for the recovery standard is recommended by adding the calibration solution to a real sample. Recoveries should be between 70 and 120%, if not, samples should be repeated.

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Acknowledgements: This technical annex was prepared by the ICES Marine Chemistry Working Group and Working Group on Marine Sediments with contributions from Patrick Roose, Lynda Webster, Franscesco Pellizato, Katrin Vorkamp, Philippe Bersuder, Jacob de Boer, Peter Lepom.

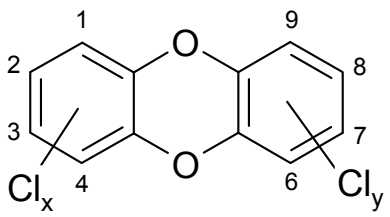
Annex 9: Technical Annex on dioxins/furans and dioxin-like PCBs in biota

1. Introduction

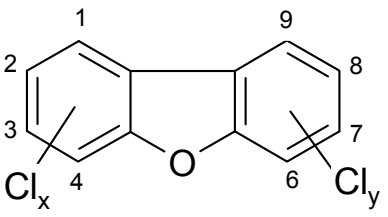
Polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (dioxins/furans - PCDD/Fs) are ubiquitous in the environment, primarily as unintentional by-products of combustion and industrial processes. They enter the aquatic environment via several routes, including atmospheric deposition. Being strongly hydrophobic compounds, sediments are the eventual sink in the aquatic environment, providing a source of potential exposure to aquatic organisms (Hurst *et al*., 2004). Generally highly resistant to metabolism, PCDD/Fs bioaccumulate and biomagnify and have reached high concentrations in e.g. fish from the Baltic Sea, which resulted in recommendations to restrict the use of those fish for human consumption (Verta *et al*., 2007). This guideline only addresses the 17 tetra- through octa-chlorinated 2,3,7,8-substituted dioxin and furan congeners, and the non- and mono-*ortho* substituted polychlorinated biphenyls (PCBs), which are able to exhibit similar effects as the 2,3,7,8-substituted dioxins and furans. The general chemical structures of PCDD/Fs and PCB congeners are given in Figure 1.

In this guideline, the term "dioxin-like PCBs" (dl-PCBs) is used for the non-*ortho* and mono-*ortho* PCB congeners listed in Table 1. The coplanar structure of non-*ortho* substituted PCB congeners allows a configuration similar to that of PCDD/Fs. Mono-*ortho* substituted PCBs may take up a steric position close to coplanarity and are consequently less toxic than non-*ortho* PCBs. Nevertheless, they have been considered due to their relatively high concentrations compared to those of non-*ortho* PCBs or dioxins/furans (Daelemans *et al*., 1992). The exposure to dl-PCBs is mainly via the food-chain, as the compounds are highly lipophilic and bioaccumulate and biomagnify in lipid-rich tissue (e.g. Dyke *et al*., 2003).

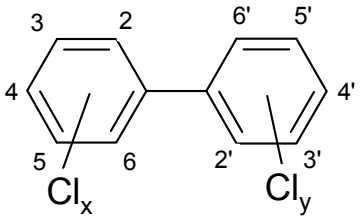
Chlorinated dioxins/furans and dl-PCBs have been shown to produce various toxic responses, including immunotoxicity, developmental and reproductive effects, neurotoxicity and carcinogenesis (OSPAR, 2005). The initial mechanism of toxicity is via the aryl hydrocarbon receptor (AhR), leading to changes in gene expression, cell growth and cell differentiation (Nebert *et al*., 1993; Hurst *et al*., 2004). Due to their persistence, high toxicity, bioaccumulation potential and ability for long-range transport, they are controlled under the Stockholm Convention for Persistent Organic Pollutants (POPs). Their spatial and temporal monitoring in the aquatic environment is important to evaluate the risk to wildlife and human health (Hurst *et al*., 2004). Due to the low concentrations at which adverse effects can be observed, the analytical methodology for the analysis of PCDD/Fs and Dl-PCBs differs from those for other organochlorine compounds, as described in this guideline.



PCDD



PCDF



PCB

Figure 1: General formula of PCDDs, PCDFs and PCBs. The possible number of chlorine atoms results in 75 PCDD congeners, 135 PCDF congeners (x=1–4, y=0–4), and 209 PCB congeners (x=1–5, y=0–5).

2. Analytes and Toxicity Equivalent Factors (TEFs)

Environmental monitoring should include the 17 tetra- through octa-chlorinated 2,3,7,8-dibenzo-p-dioxins (CDDs) and dibenzofurans (CDFs) and the dl-PCBs listed in Table 1.

In the context of food and feed analysis and compliance checks with maximum residue limits, the concept of TCDD (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) Toxicity Equivalency Factors (TEFs) is commonly used, to account for mixtures of several PCDD/Fs and other compounds with dioxin-like activity usually present in these samples. Each congener has been assigned a TEF relative to that of the most toxic dioxin congener, 2,3,7,8-TCDD that was given a TEF of 1.0. The concentrations of the individual congeners are multiplied with their respective TEFs, and the sum of this gives the total concentration of dioxin-like compounds, expressed in TCDD Equivalents (TEQs). Thus, concentrations of mixtures can be expressed in terms of their dioxin-like activity in TEQs, relative to the most potent 2,3,7,8-TCDD. Two parallel TEF systems are currently in use: TEFs established by the World Health Organization (WHO-TEF/TEQ) and TEFs developed by NATO/CCMS (International TEFs or I-TEFs/TEQ). The use of I-TEFs, however, is decreasing. The WHO-TEF-system is reviewed every five years, and Table 1 presents the most recent values, as of 2006 (Van den Berg *et al*., 2006).

According to OSPAR (2005), the scientific relevance of using TEQs to express results is greater for human exposure than for evaluation of pollution sources and emissions, for which information on congener patterns can be of more importance. Furthermore, the system assumes additive effects of the individual congeners, while both synergistic and antagonistic effects have also been reported (OSPAR, 2005). It is therefore recommended for environmental monitoring to report concentrations of individual PCDD/Fs and dl-PCB congeners in biota as absolute concentrations, i.e. pg/g wet weight (ww), with additional information on dry matter and lipid content (see chapter "Data reporting"). The TEF concept can be applied in a subsequent risk assessment, if appropriate.

As part of the TEQ approach, there are different ways of handling results below limits of quantitation:

- The concept of *upper bound* requires using the limit of quantification for the contribution of each non-quantified congener to the TEQ.
- The concept of *lower bound* requires using zero for the contribution of each non-quantified congener to the TEQ.
- The concept of *medium bound* requires using half of the limit of quantification calculating the contribution of each non-quantified congener to the TEQ.

As mentioned above, results of environmental monitoring should preferably be reported for individual congeners, in absolute concentrations. However, OSPAR (2005) mentioned the ongoing food analysis programmes which might be complementary to environmental monitoring. In this context, information on the handling of concentrations below quantitation limits will be important. Thus, results expressed as TEQ val-

ues should be reported as both upper bound and lower bound values (at least, indication of which calculation mode was used should be given). EU directive 2002/69/EC (2002) specifies that, for samples containing 1 pg WHO TEQ/g fat, the difference between upper bound and lower bound level should not exceed 20%. For lower contamination levels, this difference may be in the range of 25 to 40%.

Table 1: Chlorinated dibenzo-*p*-dioxins (CDDs), chlorinated dibenzofurans (CDFs) and dl-PCBs with their Toxicity Equivalent Factors (TEFs) according to the systems developed by the World Health Organization (WHO_{2005}-TEF[4], Van den Berg *et al.* 2006) and NATO/CCMS (I-TEF) for human risk assessment.

HOMOLOGOUS GROUP	CONGENER	I-TEF	WHO2005-TEF	IUPAC NO.
PCDDs				
TCDD	2,3,7,8	1	1	
PeCDD	1,2,3,7,8	0.5	1	
HxCDD	1,2,3,4,7,8	0.1	0.1	
	1,2,3,6,7,8	0.1	0.1	
	1,2,3,7,8,9	0.1	0.1	
HpCDD	1,2,3,4,6,7,8	0.01	0.01	
OCDD	1,2,3,4,6,7,8,9	0.001	0.0003	
PCDFs				
TCDF	2,3,7,8	0.1	0.1	
PeCDF	1,2,3,7,8	0.05	0.03	
	2,3,4,7,8	0.5	0.3	
HxCDF	1,2,3,4,7,8	0.1	0.1	
	1,2,3,6,7,8	0.1	0.1	
	1,2,3,7,8,9	0.1	0.1	
	2,3,4,6,7,8	0.1	0.1	
HpCDF	1,2,3,4,6,7,8	0.01	0.01	
	1,2,3,4,7,8,9	0.01	0.01	
OCDF	1,2,3,4,6,7,8,9	0.001	0.0003	
Non-*ortho* PCBs				
TeCB	3,3',4,4'		0.0001	77
TeCB	3,4,4',5		0.0003	81
PeCB	3,3',4,4',5		0.1	126
HxCB	3,3',4,4',5,5'		0.03	169
Mono-*ortho* PCBs				
PeCB	2,3,3',4,4'		0.00003	105
	2,3,4,4',5		0.00003	114
	2,3',4,4',5		0.00003	118
	2',3,4,4',5		0.00003	123
HxCB	2,3,3',4,4',5		0.00003	156
	2,3,3',4,4',5		0.00003	157
	2,3',4,4',5,5'		0.00003	167

4 EC Regulation 1881/2006 setting maximum levels for certain contaminants in foodstuffs calculates WHO-TEQs using 1998 WHO TEFs. Maximum limits are set for PCDD/PCDFs and PCDD/PCDFs/Dioxin-like PCBs in various foodstuffs including fish and fishery products.

HpCB	2,3,3',4,4',5,5'	0.00003	189

3. Biota samples

OSPAR (2005) presented a monitoring strategy for PCDD/Fs, which identified biota as one of the important matrices for environmental monitoring (the other one being marine sediments). Aquatic organisms can accumulate hydrophobic compounds like dioxins/furans and reach concentrations considerably above those of the surrounding waters. The ratio between the concentration in biota and in the water is the bioconcentration factor (BCF), which is between 2000 and 9000 for PCDD/Fs (OSPAR, 2005). As the BCF varies with species and compound, it is important to design a sampling programme which minimises confounding factors, i.e. to choose the same species, sampling area and sampling period.

The species selected for monitoring should fulfil certain requirements:

- Reflect concentration changes in the sampling area, i.e. ensure a link between exposure and concentration in the organisms.
- Accumulate compounds without showing adverse effects.
- Representative of and abundant in the area (to ensure sufficient sample material for analysis).
- Relatively easy to handle.

Analogous to the monitoring of other organohalogen compounds, mussels and fish are suitable and commonly used for monitoring of PCDD/Fs and dl-PCBs (OSPAR, 2005). Highest dioxin concentrations are found in fish liver and muscle tissue of fatty fish such as herring and salmon. National food agencies often analyse PCDD/Fs and dl-PCBs in commercial fish and fish products, in order to monitor compliance with EU limit values. While different approaches will be necessary in environmental analyses, OSPAR (2005) recommends the monitoring of fish and shellfish as part of the monitoring strategy for dioxins.

In general, the same recommendations are valid as described for other organochlorine compounds, i.e. in the OSPAR JAMP guideline on organic compounds (OCs) in biota, which also contains details on sample dissection and homogenization. It should be pointed out, however, that the risk of sample contamination is considerably higher, given the extremely low concentrations of PCDD/Fs and dl-PCBs in most biota samples. The staff collecting and handling the samples should be well-trained and properly instructed in how to avoid any contamination.

For mussel samples, it is important to remove any sediment particles from their intestinal system, by depuration in a glass aquarium with filtered water from the sampling location for approximately 24 hours. Mussel samples must not be frozen prior to dissection, but should be transported at temperatures between 5 and 15°C, suitable for the area of origin, in a clean container. After dissection, all samples should be stored in the dark at <–20°C prior to analysis. Under these conditions, long-term storage of tissue samples is possible (De Boer and Smedes, 1997). More details on the practical aspects of sample handling and preparation are given in the OSPAR JAMP guideline on OCs in biota.

4. Analytical methods

According to COMMISSION DIRECTIVE 2002/69/EC laying down the sampling methods and the methods of analysis for the official control of dioxins and the determination of dioxin-like PCBs in foodstuffs, a two-step approach can be chosen: This approach includes an initial screening aiming to detect the presence of PCDD/Fs and dl-PCBs in the sample (EU, 2002). Positively tested samples will then be subjected to verification methods (e.g. HRGC/HRMS), in order to identify and quantify individual congeners and compounds. This approach has not been applied in the monitoring of dioxins/furans under the OSPAR Co-ordinated Environmental Monitoring Programme (CEMP), but will be briefly described in this guideline (see "Screening methods based on bioassays"). Primarily, it is applied for the screening of a high number of samples, as the method, based on cell lines, is relatively costly to maintain or to use for lower sample numbers. In environmental monitoring, it might for instance be useful for the identification of suitable sampling locations.

An example of a suitable method for the analysis of biota samples is summarised in Figure 2.

4.1 Preparatory steps

It is essential to avoid contamination during all analytical steps. Where possible, reagents should be of high purity or cleaned by extraction or solvent rinse. All solvents used must be checked for presence of residues of target or interfering compounds (e.g. polychlorinated diphenyl ethers). The purity of standards should be checked. Reusable glassware should be rinsed with solvent, disassembled, washed with a detergent solution and further rinsed with Milli Q grade water and solvent. Baking glassware is common practice as part of the cleaning process, but the formation of active sites on the glass surface that may adsorb the target compounds has been reported (USEPA, 1994).

The preparation of stock solutions and standards can follow the guidelines developed for OCs in biota. However, care has to be taken to monitor and to avoid contamination. Furthermore, the high toxicity of the compounds might require a particularly careful handling; see comments under "Safety". Commercially available diluted stock solutions can be used to reduce safety issues. As valid for the entire analytical method, only trained personnel should perform these steps.

PCDD/Fs and dl-PCBs are normally determined by isotope dilution, using high resolution gas chromatography and high resolution mass spectrometry (HRGC/HRMS). ^{13}C-labelled standards of all the congeners to be analysed are added prior to extraction of the samples. These internal standard are used to quantify the native PCDD/Fs and PCBs and to check the method performance in each sample (recovery surrogates). Table 2 provides a list of all $^{13}C_{12}$ labelled congeners available for use as internal standards while Table 3 provides the minimum number of internal standards to be used for the quantification of PCDD/Fs congeners.

Table 2: $^{13}C_{12}$ labelled congeners that can be used as the internal standards

PCDD/F CONGENERS	PCB CONGENERS
2,3,7,8-13C12-TCDD	13C12-CB77
1,2,3,7,8-13C12-PeCDD	13C12-CB81
1,2,3,4,7,8-13C12-HxCDD	13C12-CB126
1,2,3,6,7,8-13C12-HxCDD	13C12-CB169
1,2,3,7,8,9-13C12-HxCDD	
1,2,3,4,6,7,8-13C12-HpCDD	13C12-CB105
13C12-OCDD	13C12-CB114
	13C12-CB118
2,3,7,8-13C12-TCDF	13C12-CB123
1,2,3,7,8-13C12-PeCDF	13C12-CB156
2,3,4,7,8-13C12-PeCDF	13C12-CB157
1,2,3,4,7,8-13C12-HxCDF	13C12-CB167
1,2,3,6,7,8-13C12-HxCDF	13C12-CB189
2,3,4,6,7,8-13C12-HxCDF	
1,2,3,7,8,9-13C12-HxCDF	
1,2,3,4,6,7,8-13C12-HpCDF	
1,2,3,4,7,8,9-13C12-HpCDF	
13C12-OCDF	

Table 3: Minimum number of internal standards to be used for calibration of PCDD and PCDF homologue groups

SUBSTANCE	PCDD-HOMOLOGUES		PCDF-HOMOLOGUES	
	Native	13C12-labelled	Native	13C12-labelled
Tetrachloro homologues	2,3,7,8	2,3,7,8	2,3,7,8	2,3,7,8
Pentachloro homologues	1,2,3,7,8	1,2,3,7,8	1,2,3,7,8	2,3,4,7,8 1,2,3,7,8
Hexachloro homologues	1,2,3,4,7,8 1,2,3,6,7,8 1,2,3,7,8,9	1,2,3,7,8,9	1,2,3,4,7,8 1,2,3,6,7,8 1,2,3,7,8,9 2,3,4,6,7,8	2,3,4,6,7,8
Heptachloro homologues	1,2,3,4,6,7,8	1,2,3,4,6,7,8	1,2,3,4,6,7,8 1,2,3,4,7,8,9	1,2,3,4,6,7,8

4.2 Lipid determination

The total lipid content should be determined in all biota samples, using the method of Bligh and Dyer (1959) as modified by Hanson and Olley (1963) or an equivalent method such as Smedes (1999). For normalisation purposes, the total lipid content is preferred to the extractable lipid content (De Boer, 1988).

4.3 Extraction

Soxhlet extraction is commonly used for biota samples. Immediately prior to use, the Soxhlet apparatus should be pre-extracted with e.g. dichloromethane: hexane (1:1) for

approximately 3 hours (USEPA, 1994). An adequate amount of tissue (e.g. 10 g of wet tissue) is spiked with the labelled compounds and mixed with sodium sulphate. The sample is allowed to dry for 12–24 hours and should be remixed prior to transfer to a glass Soxhlet thimble. Soxhlet extraction proceeds for 18–24 hours using e.g. fresh dichloromethane: hexane (1:1) (USEPA, 1994).

More recently, pressurised liquid extraction (PLE) has become a common and faster alternative to Soxhlet extraction (Focant *et al*., 2004). PLE uses organic solvents at temperatures above their boiling point maintained in the liquid phase under high pressure. The extraction cell which contains the sample is heated (ex: 100°C) and filled up with an appropriate solvent (example: toluene, DCM) up to a pressure of 140 bars. The minimum extraction time should be 10 minutes in static mode, and several extraction cycles are recommended (n = 2-3). To further reduce analysis time, PLE can be combined with in-line clean-up procedures using preferably sulphuric acid impregnated silica as fat retainer (Björklund *et al*., 2006). Proper fat-fat retainer ratios are important to avoid fat remaining in the sample after extraction. Mixed (polar/non-polar) solvent combinations cannot be used with this technique. It is understood that the combination of pressure and temperature is sufficient to remove all dioxins, furans and dl-PCBs from the matrix.

4.4 Clean-up

The extracts are concentrated using suitable evaporation devices, e.g. rotary evaporation, Turbovap, Syncore, Kuderna-Danish. The risk of cross-contamination is fairly high for rotary evaporation, so the evaporator should be pre-cleaned, e.g. by 100 ml of clean solvent. If the extracts are to be cleaned up by adsorption chromatography on e.g. silica gel, a solvent change to hexane is recommended. The purification procedures have two objectives: i) removal or destruction of lipids and ii) removal of interfering compounds. Due to the very low levels of PCDD/Fs in biota samples, the elimination of interferences is essential. Prior to column chromatography clean-up, the precision and recovery of this step should be assessed.

For the first part, addition of concentrated sulphuric acid is commonly applied, either in terms of a column chromatography clean-up or by direct addition of silica impregnated with sulphuric acid to the extracts. The column chromatography clean up suggested by USEPA (1994) for lipid removal in biota extracts includes 2 g of silica gel, 2 g of potassium silicate, 2 g of anhydrous Na2SO4, 10 g of silica gel (impregnated with sulphuric acid) and another 2 g of anhydrous sodium sulphate, to be packed bottom-to-top into a column of 25 mm ID. The column is pre-eluted with 100 ml of hexane and after loading of the sample, eluted with 200 ml of hexane. Ready to use, multi-layer clean-up columns are also available commercially. Alternatively, approximately 30–100 g of sulphuric acid impregnated silica gel can be added to the extract (200 ml), while stirring for 2–3 hours. The treatment with sulphuric acid impregnated silica requires strict safety procedures as the small particles can cause serious health damage after inhalation. Gel permeation chromatography (GPC) has also been applied for lipid removal, but often a series of GPC columns is needed to ensure a 100% fat removal. Alternatively, an additional cleanup step using concentrated sulphuric acid might be applied after GPC to remove residual lipids from samples having a higher lipid content.

For removal of interferences, HPLC, GPC, and column chromatography using alumina, silica gel, Florisil and activated carbon are possible alternatives. USEPA (1994) suggests adsorption chromatography on alumina or Florisil and carbon as minimum additional clean-up steps after lipid removal. Depending on whether acid or basic

alumina is chosen, the eluents should be dichloromethane: hexane (1:4) or (1:1), respectively. The material for the carbon column can be e.g. Carbopack™-C. Interferences are removed in a washing step with e.g. hexane, dichloromethane:cyclohexane and dichloromethane:toluene. Then, the column is inverted and the analytes are eluted with toluene. HPLC can also be used for purification and fractionation of the extracts. 2-(1- pyrenyl) ethyldimethylsilylated (PYE) silica columns and porous graphitised carbon are suitable columns for this purpose (Echols *et al*., 1998). When coupled in series, nitrophenylpropylsilica column (Nucleosil, 5 μm particles, 250 x 4.6 mm) and PYE (Cosmosil, 5 μm particles, 150 x 4.6 mm) enables the separation of PCDD/Fs from dl-PCBs (Bandh *et al*., 1996). Fully automated clean-up systems are also available commercially (e.g. PowerPrep™ system). The European research project DIFFERENCE recommended at least three clean up or fractionation steps to ensure sufficiently clean extracts (Van Leeuwen *et al*., 2007).

4.5 Concentration and syringe standards

After clean-up, a keeper is added (e.g. iso-octane or nonane) and the extracts are concentrated to near dryness, i.e. 10-20 μl. A syringe standard mix should also be added to evaluate the recovery of labelled internal standards. For example $^{13}C_{12}$ -1,2,3,4-TCDD can be used for recovery determinations of TCDD/Fs and PeCDD/Fs internal standards while $^{13}C_{12}$ -1,2,3,7,8,9-HxCDD can be used for recovery determinations of HxCDD/Fs, HpCDD/Fs and OCDD/F internal standards.

4.6 HRGC/HRMS

The dioxin/furan content in environmental samples is commonly monitored using high resolution gas chromatography (HRGC) and high resolution mass spectrometry (HRMS), but low resolution mass spectrometry (LRMS) may be a suitable and cost effective alternative if the required minimum performance criteria are met (see "HRGC/LRMS").

4.7 GC-analysis

The GC analysis should be optimised with regard to separation and sensitivity. Fishman *et al*. (2007) provided a comprehensive review of GC columns available for dioxins analysis. Generally, 50–60 m, 5% diphenyl 95% dimethylpolysiloxane columns are a common choice. However, these columns could exhibit multiple coelutions for both PCBs and PCDD/Fs (Reiner *et al*., 2006), depending on the matrix to be analyzed. The use of RTx-Dioxin 2 column has been reported in the literature as a suitable alternative to DB-5 columns. Combining this phase with reduced inner diameter and phase thickness (for example a 40m x 0.18mm x 0.18μm) enables the analysis of the 17 PCDD/F congeners in 40 minutes, with data fulfilling QA/QC requirements and providing better selectivity, especially for 2,3,7,8-TCDD and 2,3,7,8-TCDF(Reiner *et al*., 2006; Cochran *et al*., 2007).

Potential interferences for dl-PCBs on common GC-columns are summarized in Table 4 (Reiner *et al*., 2006). Complete separation can be achieved by multi-analysis on columns of different polarity. The GC separation of congener CB-123 from interferences is critical. Due to the minor contribution of this congener to the overall TEQ, possible interferences only lead to a marginal increase in the uncertainty of the total TEQ. Recent developments indicate possibilities of full separation of relevant PCB congeners on one column, e.g. on an SGE HT8-PCB capillary column. A full separation of all PCB congeners is also possible by using comprehensive multi-dimensional GC (GCxGC).

Table 4: Possible interferences for selected dl-PCBs using a 5% phenyl column (Reiner *et al.*, 2006)

PCB CONGENER	POTENTIAL INTERFERENCE
CB-81	CB-87
CB-77	CB-110
CB-123	CB-149
CB-126	CB-178 and CB-129
CB-156	CB-171
CB-157	CB-201

Various injection techniques are possible, e.g. on-column injection, splitless injection, pressure-pulsed splitless injection and programmed temperature vaporizing (PTV) injection. The most suitable injection volume depends on the dioxin concentrations in the sample and the sensitivity of the instrumental analysis. In HRGC/HRMS analysis, 1–2 μl are common injection volumes.

4.8 Compound identification

The HRMS system should be operated at a minimum of 10,000 resolving power throughout all the runs, and resolution should be checked regularly during the sequence of runs. The individual dl-PCBs, PCDD/Fs or labelled compound are identified by comparing the GC retention time and ion abundance ratio of two exact masses monitored (Tables 5 and 6) with the corresponding retention time of an authentic labelled internal standard and the theoretical or acquired ion abundance ratio of the two exact masses. The congeners for which there are no labelled analogues are identified when relative retention time and ion abundance ratios agree within predefined limits. The following criteria should be met for identification of an individual dl-PCB, PCDD/F or labelled compound in a standard, blank or sample:

- The signal for the two exact masses specified in Tables 5 and 6 should be present and within ± 2 s.
- The signal-to-noise ratio (S/N) for the GC peak at each exact mass has to be at least 3 for each congener detected in a sample extract, and at least 10 for all congeners in the calibration standard.
- The ratio of the integrated areas of the two exact masses specified in Tables 5 and 6 has to be within 15% of the theoretical shown in Table 7.
- The relative retention time of a native dl-PCB, PCDD/F has to be within a time window of ± 3 s based on the retention time of the corresponding $^{13}C_{12}$-labelled standard. The relative retention time of congeners for which there are no labelled analogues has to be within ± 0.002.

If interferences preclude identification, extract a new, further cleaned up aliquot and analyse again. If interferences cannot be removed flag the data to indicate results are maximum concentrations.

4.9 Compound quantification

Quantitative analysis is performed using selected ion monitoring (SIM) area, in one of the two following ways:

- For the dl-PCBs, PCDD/Fs for which labelled analogues have been added to the sample (Table 2), the GC/MS system is calibrated, and the concentration of each compound is determined using the isotope dilution technique.
- For the dl-PCBs, PCDD/Fs for which labelled analogues are not added to the sample (see Table 3 for PCDD/Fs), the GC/MS system is calibrated for each compound using a labelled isomer with the most similar structure and the concentration of each compound is determined using the internal standard technique.

Calibration curves should be based on a minimum of 5 calibration points. Mass drift correction is mandatory, usually based on a lock-mass *m*/*z* of perfluorokerosene (PFK) or perfluorotributylamine (PFTBA, FC43).

Table 5: Masses for the detection and quantification of PCDD/Fs.

	Dibenzofurans		Dibenzo-p-dioxins	
Substance	Native	13C12-labelled	Native	13C12-labelled
Tetra-CDD/F	303.9016	315.9419	319.8965	331.9368
	305.8987	317.9389	321.8937	333.9339
Penta-CDD/F	339.8598	351.9000	355.8547	367.8949
	341.8569	353.8970	357.8518	369.8919
Hexa-CDD/F	373.8208	385.8610	389.8157	401.8559
	375.8179	387.8580	391.8128	403.8529
Hepta-CDD/F	407.7818	419.8220	423.7767	435.8169
	409.7789	421.8190	425.7738	437.8140
Octa-CDD/F	441.7428	453.7830	457.7377	469.7779
	443.7399	455.7801	459.7348	471.7750

Table 6: Masses for the detection and quantification of PCBs.

Homologue groups	Native CBs	13C12-labeled CBs
Tetrachlorobiphenyls	289.9223	301.9626
	291.9194	303.9597
Pentachlorobiphenyls	325.8804	337.9207
	327.8775	339.9177
Hexachlorobiphenyls	359.8415	371.8817
	361.8385	373.8788
Heptachlorobiphenyls	393.8025	405.8427
	395.7995	407.8398

The isotope ratio between the two ions of the molecular isotope cluster, which are recorded has to match the theoretical value within ± 15 % (see Table 7).

Table 7: Tolerance limits of isotope ratios for PCDD/Fs and dl-PCBs.

Chlorine atoms	Isotope Ratio Lower Limit	Isotope Ratio Theoretical Value	Isotope Ratio Upper Limit
4	0.65	0.77 (M/M+2)	0.89
5	0.55	0.64 (M+4/M+2)	0.75
6	0.69	0.81 (M+4/M+2)	0.94
7	0.83	0.96 (M+4/M+2)	1.10
8	0.76	0.89 (M+2/M+4)	1.02

4.10 HRGC/LRMS

Low resolution mass spectrometry (LRMS) has also been applied to the analysis of PCDD/Fs and/or dl-PCBs. Limits of detections are higher than those obtained with HRMS detectors, but can be compensated by e.g. larger injection volumes. A very efficient extract clean up is of utmost experience to exclude any interferences. A technique commonly applied is GC-LRMS using ion trap mass analysers working in tandem mode (Focant *et al*., 2005; Malavia *et al*., 2008, Eppe *et al*., 2004). Table 8 provides information on precursor and product ions obtained by GC-ion trap MS. GC-LRMS (quadrupole) can be an option for dl-PCBs in particular.

Both HRMS and LRMS techniques have to demonstrate that they meet the requirements regarding separation and sensitivity described in the monitoring programme, see also comments under "Quality Assurance and Quality Control". When using LRMS the maintenance of the instrument is crucial and could be time consuming (e.g. frequent cleaning of the ion source). The sensitivity for 2,3,7,8-TCDD may be critical. For matrices with relatively high dioxin and dl-PCB levels LRMS may be very useful, and much cheaper than HRMS.

Table 8: Precursor ions and product ions for the determination of PCDD/Fs and dl-PCBs by HRGC-ion trap tandem MS.

Target Compounds	Native		13C12-labelled	
	Precursor Ion (m/z)	Product Ions (m/z)	Precursor Ion (m/z)	Product Ions (m/z)
TCDD	322 (M+2)	257 + 259	334 (M+2)	268 + 270
PeCDD	356 (M+2)	291 + 293	368 (M+2)	302 + 304
HxCDD	390 (M+2)	325 + 327	402 (M+2)	336 + 338
HpCDD	424 (M+2)	359 + 361	436 (M+2)	370 + 372
OCDD	460 (M+4)	395 + 397	472 (M+4)	406 + 408
TCDF	306 (M+2)	241 + 243	318 (M+2)	252 + 254
PeCDF	340 (M+2)	275 + 277	352 (M+2)	286 + 288
HxCDF	374 (M+2)	309 + 311	386 (M+2)	320 + 322
HpCDF	408 (M+2)	343 + 345	420 (M+2)	354 + 356
OCDF	444 (M+4)	379 + 381		
CB-81, 77	292 (M+2)	220 + 222	304 (M+2)	232 + 234

CB-123, 118, 114, 105, 126	326 (M+2)	254 + 256	338 (M+2)	266 + 268
CB-167, 156, 157, 169	360 (M+2)	288 + 290	372 (M+2)	300 + 302
CB-189	394 (M+2)	322 + 324	406 (M+2)	334 + 336

5. Quality Assurance and Quality Control

The laboratory is required to operate a formal quality assurance programme. Indicative values for accuracy and precision are given under "Verification method" in Table 9. An example of a comprehensive QA/QC approach is described in method 1613 by USEPA (1994).

The analytical method requires high sensitivity and low detection limits, usually in the pg-TEQ-range, for both dioxins/furans and dl-PCB congeners (OSPAR, 2005), and should meet the requirements for limits of quantitation specified in the monitoring programme.). For individual PCDD/Fs, limits of quantification (LoQ) of 0.3 pg/g wet weight should be achievable, with the exception of OCDD (1 pg/g wet weight). For non-*ortho* PCBs, LoQ should be as low as 5 pg/g wet weight, while for mono-*ortho* PCBs requirements on LoQ are less strict as the their concentrations in biota samples are usually higher, in particular concentrations of congeners CB-105, CB-118 and CB-156. The selectivity of the method should be sufficient to avoid interfering compounds, i.e. the individual congeners should be separated from each other and any interferences present.

All sample series should include procedural blanks and measurements of certified/laboratory reference materials. Certified reference materials should be analysed regularly, although only few are available for the determination of PCDD/Fs and dl-PCBs in biota, for example from NIST (cod liver oil), Cambridge Isotope Laboratories (fish) and the National Research Council Canada (fish) (De Boer and McGovern, 2001). The laboratory should further prove its competence by regular participation in relevant laboratory proficiency tests. It is essential that the matrix and concentration range of the proficiency testing samples are comparable with the samples routinely analysed within the monitoring programme (De Boer, 2001, Wells and de Boer, 2006).

The recovery of the individual internal standards added prior to extraction should be between 60-120%. According to Commission Directive 2002/69/EC, lower or higher recovery rates can be accepted for the hepta- and octa-PCDD/Fs, as long as their TEQ contributions are below 10% of the total TEQ value (EU, 2002). Blanks should be as low as possible, at least below 20% of the lowest concentration of interest.

6. Screening methods based on bioassays

As mentioned above, bioassays are not currently applied in the monitoring under OSPAR CEMP, but have been suggested as screening tools for monitoring PCDD/F and dl-PCB in foodstuffs (Commission Directive 2002/69/EC), with the requirement to meet the criteria given in Table 9. Screening tools might be useful in, for instance, choice of sampling sites, and will therefore be briefly discussed in this guideline. Hurst *et al*. (2004) also emphasised that monitoring programmes were moving towards effect-based monitoring, with biological relevance becoming more important. The authors list the following requirements for bioassays to be included in monitoring programmes: The tool must be capable of rapid, inexpensive and high-throughput screening producing interpretable and meaningful results (Hurst *et al*., 2004).

Table 9: Quality criteria for screening and verification methods (EU, 2002).

	SCREENING METHOD	VERIFICATION METHOD
False negatives	< 1%	
Accuracy		± 20%
Precision (expressed as the coefficient of variation between repeated measurements)	< 30%	< 15%

The dioxin responsive chemically activated luciferase expression (DR-CALUX) assay is mechanism specific and utilises the interaction of compounds with the AhR. However, it is not compound specific and produces a response with all compounds capable of interactions with the AhR. While Commission Directive 2002/69/EC demands that the TEQ-values determined by bioassays should be the sum of PCDD/Fs and dl-PCBs in the sample, Hurst *et al*. (2004) and Van Leeuwen *et al*. (2007) demonstrated some disagreement between the results of the bioassay and the conventional targeted HRGC/HRMS analysis. The differences may be caused by unknown compounds producing a dioxin-like response in the CALUX assay (e.g. brominated or mixed halogenated dioxin analogues, polychlorinated naphthalenes, PAHs etc.) – or compounds antagonising the AhR (e.g. di-*ortho*-substituted PCBs). These deviations from results of chemical analysis were also considered as an advantage by Hurst *et al*. (2004), as the assay allows a more accurate assessment of the true potency of dioxin-like compounds present in the samples. In order to obtain specific responses to PCDD/Fs and dl-PCBs in the sample, the extracts require specific clean up methods to exclude interferences from other dioxin-like compounds. As mentioned above, environmental monitoring aims at presenting concentrations of individual compounds in the respective samples, rather than toxicity assessments.

7. Safety

The chemical compounds dealt with in this guideline are hazardous and must only be handled by trained personnel familiar with handling of PCDD/F and dl-PCBs, and associated risks as well as precautionary measures. USEPA (1994) recommends that laboratories purchase diluted standard solutions instead of preparing primary solutions.

8. Data reporting

Results are reported on a congener basis in pg g^{-1} ww. The lipid content and water content of the samples should be reported as well. For normalizing purposes the total lipid content should be determined, rather than the extractable lipid content (De Boer, 1988). Concentrations are reported to two significant figures. Minimum performance criteria such as LoQ and measurement uncertainty along with information on blanks and reference materials should be included in the report.

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Acknowledgements: This technical annex was prepared by the ICES Marine Chemistry Working Group with contributions from Katrin Vorkamp, Philippe Bersuder, Jacob de Boer, Rossana Bossi, Peter Lepom, Patrick Roose, Lynda Webster and Catherine Munschy.

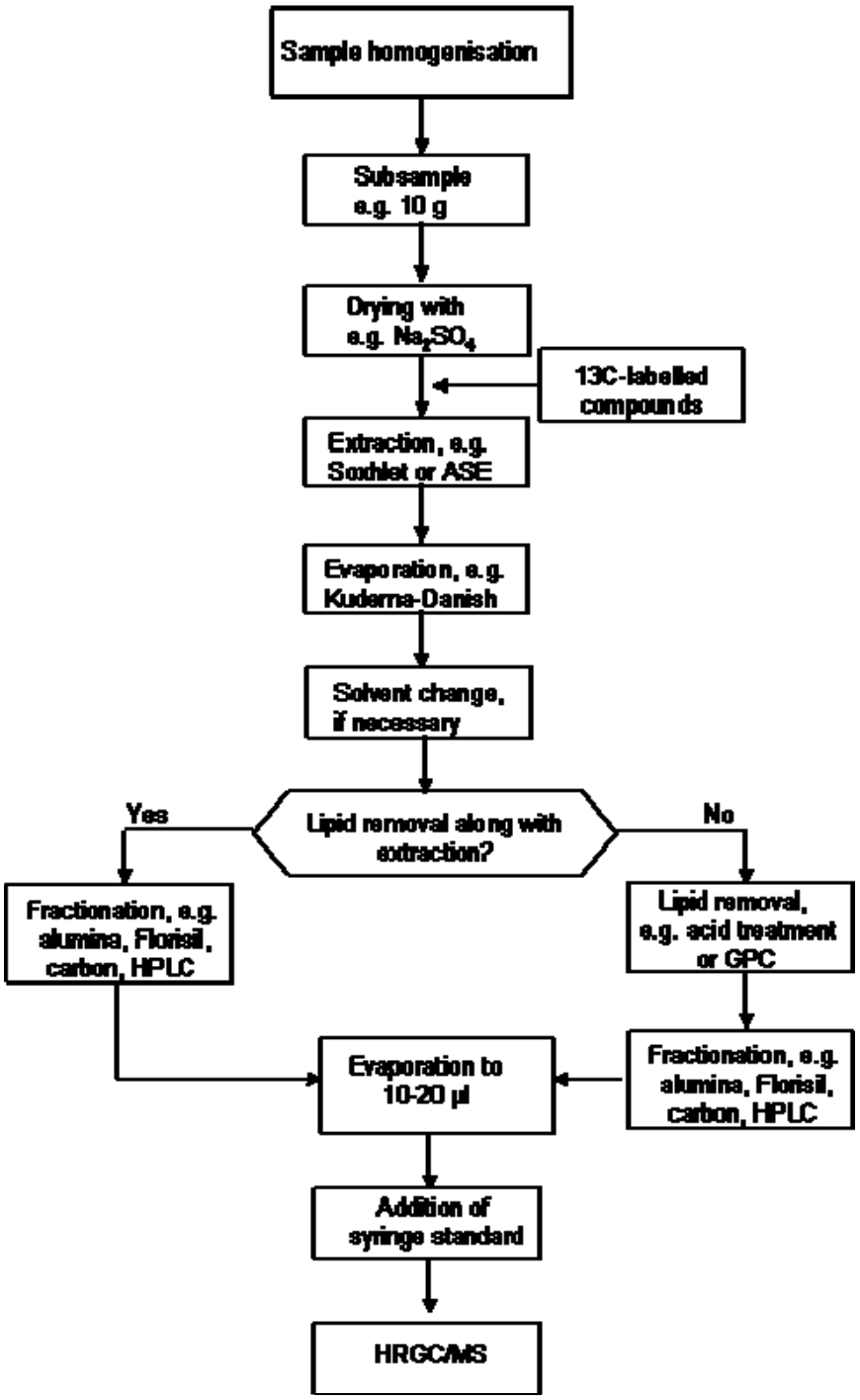

Figure 2: Analytical method recommended for analysis of biota samples within environmental monitoring.

Annex 10: Technical annex on PFCs in sediment

1. Introduction

Per- and polyfluorinated compounds (PFCs) are man-made chemicals and are ubiquitous in the environment (Giesy and Kannan, 2001). PFCs are widely used as processing additives during fluoropolymer production and as surfactants in consumer applications, including surface coatings for carpets, furniture and paper products. They are also components in breathable, waterproof fabrics, fire-fighting foams and insulators for electric wires (Kissa, 2001). From the production and use of these products, PFCs can be released into the environment.

In this document, the name PFCs refers to compounds with a hydrophilic functional group and a hydrophobic fully fluorinated chain which can vary in chain length. The polyfluorinated acids have moderate water solubilities, low pK_a values and are therefore dissociated at environmentally relevant pH values (Kissa, 2001). Perfluorinated sulfonamides and fluorotelomer alcohols (FTOHs) are neutral compounds with a moderate vapour pressure currently discussed as precursors to perfluorooctane sulfonate (PFOS) and perfluorinated carboxylic acids (PFCAs) (Ellis *et al*., 2004).

The objective of this technical annex is to provide advice on the analysis of PFCs in sediment. The detection of PFCs at ppb to ppt levels is complex because of a risk of contamination during sample handling, storage, preparation and instrumental analysis. Only a few methods to determine PFCs in sediment are applied in various laboratories, but they generally apply extraction with polar solvents, clean-up steps and liquid-chromatography (LC) with mass spectrometric detection (MS). International Standards Organisation (ISO) has already promoted a standard for the determination of PFOS and perfluorooctanoic acid (PFOA) in water (ISO 25101, 2009), but at present no standard is available for the analysis of these compounds in sediment samples.

2. Analytes

Table 1 gives an overview of relevant PFCs for analysis in sediments. They are chosen from the following groups: Perfluorinated sulfonates (PFSAs), perfluorinated sulfinates (PFSiAs), PFCAs and perfluorinated sulfonamides. For monitoring purposes, the high-volume chemicals PFOS and PFOA are considered the most important PFCs and are highly recommended to be included in sediment monitoring. Although most studies have focused mainly on PFOS, it is suggested that long-chained PFCs ($\geq C_8$) should be included in analysis due to their adsorption potential to sediment.

Table 1: Full names, acronyms, formulas and Chemical Abstract System (CAS) numbers of native and mass-labelled PFCs relevant for sediment analysis.

Analyte	Acronym	Formula	CASa-Number
Perfluorobutanoic acid	PFBA	C3F7COOH	375-22-4
Perfluoropentanoic acid	PFPA	C4F9COOH	2706-90-3
Perfluorohexanoic acid	PFHxA	C5F11COOH	307-24-4
Perfluoroheptanoic acid	PFHpA	C6F13COOH	375-85-9
Perfluorooctanoic acid	PFOA	C7F15COOH	335-67-1
Perfluorononanoic acid	PFNA	C8F17COOH	375-95-1
Perfluorodecanoic acid	PFDA	C9F19COOH	335-76-2

Perfluoroundecanoic acid	PFUnDA	C10F21COOH	4234-23-5
Perfluorododecanoic acid	PFDoDA	C11F23COOH	307-55-1
Perfluorotridecanoic acid	PFTriDA	C15F25COOH	72629-94-8
Perfluorotetradecanoic acid	PFTeDA	C13F27COOH	376-06-7
Perfluorohexadecanoic acid	PFHxDA	C15F31COOH	67905-19-5
Perfluorobutane sulfonate	PFBS	C4F9SO2O-	29420-49-3 (potassium salt)
Perfluorohexane sulfonate	PFHxS	C6F13SO2O-	3871-99-6 (potassium salt)
Perfluoroheptane sulfonate	PFHpS	C7F15SO2O-	n.a.
Perfluorooctane sulfonate	PFOS	C8F17SO2O-	1763-23-1 (sodium salt)
Perfluoro-1-decanesulfonate	PFDS	C10F21SO2O-	13419-61-9 (sodium salt)
1H,1H,2H,2H-perfluorooctane sulfonate	THPFOS (6:2 FTS)	C6F13C2H4SO3-	27619-97-2
Perfluorooctane sulfinate	PFOSi	C8F17SO2-	n.a.
Perfluorooctane sulfonamide	PFOSA	C8F17SO2NH2	754-91-6
Internal Standards			
Perfluoro-n-(1,2,3,4-13C4)butanoic acid	[13C4]-PFBA	[2,3,4-13C3]F713COOH	n.a.
Perfluoro-n-(1,2-13C2)hexanoic acid	[13C2]-PFHxA	C4F9[2-13C]F213COOH	n.a.
Perfluoro-n-[1,2,3,4-13C4]octanoic acid	[13C4]-PFOA	C4F9[2,3,4-13C3]F613COOH	n.a.
Perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid	[13C5]-PFNA	C4F9[2,3,4,5-13C4]F813COOH	n.a.
Perfluoro-n-[1,2-13C2]decanoic acid	[13C2]-PFDA	C8F1713CF213COOH	n.a.
Perfluoro-n-[1,2-13C2]undecanoic acid	[13C2]-PFUnDA	C9F1913CF213COOH	n.a.
Perfluoro-n-[1,2-13C2]dodecanoic acid	[13C2]-PFDoDA	C10F2113CF213COOH	n.a.
Perfluoro-1-hexane[18O2]sulfonate	[18O2]-PFHxS	C6F13S[18O2]O-	n.a.
Perfluoro-1-[1,2,3,4-13C4]octanesulfonate	[13C4]-PFOS	C4F9[1,2,3,4-13C4]F8SO2O-	n.a.
Perfluoro-1-[1,2,3,4-13C4]octanesulfinate	[13C4]-PFOSi	C4F9[1,2,3,4-13C4]F8SO2-	n.a.

a, Chemical Abstract System; n.a., not available

3. Sampling, transportation and storage

The sampling should be carried out by trained personnel being aware of the risk of contamination of samples if incorrectly handled. Materials and clothes that contain or can adsorb fluorinated compounds must be avoided. In particular the containers or bags that come in direct contact with the sample should not contain fluorinated polymers like Teflon™. Instead, containers and equipment made of polypropylene, glass or stainless steel should be used. After collection, samples should be stored in closed containers at a temperature lower than -20°C until sample preparation. The

handling time at room temperature should be short to minimize possible degradation of precursors to PFCAs and PFSAs.

4. Sample preparation

Sample preparation requires clean conditions on a clean bench. The laboratory should be free from any material that can contain fluorinated compounds (e.g. Teflon™). Every material that can come in contact with the sample must be free of fluorinated compounds. Materials used in the PFC analysis should be cleaned with solvents such as methanol and acetone and covered with solvent rinsed aluminium foil to keep out any dust. The septa of vials should be Teflon™-free such as Barrier™ septa made of silicone polymer and aluminium. Solvents including water should be of highest purity and must be tested for residues of PFCs prior to use.

Within each sample batch, a method blank should be analysed. If measurable blanks occur, the analytical instrumentation and every sample preparation step have to be checked for contamination and appropriate measures have to be taken before continuation of analysis.

4.1 Pre-treatment

It is advised to determine the water content or to dry samples before extraction. Freeze-drying is becoming available in an increasing number of laboratories. However, its application should be carefully considered, since losses of volatile PFCs or contamination may occur. Losses through evaporation are diminished by keeping the temperature in the evaporation chamber below 0°C. Alternatively, air-drying to constant weight at room temperature on a clean bench or drying in an oven may be considered. However, degradation of precursor compounds can change the composition profile of PFCs in the sediment sample. In addition, PFCs can be lost by volatilization depending on temperature and drying time.

Before taking a subsample for analysis, the samples should be sufficiently homogenised. Depending on matrix and expected concentrations, an appropriate sample amount is weighed in polypropylene tubes for extraction. Then, the extract is spiked with a mass-labelled internal standard (IS) mixture at concentrations close to the environmental level. Before extraction, the sample should be incubated with the IS for about 12 h at 4° C so that the IS can interact with the matrix.

4.2 Extraction

Three methods have been described in the scientific literature for the extraction of PFCs from sediments (Powley et. al, 2005, Higgins *et al*. 2005; Washington *et al*. 2008). Powley *et al*. (2005) used a wrist-action shaker operated at maximum deflection to extract PFCs from sediments with methanol followed by a graphitized carbon adsorbent clean-up. Higgins *et al*. (2005) used an acetic acid wash, followed by repeated extraction with methanol/1% acetic acid in water (90:10, v/v) in a heated (60 °C) sonication bath and subsequent clean-up using C_{18} cartridges. The method is described by Washington *et al*. (2008) includes sonication with acetonitrile/water (60:40, v/v) and ion pairing clean-up.

Alternatively, extraction can be performed by shaking placed on a wrist-action shaker set at maximum deflection. However, all extraction methods should include a minimum of three extractions, each with a solvent volume that corresponds to ten times the sample volume and 30 min extraction time. After the extraction, the three extracts are combined for clean-up.

4.3 Clean-up

Because of matrix effects on ionisation enhancement/suppression in electrospray tandem mass spectrometry (ESI-MS-MS), a clean-up of the extracts is necessary. Different methods can be used, either separately or in combination, depending on extraction solvent and concentration level.

An appropriate clean-up method is described by Powley *et al*. (2005). Briefly, 25 mg of graphitized carbon adsorbent (e.g. ENVI-Carb™, 100 m^2 g^{-1}, 120/400 mesh) and 50 µL acetic acid are added into a small tube. The extract is concentrated to 1 mL and transferred into this tube. The extract is mixed, centrifuged and finally, 0.5 mL of the supernatant is transferred to another flask. Additional clean-up might be required, depending on sample type and concentration levels (Higgins *et al*. 2005; Washington *et al*. 2008).

Sample extracts should be concentrated according to the required sensitivity. Concentration techniques at low temperature (< 40°C) and controlled pressure conditions are preferred in order to avoid losses of the more volatile PFCs. Evaporation to dryness should be avoided in any case.

An injection standard (InjS) can be added to the final extract for correction of the injection volumes and calculation of the recoveries of the mass-labelled IS. The InjS should not occur in environmental samples, hence, the use of a mass-labelled InjS is recommended.

The solvent composition of the final extract should correspond to the mobile phase of the LC method in order to obtain a satisfactory peak shape of the compounds, in particular of early eluting short-chain PFCs. Unless the samples are analysed immediately, the vials should be kept at <4°C and analysed within one week.

5. Instrumental analysis

LC coupled with a tandem mass spectrometer and interfaced with an electrospray ionisation source in a negative-ion mode (LC-(-)ESI-MS/MS) (Hansen *et al*., 2001) or LC coupled with an (-)ESI time-of-flight mass spectrometer (LC-ESI-QTOF-MS) (Berger and Haukas, 2005) can be used for PFC analysis. Tandem MS and QTOF-MS have the advantage of low instrumental noise with a high selectivity.

5.1 Liquid chromatography

For the liquid-chromatography C_8 or C_{18} reserved phase columns can be used. A guard column may improve the peak performance and extend the lifetime of the chromatographic column. Mixtures of water and methanol or acetonitrile can be used as mobile phase, both with 2-10 mM ammonium acetate as an ionisation aid. Gradients from 10% to 100% methanol or acetonitrile are required for the separation of the compounds listed in Table 1.

Modifications of the instrument might be necessary to minimise contact with fluorine-containing materials (Yamashita *et al*., 2004). For example, Teflon™-containing tubing, filters for the mobile phase solvents and degassers can be sources for contaminations. A scavenger cartridge can be installed between the pump and injector to trap contaminants originating from the degasser, connecting tubes and mobile phase.

To ensure stability of retention times, the use of a temperature controlled column oven is strongly recommended.

5.2 Detection methods

The most widely used technique for detection of PFCs is by tandem MS operated in the MRM mode. Typical precursor and product ions are given in Table 2. MS-parameters for the individual compounds, such as collision energy, declustering potential and cone voltage, have to be optimised for each instrument. The sensitivity of tandem MS is usually about one order of magnitude higher than that of QTOF-MS (Berger *et al*., 2004).

Table 2: Precursor and product ions for PFCs analysed using LC-(-)ESI-MS/MS.

Analyte	Precursor ion (m/z)	Product ion (m/z)
PFBA	112.9	168.7
PFPA	262.8	218.9
PFHxA	312.9	268.8
PFHpA	362.9	318.9
PFOA	413.0	368.9
PFNA	462.9	418.9
PFDA	512.9	469.0
PFUnDA	562.9	519.0
PFDoDA	613.0	568.9
PFTriDA	663.1	618.9
PFTeDA	713.0	669.0
PFHxDA	812.8	769.1
PFBS	298.9	79.8
PFHxS	398.9	79.8
PFHpS	449.0	79.3
PFOS	499.0	79.7
PFDS	598.9	79.5
THPFOS (6:2 FTS)	426.9	406.7
PFOSi	482.8	418.9
PFOSA	497.9	77.9
[$^{13}C_4$]-PFBA	216.8	171.8
[$^{13}C_2$]-PFHxA	314.9	269.9
[$^{13}C_4$]-PFOA	417.0	371.8
[$^{13}C_5$]-PFNA	467.9	423.0
[$^{13}C_2$]-PFDA	514.9	469.8
[$^{13}C_2$]-PFUnDA	565.0	519.8
[$^{13}C_2$]-PFDoDA	614.9	569.9
[$^{18}O_2$]-PFHxS	403.0	83.9
[$^{13}C_4$]-PFOS	502.9	79.5
[$^{13}C_4$]-PFOSi	486.9	422.9

6. Calibration and quantification

6.1 Standards

The use of commercially available standards with a purity of > 99% is recommended. The purity of standards should be verified, as impurities from the same homologue group and isomers can occur.

Suggestions for mass-labelled IS are given in Table 1. It is strongly recommended to use IS for PFC-analysis, to compensate for signal enhancement/suppression or losses during sample preparation. The IS and InjS have to be added before the extraction and the measurement, respectively. If possible, the corresponding mass-labelled IS should be used for each target analyte. In case a mass-labelled standard is not available, an IS with physicochemical characteristics and recovery rates similar to that of the target compound can be used but matrix suppression/ enhancement effects must first be checked in LC-ESI-MS/MS.

6.2 Calibration

The calibration curves must include the IS and InjS in the same range as the spike level for the samples. Linearity has to be checked for the calibration range and the correlation coefficient (R) should be better than 0.99. The lower end of linear range is determined by the quantification limits and blank levels. The blank response should be lower than 20 % of the lowest calibration standard. A multilevel calibration should have at least five calibration levels.

In case of matrix effects, standard addition may be an alternative calibration option.

6.3 Quantification

Every detection and quantification must comply with defined criteria for quality assurance (U.S. DHHS/FDA/CDER/CVM, 2001). If possible, two mass transitions should be recorded for each target analyte, one for quantification (quantifier) and one for identification (qualifier). The abundance ratio of these two masses in the sample is compared with that of the calibration standards obtained under identical chromatographic conditions. A substance is considered identified

- if the relative retention time of the target compound in the sample is within ± 0.3 min of that in the calibration standard and
- if the abundance ratio of the two masses in the sample deviates less than 30 % from the average abundance ratio calculated from the calibration standards.

For quantification, the signal to noise ratio for the HPLC-peak has to be at least 10 for all target compounds. The peak height of the target compound should exceed the measured blank as a minimum by a factor of 5.

Some PFSAs and sulfonamides show more than one peak in the chromatogram, which is due to the presence of branched isomers. The ratio of linear and branched isomers can differ between the calibration standard and environmental samples. Branched isomers cannot be quantified precisely because of the lack of proper calibration standards. If the peak area of the branched isomer exceeds 10 % of that of the linear isomer, it is recommended to estimate its concentration based on the response factor of the linear standard.

The PFCAs and PFSAs are almost dissociated completely in environmental matrices. If salts are used for the preparation of calibration standards, quantification results should be calculated for the corresponding acids.

7. Quality Assurance and Quality Control

Prior to the analysis of environmental samples, the method should be subject to a full in-house validation according to the requirements of the monitoring programme.

This should include the determination of limits of detection, limits of quantification, trueness, precision, linearity of calibration, measurement uncertainty and robustness.

Every sample batch should include a procedural blank that is prepared in the same way as the samples. The number of samples per batch may differ between laboratories and depend on how many samples can be processed under comparable conditions.

If mass labelled internal standards are used, absolute recoveries between 50% and 150% are acceptable. In all other cases, recoveries should be between 70% and 120%.

Within each sample batch, at least one sample should be extracted in duplicate and a laboratory control samples should be included. The results should be recorded and monitored in control charts.

At present, no certified reference material is available for PFCs in sediment. Possible bias in the analytical method should be checked by the analysis of spiked laboratory control samples.

Laboratories should demonstrate their competence by participation in laboratory proficiency testing schemes relevant for the monitoring programme. Such exercises are still rarely offered by proficiency testing providers, but a recent interlaboratory study aiming at method validation demonstrated acceptable performance of laboratories in analysing PFC in biota and water (van Leeuwen *et al*., 2009).

8. Data Reporting

For routine analysis, the data report should be in accordance with the relevant monitoring programme; it should e.g. include information about sampling, sample processing, storage and analysis. Results should be reported along with the associated measurement uncertainty.

9. References

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Acknowledgements: This technical annex was prepared by the ICES Marine Chemistry Working Group with contributions from Lutz Ahrens, Christiaan Kwadijk, Katrin Vorkamp, Peter Lepom, Philippe Bersuder, Ralf Ebinghaus and Evin McGovern.

Annex 11: Technical annex on PFCs in seawater

1. Introduction

Per- and polyfluorinated compounds (PFCs) are man-made chemicals and are ubiquitous in the environment (Giesy and Kannan, 2001). PFCs are widely used as processing additives during fluoropolymer production and as surfactants in consumer applications, including surface coatings for carpets, furniture and paper products. They are also components in breathable, waterproof fabrics, fire-fighting foams and insulators for electric wires (Kissa, 2001). From the production and use of these products, PFCs can be released into the environment.

In this document, the name PFCs refers to compounds with a hydrophilic functional group and a hydrophobic fully fluorinated chain which can vary in chain length. The polyfluorinated acids have moderate water solubilities, low pK_a values and are therefore dissociated at environmentally relevant pH values (Kissa, 2001). Perfluorinated sulfonamides and fluorotelomer alcohols (FTOHs) are neutral compounds with a moderate vapour pressure currently discussed as precursors to perfluorooctane sulfonate (PFOS) and perfluorinated carboxylic acids (PFCAs) (Ellis *et al*., 2004).

A method for the determination of PFOS and perfluorooctanoic acid (PFOA) in unfiltered water is already described in the International Standard ISO 25101 (ISO 25101, 2009). The objective of this technical annex is to provide advice on the analysis of PFCs in water based on the ISO 25101. This method should be extended to more PFCs and to water samples with a higher amount of suspended particle matter (SPM).

2. Analytes

Table 1 gives an overview of relevant PFCs for analysis in water. They are chosen from the following groups: Perfluorinated sulfonates (PFSAs), perfluorinated sulfinates (PFSiAs), PFCAs and perfluorinated sulfonamides. For monitoring purposes, the high-volume chemicals PFOS and PFOA are considered the most important PFCs and are highly recommended to be included in water monitoring. Although most studies have focused mainly on PFOA and PFOS, it is suggested that short-chained PFCs ($\leq C_8$), in particular perfluorobutane sulfonate (PFBS) and perfluorobutanoic acid (PFBA), should be included in monitoring programmes due to their good water solubility and their use as substitutes for PFOS in various applications.

Table 1: Full names, acronyms, formulas and Chemical Abstract System (CAS) numbers of native and mass-labelled PFCs relevant for water analysis.

Analyte	Acronym	Formula	CASa-Number
Perfluorobutanoic acid	PFBA	C3F7COOH	375-22-4
Perfluoropentanoic acid	PFPA	C4F9COOH	2706-90-3
Perfluorohexanoic acid	PFHxA	C5F11COOH	307-24-4
Perfluoroheptanoic acid	PFHpA	C6F13COOH	375-85-9
Perfluorooctanoic acid	PFOA	C7F15COOH	335-67-1
Perfluorononanoic acid	PFNA	C8F17COOH	375-95-1
Perfluorodecanoic acid	PFDA	C9F19COOH	335-76-2
Perfluoroundecanoic acid	PFUnDA	C10F21COOH	4234-23-5
Perfluorododecanoic acid	PFDoDA	C11F23COOH	307-55-1
Perfluorotridecanoic acid	PFTriDA	C15F25COOH	72629-94-8

Perfluorotetradecanoic acid	PFTeDA	C13F27COOH	376-06-7
Perfluorohexadecanoic acid	PFHxDA	C15F31COOH	67905-19-5
Perfluorobutane sulfonate	PFBS	C4F9SO2O-	29420-49-3 (potassium salt)
Perfluorohexane sulfonate	PFHxS	C6F13SO2O-	3871-99-6 (potassium salt)
Perfluoroheptane sulfonate	PFHpS	C7F15SO2O-	n.a.
Perfluorooctane sulfonate	PFOS	C8F17SO2O-	1763-23-1 (sodium salt)
Perfluoro-1-decanesulfonate	PFDS	C10F21SO2O-	13419-61-9 (sodium salt)
1H,1H,2H,2H-perfluorooctane sulfonate	THPFOS (6:2 FTS)	C6F13C2H4SO3-	27619-97-2
Perfluorooctane sulfinate	PFOSi	C8F17SO2-	n.a.
Perfluorooctane sulfonamide	PFOSA	C8F17SO2NH2	754-91-6
Internal Standards			
Perfluoro-n-(1,2,3,4-13C4)butanoic acid	[13C4]-PFBA	[2,3,4-13C3]F713COOH	n.a.
Perfluoro-n-(1,2-13C2)hexanoic acid	[13C2]-PFHxA	C4F9[2-13C]F213COOH	n.a.
Perfluoro-n-[1,2,3,4-13C4]octanoic acid	[13C4]-PFOA	C4F9[2,3,4-13C3]F613COOH	n.a.
Perfluoro-n-[1,2,3,4,5-13C5]nonanoic acid	[13C5]-PFNA	C4F9[2,3,4,5-13C4]F813COOH	n.a.
Perfluoro-n-[1,2-13C2]decanoic acid	[13C2]-PFDA	C8F1713CF213COOH	n.a.
Perfluoro-n-[1,2-13C2]undecanoic acid	[13C2]-PFUnDA	C9F1913CF213COOH	n.a.
Perfluoro-n-[1,2-13C2]dodecanoic acid	[13C2]-PFDoDA	C10F2113CF213COOH	n.a.
Perfluoro-1-hexane[18O2]sulfonate	[18O2]-PFHxS	C6F13S[18O2]O-	n.a.
Perfluoro-1-[1,2,3,4-13C4]octanesulfonate	[13C4]-PFOS	C4F9[1,2,3,4-13C4]F8SO2O-	n.a.
Perfluoro-1-[1,2,3,4-13C4]octanesulfinate	[13C4]-PFOSi	C4F9[1,2,3,4-13C4]F8SO2-	n.a.

a, Chemical Abstract System; n.a., not available

3. Sampling, transportation and storage

The sampling should be carried out by trained personnel being aware of the risk of contamination of samples if incorrect handled. It is recommended using sampling containers made of polypropylene, glass or stainless steel (for further details see ISO 25101).

4. Sample preparation

4.1 Pre-treatment

It is recommended filtering the samples before extraction to remove SPM using glass fibre filters (GFFs), and after filtration, rinsing the equipment with a polar solvent.

After filtration the sample should be spiked with a mass-labelled internal standard (IS) mixture at concentrations close to the environmental level. Before extraction the

sample should be incubated with the IS for about 1 h at 4° C so that the IS can interact with the matrix.

4.2 Extraction

The extraction is carried out by solid phase extraction (SPE) according to the ISO 25101.

4.3 Clean-up

Sample matrix may affect ionisation yield (enhancement/suppression) in electrospray tandem mass spectrometry (ESI-MS-MS), hence, a clean-up of the extracts is often necessary. An appropriate clean-up method has been described by Powley *et al.* (2005). Briefly, 25 mg of graphitized carbon adsorbent (e.g., ENVI-Carb™, 100 m^2 g^{-1}, 120/400 mesh) and 50 μL acetic acid are added into a small tube. The extract is concentrated to 1 mL and transferred into this tube. The suspension is mixed vigorously, centrifuged and finally, 0.5 mL of the supernatant is transferred to another flask.

An injection standard (InjS) can be added to the final extract for correction of the injection volumes and calculation of the recoveries of the mass-labelled IS. The InjS should not occur in environmental samples, hence, the use of a mass-labelled InjS is suggested.

The solvent composition of the final extract should correspond to the mobile phase of the liquid-chromatography (LC) method in order to obtain a satisfactory peak shape of the compounds, in particular of early eluting short-chain PFCs. Unless the samples are analysed immediately, the vials should be kept at <4°C and analysed within one week.

5. Instrumental analysis

LC coupled with a tandem mass spectrometer and interfaced with an electrospray ionisation source in a negative-ion mode (LC-(-)ESI-MS/MS) (Hansen *et al.*, 2001) or LC coupled with an (-)ESI time-of-flight mass spectrometer (LC-ESI-QTOF-MS) (Berger and Haukas, 2005) can be used for PFC analysis. Tandem MS and QTOF-MS have the advantage of low instrumental noise with a high selectivity (for further details see ISO 25101).

5.1 Liquid chromatography

Modifications of the instrument might be necessary to minimise contact with fluorine-containing materials (Yamashita *et al.*, 2004). A scavenger cartridge can be installed between the pump and injector to trap contaminants originating from the degasser, connecting tubes and the mobile phase.

5.2 Detection methods

The most widely used technique for detection of PFCs is tandem MS operated in the MRM mode. Typical precursor and product ions are given in Table 2. The sensitivity of tandem MS is usually about one order of magnitude higher than that of QTOF-MS (Berger *et al.*, 2004).

Table 2: Precursor and product ions for PFCs analysed using LC-(-)ESI-MS/MS.

Analyte	Precursor ion (m/z)	Product ion (m/z)
PFBA	112.9	168.7
PFPA	262.8	218.9
PFHxA	312.9	268.8
PFHpA	362.9	318.9
PFOA	413.0	368.9
PFNA	462.9	418.9
PFDA	512.9	469.0
PFUnDA	562.9	519.0
PFDoDA	613.0	568.9
PFTriDA	663.1	618.9
PFTeDA	713.0	669.0
PFHxDA	812.8	769.1
PFBS	298.9	79.8
PFHxS	398.9	79.8
PFHpS	449.0	79.3
PFOS	499.0	79.7
PFDS	598.9	79.5
THPFOS (6:2 FTS)	426.9	406.7
PFOSi	482.8	418.9
PFOSA	497.9	77.9
[13C4]-PFBA	216.8	171.8
[13C2]-PFHxA	314.9	269.9
[13C4]-PFOA	417.0	371.8
[13C5]-PFNA	467.9	423.0
[13C2]-PFDA	514.9	469.8
[13C2]-PFUnDA	565.0	519.8
[13C2]-PFDoDA	614.9	569.9
[18O2]-PFHxS	403.0	83.9
[13C4]-PFOS	502.9	79.5
[13C4]-PFOSi	486.9	422.9

6. Calibration and quantification

6.1 Standards

The use of commercially available standards with a purity of > 99% is recommended. Suggestions for mass-labelled IS are given in Table 1. It is strongly recommended to use IS for PFC-analysis, to compensate for signal enhancement/suppression or losses during sample preparation. If possible, the corresponding mass-labelled IS should be used for each target analyte. In case a mass-labelled standard is not available, an IS with physicochemical characteristics and recovery rates similar to that of the target compound can be used but matrix suppression/ enhancement effects must first be checked in LC-ESI-MS/MS (for further details see ISO 25101).

6.2 Calibration and quantification

The calibration and quantification should be carried out using the method of internal standard according to the ISO 25101. In case of matrix effects, standard addition may be an alternative calibration option.

Some PFCs show more than one peak in the chromatogram, which is due to the presence of branched isomers. The ratio of linear to branched isomers can differ between the calibration standard and environmental samples. Branched isomers cannot be quantified precisely because of the lack of proper calibration standards. If the peak area of the branched isomer exceeds 10 % of that of the linear isomer, it is recommended to estimate its concentration based on the response factor of the linear standard.

The PFCAs and PFSAs are almost dissociated completely in environmental matrices. If salts are used for the preparation of calibration standards, quantification results should be calculated for the corresponding acids.

7. Quality Assurance and Quality Control

Prior to the analysis of environmental samples, the method should be subject to a full in-house validation according to the requirements of the monitoring programme. This should include the determination of limits of detection, limits of quantification, trueness, precision, linearity of calibration, measurement uncertainty and robustness (for further details see ISO 25101).

Within each sample batch, at least one sample should be extracted in duplicate and a laboratory control samples should be included. The results should be recorded and monitored in control charts.

Laboratories should demonstrate their competence by participation in laboratory proficiency testing schemes relevant for the monitoring programme. Such exercises are still rarely offered by proficiency testing providers, but a recent interlaboratory study aiming at method validation demonstrated acceptable performance of laboratories in analysing PFC in biota and water (van Leeuwen *et al.*, 2009).

8. Data Reporting

For routine analysis, the data report should be in accordance with the relevant monitoring program; it should e.g. include information about sampling, sample processing, storage and analysis. Results should be reported along with the associated measurement uncertainty.

9. References

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Acknowledgements: This technical annex was prepared by the ICES Marine Chemistry Working Group with contributions from Lutz Ahrens, Christiaan Kwadijk, Katrin Vorkamp, Peter Lepom, Philippe Bersuder, Ralf Ebinghaus and Evin McGovern

Annex 12: Minimum PCDD/F concentrations from various studies

Sample	Sampling area	Source	Concentrations, ng TE/kg ww			(29 compounds)				
Fish			MIN PCDD/PCDF	MIN dlPCB	Min Sum PCDD/F+dlPCB	Comment	PCDD	PCDF	Non-*ortho* PCB	Mono-*ortho* PCB
Mackerel filet	Northern North Sea	NIFES report, 2008	0.15	0.2	0.35		0,10-0,69	0,05-0,32	0,15-0,73	0,05-1,2
Greenland halibut filet	Muleegga area, North Sea	NIFES report, 2008	0.39	1.21	1.6		0,10-2,02	0,29-6,2	0,91-6,95	0,30-3,50
Atlantic halibut, muscle	Norwegian Sea	NIFES report, 2008	0.4	0.64	1.04	Contaminated?	0,11-1,3	0,29-3,4	0,46-13,8	0,17-8,1
Atlantic halibut, belly	Norwegian Sea	NIFES report, 2008	1.13	2.57	3.7	Contaminated?	0,35-3,1	0,78-8,1	1,9-33	0,67-19
Fish available commercially	8 estuarine locations, Australian coast	Australian report 2004 (6.b.1-6.b.2)	0.015	0	0.015					
Fish available commercially	12 marine (mainly coastal) locations, Australian seas	Australian report 2004 (6.b.1-6.b.2)	0.011	0	0.011					
Whiting	Australia	Mosse and Haynes, 1993	0.05							
Flathead	Australia	Mosse and Haynes, 1993	0.15							
consumer fish from the Pacific ocean	Spanish shops	Abad *et al*. 2003	0.37							
Wild Atlantic salmon	Irish North Atlantic	Irish report 2007	0.13	0.28	0.41					
Fresh tuna (albacore)	Irish North Atlantic	Irish report 2007	0.11	0.5	0.61					
Fresh herring	Irish North Atlantic	Irish report 2007	0.38	0.55	0.93					
Fresh mackerel	Irish North Atlantic	Irish report 2007	0.21	0.76	0.97					
Bartailed Flathead	Tokyo Bay	Sakuari *et al*. 2001	0.32							
Farmed char	Canada	Rawn *et al*. 2006			0.263					
Wild char	Canada	Rawn *et al*. 2006			0.305					
Farmed salmon	Canada	Rawn *et al*. 2006			0.468					
Wild salmon	Canada	Rawn *et al*. 2006			0.113					
Farmed Tilapia	Canada	Rawn *et al*. 2006			0.023					
Wild tilapia	Canada	Rawn *et al*. 2006			0.048					
Farmed trout	Canada	Rawn *et al*. 2006			0.26					
Sardine	Catalonia	Bocio *et al*. 2007	0.22	1.19	1.41					
Tuna	Catalonia	Bocio *et al*. 2007	0.19	1.17	1.36					
Anchovy	Catalonia	Bocio *et al*. 2007	0.19	1.24	1.43					
Mackerel	Catalonia	Bocio *et al*. 2007	0.23	0.89	1.12					
Swordfish	Catalonia	Bocio *et al*. 2007	0.06	0.42	0.48					
Salmon	Catalonia	Bocio *et al*. 2007	0.24	0.87	1.11					
Hake	Catalonia	Bocio *et al*. 2007	0.04	0.34	0.38					
Red mullet	Catalonia	Bocio *et al*. 2007	0.5	4.15	4.65					
Sole	Catalonia	Bocio *et al*. 2007	0.13	0.24	0.37					
Cuttlefish	Catalonia	Bocio *et al*. 2007	0.03	0.02	0.05					
Common cuttlefish	Spain	Bordajandi *et al*. 2006	0.124	0.086	0.21					
Wedge sole	Spain	Bordajandi *et al*. 2006	0.085	0.083	0.168					
Common sole	Spain	Bordajandi *et al*. 2006	0.076	0.081	0.157					
White sea bream	Spain	Bordajandi *et al*. 2006	0.124	0.438	0.562					
Angler fish	Spain	Bordajandi *et al*. 2006	0.043	0.049	0.092					
Sardine	Spain	Bordajandi *et al*. 2006	0.173	0.757	0.93					
Salmon	Baltic sea Polish coast	PISKORSKA–PLISZCZYŃSKA *et al*. 2004	2.01							
Cod	Baltic sea Polish coast	PISKORSKA–PLISZCZYŃSKA *et al*. 2004	0.82							
Flounder	Baltic sea Polish coast	PISKORSKA–PLISZCZYŃSKA *et al*. 2004	1.52							
Herring	Baltic sea Polish coast	PISKORSKA–PLISZCZYŃSKA *et al*. 2004	1.1							
Fish	Pearl river delta area, China	Zhang *et al*. 2007	0.063							
Sea bass	France	Bodin *et al*. 2007	0.38	6.08	6.46					
Garfish (*Hemiramphus*	Australia	Matthews *et al*. 2008	0.0621	0	0.06					

robustus)	Australia	Matthews *et al*. 2008				
Mullet (*Mugil cephalus*) n =	Australia	Matthews *et al*. 2008	0.0365	0.0135	0.05	
Flounder	Australia	Matthews *et al*. 2008	0.1656	0.0344	0.2	
(Pseudorhombus	Australia	Matthews *et al*. 2008				
jenynsii)	Australia	Matthews *et al*. 2008				
Summer Whiting	Australia	Matthews *et al*. 2008	0.01008	0	0.01	
(Sillago ciliata)	Australia	Matthews *et al*. 2008		0		
Butter Bream	Australia	Matthews *et al*. 2008	0.252	0.098	0.35	
(Monodactylus	Australia	Matthews *et al*. 2008				
argenteus)	Australia	Matthews *et al*. 2008				
Moses Perch (*Lutjanus*	Australia	Matthews *et al*. 2008	0.288	0.062	0.35	
russelli)	Australia	Matthews *et al*. 2008				
Snapper (*Pagrus*	Australia	Matthews *et al*. 2008	0.1139	0.0061	0.12	
auratus)	Australia	Matthews *et al*. 2008				
Yellowfin Bream	Australia	Matthews *et al*. 2008	0.01584	0.00416	0.02	
(*Acanthopagrus*	Australia	Matthews *et al*. 2008				
australis)	Australia	Matthews *et al*. 2008				
Flathead	Australia	Matthews *et al*. 2008	0.01273	0.01727	0.03	
(*Platycephalus*	Australia	Matthews *et al*. 2008				
fuscus)	Australia	Matthews *et al*. 2008				
Tailor (*Pomatomus*	Australia	Matthews *et al*. 2008	0.306	0.354	0.66	
saltatrix)		Matthews *et al*. 2008				
Mackerel		Matthews *et al*. 2008	0.31437	0.23563	0.55	
Anchovy	Mediterranean	van Leeuwen *et al*. 2007	0.6	7.9	8.5	
Coalfish	North Sea	van Leeuwen *et al*. 2007	0.1	0.2	0.3	
Cod	North Sea	van Leeuwen *et al*. 2007	0.07	0.23	0.3	
Eel farmed	The Netherlands, Italy	van Leeuwen *et al*. 2007	0.7	3.2	3.9	
Eel wild	IJsselmeer	van Leeuwen *et al*. 2007	1.4	7.3	8.7	
Herring	The English Channel, North Sea	van Leeuwen *et al*. 2007	1.3	1.1	2.4	
	The English Channel, North Sea, Shetland Islands	van Leeuwen *et al*. 2007	0.7	0.6	1.3	
	The English Channel, North Sea, Skagerrak	van Leeuwen *et al*. 2007	0.7	0.9	1.6	
Mackerel	Skagerrak, Atlantic Ocean, Celtic Sea	van Leeuwen *et al*. 2007	0.3	1	1.3	
	North Sea, Shetland Islands, Atlantic Ocean	van Leeuwen *et al*. 2007	0.2	0.6	0.8	
	North Sea, Shetland Islands	van Leeuwen *et al*. 2007	0.3	1.1	1.4	
Pike perch	Nieuwe Merwede, rivers Lek, Amer, Rhine, Waal	van Leeuwen *et al*. 2007	0.8	1.9	2.7	
Salmon	Norway, Scotland	van Leeuwen *et al*. 2007	1.1	2.2	3.3	
	Norway, Scotland	van Leeuwen *et al*. 2007	0.3	1.6	1.9	
Seabass	Mediterranean	van Leeuwen *et al*. 2007	1	14	15	
Tuna	Sri Lanka, Mediterranean	van Leeuwen *et al*. 2007	0.01	0.02	0.03	
Bivalves						
Various bivalves	11 estuarine locations, Australian coast	Australian report 2004 (6.b.1-6.b.2)	0.0068	0	0.0068	
Various bivalves	6 marine locations, Australian seas	Australian report 2004 (6.b.1-6.b.2)	0.022	0	0.022	
Various bivalves	Western Adriatic Sea	Bayarri *et al*. 2001			0.07	
Various bivalves	Spanish Atlantic&Mediterranean coast	Abad *et al*. 2004	0.11			
Oysters	Japan	Tsutsumi *et al*., 2003	0.22			
Various bivalves	Korea	Choi *et al*. 2001	0.001			
Various bivalves	New Zealand	New Zealand report 1999	0.015			Only PCDD/PCDF

Oysters (*C.gigas*)	Irish North Atlantic	Irish report 2010	0.09	0.1	0.19				
Oysters and mussels	Korean sea waters	Oh *et al*. 2003	0.129	0.171	0.3				
Farmed mussels	Canada	Rawn *et al*. 2006			0.083				
Farmed oysters	Canada	Rawn *et al*. 2007			0.078				
Wild oysters	Canada	Rawn *et al*. 2008			0.101				
Clam	Catalonia	Bocio *et al*. 2007	0.04	0.05	0.09				
Mussel	Catalonia	Bocio *et al*. 2007	0.14	0.24	0.38				
Mussel	France	Bodin *et al*. 2007	1.12	1.33	2.45				
D. trunculus	Spain	Bordajandi *et al*. 2006	0.059	0.037	0.096				
C. gallina	Spain	Bordajandi *et al*. 2006	0.054	0.04	0.094				
Pacific Oyster	Australia	Matthews *et al*. 2008	0.323	0.037	0.36				
(*Crassostrea gigas*)	Australia	Matthews *et al*. 2008							
Pearl Oyster (*Pinctada*	Australia	Matthews *et al*. 2008	0.0704	0	0.0704				
margaritifera)	Australia	Matthews *et al*. 2008							
Pipi (*Donax deltoides*)	Australia	Matthews *et al*. 2008	0.1863	0.0037	0.19				
Mussel	Eastern Scheldt, Wadden Sea	van Leeuwen *et al*. 2007	1.1	1.2	2.3				
Mussel	Greenland	Asmund, unpublished data	0.019	0.030	0.049				
Mussel	Greenland	Asmund, unpublished data	0.033	0.050	0.083				
Other									
Fish and bivalves together	Japan	Environment Agency of Japan, 1999	0.002			NB: aquatic biota (fish and bivalvs together)			
Fish oil (16 types)	Various shops in Bergen area	NIFES report, 2008	0.1	0.04	0.14	0,08-0,34	0,02-0,24	0,02-0,73	0,02-1,19
Farmed shrimp	Canada	Rawn *et al*. 2006			0.025				
Wild shrimp	Canada	Rawn *et al*. 2007			0.04				
Wild crabs	Canada	Rawn *et al*. 2008			0.114				
Squid	Catalonia	Bocio *et al*. 2007	0.1	0.61	0.71				
Shrimp	Catalonia	Bocio *et al*. 2007	0.06	0.03	0.09				
Spider crab	France	Bodin *et al*. 2007	0.3	0.38	0.68				
Edible crab	France	Bodin *et al*. 2007	0.25	0.25	0.5				
Velvet swimming crab	France	Bodin *et al*. 2007	4.77	8.55	13.32				
Norway lobster	France	Bodin *et al*. 2007	0.68	0.47	1.15				
Prawns A	Spain	Bordajandi *et al*. 2006	0.092	0.062	0.154				
Bay Prawn	Australia	Matthews *et al*. 2008	0.2156	0.1544	0.37				
(*Metapenaeus*									
macleayi)									
Banana Prawn	Australia	Matthews *et al*. 2008	0.2496	0.1504	0.4				
(*Penaeus*	Australia	Matthews *et al*. 2008							
merguiensis)	Australia	Matthews *et al*. 2008							
Tiger Prawn (*Penaeus*	Australia	Matthews *et al*. 2008	0.1674	0.0026	0.17				
esculentus)	Australia	Matthews *et al*. 2008							
Mudcrab (*Scylla*	Australia	Matthews *et al*. 2008	0.13	0	0.13				
serrata)									
Shrimp	Norway, Western Scheldt, Wadden Sea	van Leeuwen *et al*. 2007	0.1	0.1	0.2				

Annex 13: Revised guidelines for monitoring nutrients

JAMP Eutrophication Monitoring Guidelines: Nutrients

JAMP Eutrophication Monitoring Guidelines: Nutrients

1. Introduction

Nutrient enrichment may give rise to eutrophication if other conditions are favourable. Nutrient concentrations may be used to help assess the trophic status of marine waters and to determine the cause of eutrophication problems. These guidelines are intended to support the minimum monitoring requirements of the Eutrophication Monitoring Programme. In addition they will support the Water Framework Directive and the European Marine Strategy Framework Directive.

2. Purposes

The measurement of nutrients in seawater is carried out for, *inter alia*, the following purposes:

- to monitor the spatial distribution of nutrient concentrations within the Maritime Area which are influenced by anthropogenic nutrient inputs, taking into account the minimum monitoring requirements of the Eutrophication Monitoring Programme;
- to monitor temporal trends in nutrient concentrations, over periods of several years, (in areas identified under purpose 1) in order to assess whether there are increasing or decreasing trends in concentrations as a result of changes in inputs, taking into account the minimum monitoring requirements of the Eutrophication Monitoring Programme;
- to support an assessment of the degree of nutrient enrichment within the Maritime Area, within the context of the work on the development and implementation of a Common Procedure for the Identification of the Eutrophication Status of the Maritime Area;
- to further the work on understanding the relationship between nutrient concentrations and/or fluxes and the eutrophication effect parameters specified in the minimum monitoring requirements of the Eutrophication Monitoring Programme.

3. Quantitative objectives

The quantitative objectives must take into account the characteristics (*e.g.* variability) of the marine areas concerned.

It is intended that the region-specific temporal trend monitoring programme should have the power (*e.g.* 90%) to detect a change in concentration (*e.g.* 50%) over a selected period (*e.g.* 10 years). To clarify the situation and to help define objectives Contracting Parties should undertake statistical analyses of their existing data sets. This would help to determine the representativeness of the monitoring stations and would also help to determine the selection of suitable sampling stations and sampling frequencies.

The spatial distribution monitoring programme should enable Contracting Parties to determine the representativeness of their monitoring stations with regard to spatial variability in nutrient concentrations. This would include a definition of the extent of the monitoring area and some understanding of the randomness of the monitoring stations.

4. Sampling strategy

Monitoring should consider the following nutrient species[5]:

- ammonium, nitrate, nitrite, particulate nitrogen, total nitrogen;
- *ortho*phosphate, total phosphorus, particulate phosphorus;
- dissolved reactive silicate;

From these parameters the dissolved organic fractions of nitrogen (DON) and phosphorus (DOP) can be calculated.

Apart from station information, temperature and salinity are essential supporting parameters. Additional parameters, including chlorophyll pigments, Secchi depth, turbidity, current speed or information about tides, may be needed depending on site and purpose of the investigation.

The most important inorganic nutrients with respect to eutrophication problems are phosphate and the sum of nitrite and nitrate. Silicate and ammonia are important mainly in relation to particular events and situations. Ammonia is often present in high concentrations in low oxygen waters, e.g. anoxic stagnant bottom waters. Total phosphorus, total nitrogen and particulate nitrogen and phosphorus are important in relation to temporal trends, ecosystem analysis and nutrient budgets. The dissolved organic fractions should also be recognized as a significant source of matter for the recycling of inorganic nutrient species within the system. Dissolved organic carbon concentration is necessary for the interpretation of organic nutrient concentrations.

4.1 Monitoring for purposes 1, 2 and 3

Monitoring for purposes 1, 2 and 3 should take place at the time of lowest algal activity, which is usually winter. This is because surface waters become progressively depleted in inorganic nutrients during spring, summer and autumn due to their removal by phytoplankton. Therefore, for the Maritime Area as a whole, the sampling period and the sampling frequency cannot be specified in terms of months or dates; the period is dependent on regional and interannual differences.

Monitoring for nutrients should take place along salinity gradients in order to account for freshwater run-off from land to sea and as a measure to improve consistency. Monitoring for nutrients should take account of inputs, including terrestrial and atmospheric inputs, and the oceanographic characteristics of each region.

A nutrient-salinity relationship for a coastal area can provide information about processes involved in regulating nutrient concentrations and total amounts of nutrients. A linear relationship indicates that physical mixing is the dominant process regulating the nutrient concentration, while non-linearity indicates the additional influence of chemical and/or biological processes. Several sources of freshwater or offshore water may add complexity to nutrient-salinity mixing diagrams, and temporal variability in the nutrient concentrations of the sources may contribute additional scatter and variability to the relationship.

The temporal trend monitoring strategy should ensure that sufficient data are collected in order to confirm that maximum winter nutrient concentrations are covered

[5] The nutrient species specified in the minimum monitoring requirements of the Eutrophication Monitoring Programme are as follows: ammonium, nitrite, nitrate and phosphate. Silicate is a required parameter in problem and potential problem areas.

and that a nutrient-salinity curve can be constructed from which an adequate concentration normalised to a specified salinity (*e.g.* 30) can be calculated.

In most cases it will be possible to decide only after sampling with suitable temporal and spatial resolution, and with the assistance of supporting parameters which prove lowest algal activity (*e.g.* chlorophyll a) that the data are suitable for temporal trend studies.

4.2 Monitoring for purpose 4

For purpose 4, the sampling strategy for nutrients should be in accordance with the sampling strategy for the eutrophication effect parameters *i.e.* phytoplankton and benthos.

5. Sampling equipment

5.1 Equipment

A variety of sampling bottles can be used for the collection of nutrient samples. These are deployed on either a CTD-rosette or are clamped to a hydrowire and lowered to the prescribed depth. Reliability of CTD and depth measurements should be ensured and documented.

Working in (shallow) estuaries and coastal areas sometimes require special equipment and sampling, e.g. samples collected by pumping water through a flexible plastic hose deployed over the side of the ship. It is however essential to validate that the equipment used is demonstrated as adequate for the desired purpose.

It is important to use suitable bottles to collect and store samples, i.e. glass bottles may leach silicate and phosphate into samples. Polyethylene or polypropylene bottles may be used. The sample bottles and containers should always be rinsed with sample water before filling.

5.2 Contamination

Sampling activities always include the risk of contamination, which may have various sources depending on specific sampling situations. Care should be taken to ensure good laboratory practice during sampling procedure (e.g. avoidance of contamination from ship, cleaning of instrumentation and bottles, etc.). It is recommended that laboratories performing measurements check contamination risks and document how to minimize and control potential contamination during sampling. Among the common nutrients ammonia is usually the most challenging to determine due to airborne contamination. Avoid contact with cigarette smoke (both in the air and on workers fingers). Analysis of ammonia on ship can be challenging as it is more difficult to control the environment in which the analysis occurs.

6. Storage and pre-treatment of samples

6.1 Storage

Nutrient determinations should be carried out as soon as possible after sampling. Ammonia should be determined immediately after sampling, while nitrate, phosphate and silicate should be determined within a few hours after sampling with samples protected from light and stored in a refrigerator between sampling and analysis.

If immediate analysis is not possible samples must be preserved. Commonly used preservation methods are freezing the samples or adding a preservative, e.g. $HgCl_2$. If

the sample contains amounts of particulate matter which may compromise the analysis, it should be filtered to remove the particles before freezing. Samples for the determination of silicate, which have been frozen, should be defrosted for sufficient time for de-polymerisation to occur. This is particularly important when there are high silicate concentrations in the water.

Since no preservation method for nutrients can, at present, be recommended for general use, each laboratory must validate, and document, its storage methods for each nutrient before they are used routinely. The validation should be done over the whole seasonal cycle to investigate varying conditions e.g. during high and low nutrient concentrations and during high and low primary productivity. The QUASH (Quality Assurance of Sampling and Sample Handling) project (1996-2000) carried out an intercomparison of sampling handling and preservation methods for nutrients in seawater for a number of laboratories. The outcome demonstrated the need for laboratories to validate and document their procedures and highlighted the particular challenges of preserving samples for subsequent ammonia analysis. (QUASH 2000)

6.2 Pre-treatment

Unnecessary manipulation of the samples should be avoided, however filtration or centrifugation become necessary in particle-rich waters (*i.e.* in coastal zones, estuaries, or during phytoplankton blooms). Filtration with glass fibre filters (*e.g.* Whatman GF/F) or hydrophilic cellulose acetate filters (*e.g.* Sartorius Minisart 0.45 μm pore size) should generally be adequate, but each laboratory should prove the filtration methodology on test samples before using it for natural samples. If unfiltered samples are analysed there should be a correction for turbidity, when using a manual method for determination.

7. Analytical procedures

The determination of nutrients is largely based on colorimetric methods (cf. Grasshoff *et al*., 1999 and Kirkwood, 1996). There are also fluorometric methods available, e.g for the analysis of ammonia in seawater (Holmes *et al.*, 1999; Aminot *et al.*, 2001) or UV -spectrophotometric methods for the direct determination of nitrate.

Most methods commonly used are manual methods or manual methods adjusted to automated analytical equipment (continuous flow analysis or flow injection analysis). In addition to the validation of the chemical method itself, the validation of the handling procedures and maintenance of the automatic equipment is important.

Manuals are available or are in preparation, which detail what to consider especially when working at sea with continuous flow analysis of nutrients (Hydes *et al*, 2009).

8. Analytical quality assurance

The quality assurance programme should ensure that the data are fit for the purpose for which they have been collected, *i.e.* that they satisfy detection limits and levels of accuracy compatible with the objectives of the monitoring programme (*cf.* Table 1). The quality assurance procedures must cover all steps of the nutrient determinations, including sampling, storage of samples, analytical procedures, maintenance and handling of the equipment, training of the personnel, as well as an audit trail. It is recommended that the laboratory is accredited. The laboratory should also take part in intercalibration exercises and laboratory performance exercises to provide external verification of results.

Specific technical information on QA is to be found in Kirkwood (1996) and Vijverber and Cofino (1987) and in the Nordtest report (2006). Activities introducing Certified Reference Materials (CRMs) for nutrients in seawater are in process but the material is not yet commercially available on a large scale. CRM´s for the following nutrients in seawater are available in limited amounts: *Ortho*phosphate, Silicate, Nitrite, Nitrite and Nitrate (National Research Council of Canada. http://inms-ienm.nrc-cnrc.gc.ca/calserv/crm_files_e/MOOS-1_e.pdf). VKI CRMs for marine waters also include NH_4, TN and TP. (Eurofins, Denmark).

Table 1: Minimum analytical requirements for the minimum monitoring requirements

Determinand	Detection limit µmol/l	Accuracy requirement (±Z± score ± 2) low concentrations	medium concentrations - high concentrations
phosphate	0.02	± 25% (max)	± 12% (max)
nitrate + nitrite	0.1	± 25% (max)	± 12% (max)
nitrite	0.02	± 25% (max)	± 12% (max)
ammonia	0.1	± 25% (max)	± 12% (max)
silicate	0.1	± 25% (max)	± 12% (max)

Notes on table

1. "Low" concentrations are defined as being within a factor of ~20 of the respective detection limits.

2. Use of the term "Z score" is well understood by all QUASIMEME laboratories. A $|Z|$ score ≤ 2 (*i.e.* from -2 to +2) in a laboratory performance test is considered a minimum requirement for a satisfactory analysis. As an illustration in Round I of QUASIMEME (1993) a test sample had an assigned nitrate + nitrite concentration of 27,4 µmol/l. Laboratory A's result of 30,7 µmol/l was 27,4 µmol/l +12 % (Z = +2), and laboratory B's result of 24,1 µmol/l was 27,4 µmol/l -12% (Z = -2). Both were considered "acceptable", but were at the limits of the "acceptable" range. A $|Z|$ score ≤ 1 should be attainable.

3. Table 1 refers to the expected performance levels. In practice and at present, detection limits 2-3 times higher would be acceptable.

9. The use of in situ nutrient analysers

Platform types

Autonomous nutrient analysers have been increasingly used for providing *in situ* semi-continuous measurements of nutrient concentrations. Where a static platform is used (such as on a mooring), high frequency measurements of nutrient concentrations at a single point may be obtained. When used with a ships pumped seawater supply (such as Ferrybox), a map of nutrient concentrations over a wide area may be obtained. A Ferrybox system allows samples from a fixed depth to be obtained. A mooring may allow numerous depths to be monitored where analysers can be deployed at multiple depths. These techniques are especially useful in environments where there is a substantial temporal and spatial variability of nutrient concentrations. Such platforms should be considered to be part of a wider monitoring programme which includes ship based observations which provide a wide spatial coverage as required within section 3.

Instrument selection

Sensors for *in-situ* applications are based on wet chemistry colorimetric methods or a direct optical UV spectrophotometric measurement (nitrate only). Reviews by Moore *et al* (2009) and Johnson *et al*. (2007) discuss different types of sensor. Potential problems faced with *in-situ* sensors are biofouling, and power constraints. Biofouling may be more readily overcome on a Ferrybox system where cleaning of the measurement

system may be programmed into the routine cycle. Controls implemented on some *in-situ* optical sensors include wiped sensors, guarding with copper mesh and chlorination. Power constraints on a Ferrybox system will not usually be a problem but may be a consideration on a mooring. The extent of biofouling and power considerations will contribute to determining the length of time sensors can be left *in situ*.Coloured dissolved organic matter (CDOM) has a spectral componed to its absorption curve and thus appreciable CDOM may interfere with nitrate measurement when using optical sensors, although in general this is unlikely to be an issue in many marine applications.

Quality Assurance

Appropriate calibration and ongoing quality control must be implemented to ensure that data collected are fit for purpose. Routine laboratory testing and validation of results against discrete samples analysed in the laboratory must be undertaken to ensure that comparable results of known and acceptable quality are obtained. To date, limits of detection obtained with *in-situ* nutrient analysers are an order of magnitude higher than for conventional laboratory-based techniques, at best 1μmol l^{-1}.

10. Reporting requirements

Data reporting should be in accordance with the requirements for the latest ICES reporting formats, together with information on methods used, detection limits, reference values and any other comments or information relevant to an ultimate assessment of the data. In order to establish the acceptability of the data, they should be reported together with the dates and results of participation in intercalibration exercises and summary information from recent control charts, including dates, sample sizes, means and standard deviations.

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Acknowledgements: This technical annex was revised by the ICES Marine Chemistry Working Group Chemical Oceanography Subgroup with contributions from Klaus Nagel

Annex 14: Revised Guidelines for Monitoring Dissolved Oxygen

Eutrophication Monitoring Guidelines: Oxygen

JAMP Eutrophication Monitoring Guidelines: Oxygen

1. Introduction

Biological activity and hydrodynamic processes are the main causes of change in the oxygen concentration in seawater. Nutrient enrichment/eutrophication may give rise to decreased oxygen concentrations and saturation percentages, increased frequency of low oxygen concentrations and increased rate of oxygen consumption, mainly in deeper layers of stratified waters. Dissolved oxygen concentration is used as an indicator of ecosystem health. The use of dissolved oxygen concentration as an Ecological Quality Objective (EcoQO) has been established within an Ecological Quality Framework for an eco-system based approach to the management of the North Sea. It is also used within the Water Framework Directive and European Marine Strategy Framework Directive. These guidelines are intended to support the minimum monitoring requirements of the OSPAR Eutrophication Monitoring Programme and will also support monitoring in accordance with the above directives. The reader should refer to detailed guidance on sampling and measurement of dissolved oxygen in marine waters provided in Aminot, 1997.

2. Purposes

The measurement of oxygen concentrations in water is carried out for, *inter alia*, the following purposes:

1) to establish the spatial distribution and frequency of low oxygen concentrations;

2) to establish temporal trends in oxygen concentration over periods of several years;

3) as a component of the Eutrophication Monitoring Programme.

3. Quantitative objectives

The quantitative objectives must take into account the characteristics (*e.g.* variability) of the marine areas concerned.

It is intended that the region-specific temporal trend monitoring programme should have the power (*e.g.* 90%) to detect a change in concentration (*e.g.* 50%) over a selected period (*e.g.* 10 years). To clarify the situation and to help define objectives Contracting Parties should undertake statistical analyses of their existing data sets. This would help to determine the representativeness of the monitoring stations and thus the selection of suitable sampling stations and sampling frequencies.

The spatial distribution monitoring programme should enable Contracting Parties to determine the representativeness of their monitoring stations with regard to spatial variability in oxygen concentrations. This would include a definition of the extent of the monitoring area and some understanding of the randomness of the monitoring stations.

4. Sampling strategy

Oxygen deficits occur in the deeper layers of stratified water, including semi-enclosed basins. Low oxygen concentrations can also be found at times of increased oxygen consumption following maximum primary production and are concomitant with certain meteorological and hydrographic conditions (including temperature and wind speed). Oxygen concentrations may vary considerably from year to year as a

result of many influences and therefore trends may be difficult to establish. However, it may be possible to establish trends in some semi-enclosed basins.

Frequent measurement during and after the production season should take place in relation to phytoplankton bloom events, at stations suitable for this purpose, e.g. at stations characterised by vertical stratification or sited in semi-enclosed basins. Sampling should be conducted so that oxygen concentration gradients are resolved, especially those near to the seabed. In order to assess oxygen consumption rates, time-series measurements are required covering appropriate periods of time with high oxygen consumption. If hydrogen sulphide occurs, the concentration should be determined using the methylene blue method (Fonselius *et al.*, 1999). Concentrations should be given in μmole l^{-1} rather than in negative oxygen equivalents.

For the interpretation of oxygen measurements it is essential to have corresponding measurements of temperature and salinity. For some areas additional information including chlorophyll pigments, turbidity, hydrographic characteristics of the water column at the sampling site may be necessary in addition.

5. Sampling equipment

Many different water samplers may be used to collect discrete samples for oxygen determination. It is essential however, that the water sampler used completely isolates the sample from the surroundings so that no leakage or exchange occurs. In particular circumstances it may be necessary to use a special bottom water sampler.

Immediately after taking the water sample, an aliquot has to be transferred into a calibrated Winkler bottle. Care must be taken to minimize contact between the water sample and atmosphere, especially in samples with low oxygen concentrations. This includes the process of transferring the water from the sample bottle into the Winkler bottle as well as by introducing air into the sample bottle due to leakage. As this transfer of the sample is one of the steps in the whole determination procedure which is responsible for the greatest error, only well trained personnel should be allowed to take the samples.

Oxygen may also be determined using sensors. These sensors may be used attached to a CTD system, as part of an autonomous system on moored platforms or installed on ships for continuous measurements. The advantage of sensor measurements is the provision of high resolution data in space or time, depending on the instrumentation used. Sensors can be particularly useful, compared with conventional discrete sampling techniques, for determining temporal and spatial variability and for capturing short term oxygen deficiency or supersaturation events. On a commercial basis Clark type and Optode type sensors are widely available (Moore *et al.*, 2009).

As all sensors have limitations in their performance, no type of sensor can be generally recommended. These limitations may include the sensitivity, the precision of measurement, low response time, instability of measured results, instability due to varying environmental conditions, poisoning in anoxic waters, etc. Therefore it is necessary to test different sensors and select the one most suitable for measurements in the area to be observed.

Apart from a proper selection of a sensor, the calibration and handling of any sensor has to be validated. Furthermore, regular control, using the Winkler method as reference, is essential. Intervals of calibration, control of measurements and maintenance depend on the type of sensor and the environmental conditions in which the sensor is used. These intervals have to be evaluated and controlled as one element in the vali-

dation process of the sensor. The validation process also includes a description of the handling of the sensor in order to obtain the specified precision of the sensor.

6. Storage and pre-treatment of samples

Oxygen in discrete samples must be fixed immediately after collection to bind the oxygen in the sample. The precautions mentioned above must be maintained. After fixation, samples have to be kept in a dark place at a constant temperature - if possible the same as the in situ temperature - for at least one hour. The fixed sample should be titrated within 24 hours of collection. In some cases longer storage of the fixed sample may be necessary. Although not recommended, longer storage is possible, provided that storage conditions and handling procedures are validated and clearly documented. Zhang *et al*., (2002) noted that storage under seawater is advisable in such circumstances. Sensors for oxygen determination are designed for *in situ* measurements and should not be used for analysis of discrete samples.

7. Analytical procedures

Standard procedures for the determination of oxygen in discrete water samples are based on the Winkler method. Modifications of this method, which have been verified in intercalibration exercises, are described elsewhere (e.g. Carpenter, 1965; Grasshoff *et al*, 1999; Strickland and Parsons, 1968). Modifications mainly concern composition of the reagents, titration devices (manual titration, automatic systems) and the method used for detecting the end point of the titration step (e.g. visible colour change of indicator dyes, conductivity measurement, photometric detection). As verified by intercalibration exercises, reliable results can be obtained with all methods, if validated procedures are used by well trained personnel.

Oxygen sensors should only be used if their calibration, handling and maintenance is properly validated (see section 5), including procedures for regular checks of calibration and correct functioning of the sensor (stability, reproducibility, precision of results). The Winkler method should be used as reference method for this purpose. Care should also be taken to avoid unreliable results caused by ignoring the technical limitations of the sensor used for the measurements (see section 5) or by calibrating over an inappropriate range.

8. Analytical quality assurance

At present there is no Certified Reference Material available for oxygen in water. For the Winkler method it is therefore recommended to use internal laboratory procedures according to *Grasshoff at al.* (1999). In order to demonstrate reliable results, each laboratory must establish, validate and document a quality assurance system, with is adequate for the samples to be analysed. Specific technical information on quality assurance is to be found in Carpenter (1965b), Vijverber and Cofino (1987), Aminot (1997), and in the Nordtest report (2006). The effectiveness of the quality assurance system should be verified by participation in appropriate intercalibration exercises, where available, as often as possible.

9. Reporting requirements

Data reporting should be in accordance with the requirements of the latest ICES reporting formats, together with QA information on methods used, detection limits, reference values and any other comments or information relevant to an assessment of the data (e.g. participation in intercalibration exercises).

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Annex 15: Guidelines for passive sampling of hydrophobic contaminants in water using silicone strip samplers

1 Introduction

Passive sampling with silicone sheets is used to estimate concentrations of dissolved contaminants in the water. The sampling process is based on the passive diffusion of hydrophobic contaminants from the water phase into the polymer. At the initial stage of the sampler deployment, contaminants are absorbed at a constant rate that is directly proportional to the water concentration. As the sampling continues, the contaminant gradually approaches its equilibrium concentration in the sampler. Sampling kinetics generally is faster at higher flow rate and at higher temperature. The sampling kinetics can be quantified using the dissipation of performance reference compounds (PRCs) that are spiked into the sampler prior to deployment (details are given below).

More information on the working principles of passive samplers can be found in Huckins *et al*., 2006, Greenwood *et al*., 2007. The advantages and limitations of passive sampling compared with biomonitoring are also discussed in these references.

The present guideline summarises the use of passive sampling using silicone sheets. It is assumed that readers have made a choice to use passive sampling rather than biomonitoring based on considerations that fall outside the scope of these guidelines. It is also assumed that readers have made a choice for silicone rubber sheets (poydimethylsiloxane) rather than other passive sampler systems. Silicone rubber sheets have been used for the monitoring of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and hexachlorobenzene (HCB) in coastal and estuarine waters in the Netherlands, parallel to biomonitoring using mussels (Smedes, 2007a). In addition, these samplers have been used in a marine trial survey in which 13 laboratories from 9 European countries as well as one from Australia participated (Smedes, 2007b-d).

2 Preparation, deployment, and retrieval

2.1 Preparation of the sampler sheets

MCWG and WGMS recommend using the Altesil translucent rubbers from www.altecweb.com. Partition coefficients for HCB, PCBs and most parent PAHs are available for this polymer (Smedes *et al*., 2009). This includes also a number of PCBs not occurring in technical mixtures and deuterated PAHs applicable as PRCs.

The most commonly used thickness is 0.5 mm. Other thicknesses are possible but may require different conditions for pre-extraction and analysis. Polydimethylsiloxane (PDMS) films from Altecweb are available in either 30x30 or 60x60 cm sheets that can be cut in the appropriate sizes. To allow exchange of samplers between laboratories it is convenient to have sampler holders with equal sized stems positioned at equal distance (Fig 1). The preferred stem diameter is 5 mm and with stems positioned 35 mm apart. With a sampler sheet width at 5.5 cm, the fixing holes can be conveniently made using a paper punch. Fixing holes can be conveniently made by a paper punch. Punching a 55 mm wide sheet from both sides gives exactly 35 mm heart to heart distance between the holes. A practical length is 9 cm giving a surface area of approximately 100 cm^2. Longer sampler sheets are possible but the suggested size will not fold double during exposure.

After cutting, the samplers are soxhlet extracted with ethylacetate for a minimum of 100 hr. This will remove all oligomers (unpolymerized material) from the silicon rubber. Note that if sheets are packed closely, extraction efficiency will decrease and it is recommended extending extraction time to about a week. After soxhlet extraction, the sheets are transferred to a wide mouth bottle. Ethylacetate is removed from the sheets by washing them twice for 8 hours with methanol, using approximately 4 mL per sheet.

2.2. Spiking of PRCs

Candidate PRCs are all deuterated PAHs and several PCBs (3, *4, 10, 14, 21, 29, 30, 50, 55, 78, 104*, 112, 143, *145, 155*, 198 and *204*[6]). It is suggested to have a minimum of 6 PRCs covering the range of $\log K_{sw}$ 3.5 -5.5 at increments no more than 0.3 log units. Additionally, one PRC can be added that will not release at all ($\log K_{sw}$ >6) and one that is expected to be completely depleted ($\log K_{sw}$ <3.3).

The amount of PCR should be chosen in such a way that the concentration in the extract does not exceed the calibration range, but it should be high enough that after release from the sampler a residual 10% can still be accurately measured. The amount of spike substance to be added to the samplers is calculated as the amount per sampler times the number of samplers. The amounts of PRCs that are finally detected in the preparation controls can be up to ~10% less than the dosed amounts due to losses during the spiking procedure. This spiking procedure, described below, is for a convenient amount of samplers, but can be proportionally adjusted for other amounts of samplers.

To spike the sheets, 0.6 L of methanol is added to not more than 0.6 kg of sheets in a wide-mouth glass bottle of 2.5 L and the spike solution is added. This bottle is shaken under stepwise addition of water ending in 50% methanol following the scheme in table 1. Although a high percentage of the added PRCs is absorbed by the silicone sheets, minor amounts are still present in the solution (50% MeOH) that is discarded, particularly of PRCs with small partition coefficients. The spike repeatability between sheets is about 5 %.

Table 1: Dilution scheme after spiking of 0.6 kg sheets.

Time (hrs)	% water	Volume MeOH (mls)	Water total (mls)	Add water (mls)	Total volume (mls)	Step Time
0	0	600	0	0		
24	11	600	74	74	674	24hr
48	20	600	150	76	750	24hr
72 +	30	600	257	107	857	24hr or longer
120+	40	600	400	143	1000	48hr or longer
168+	50	600	600	200	1200	48hr or longer

After spiking, the sheets are packed in diffusion-proof jars (100 mL) without solvent. Conventional (amber) glass jars can be made diffusion-proof by covering an insert in

66 Measured K_{sw} values are available for PCB numbers in italic and for others modeled values are given (Smedes et al., 2009)

the lid with aluminium tape. The lids are tightened, and the jars stored at −20 °C. The samplers do not need to be frozen during transport, but should be kept in the dark.

A single sampler consists of 4–6 sheets, and has a total surface of about 400–600 cm^2. Each batch should consist of samplers for all planned stations. This number is increased by 10% (but at least 4) preparation controls, which are not deployed, but are used to assess any uptake by samplers during preparation, to determine the reference concentration of PRCs before exposure, and to calculate LOD and LOQ. In addition, the inclusion of about 20% field control samplers is suggested. Field controls are samplers that are not deployed, but exposed to air during deployment and recovery of exposed samplers.

The required number of samplers is then $1.2 \times n_{Station} + \max(n_{Station}/10 , 4)$.

2.3 Sampler Frame

The sampler frame is made of stainless steel and has a fixing eye that allows the frame to turn around and gives flexibility. A shackle or rope can be put through this eye to fix the sampler frame to whatever object is selected in the field (*e.g.*, jetty, buoy, tree branch, bottom lander). The shackle is secured with a pin, cable strap or stainless steel wire. Knots in ropes are secured with cable straps and tape. Figure 1 shows the RIKZ system used in the ICES trial survey.

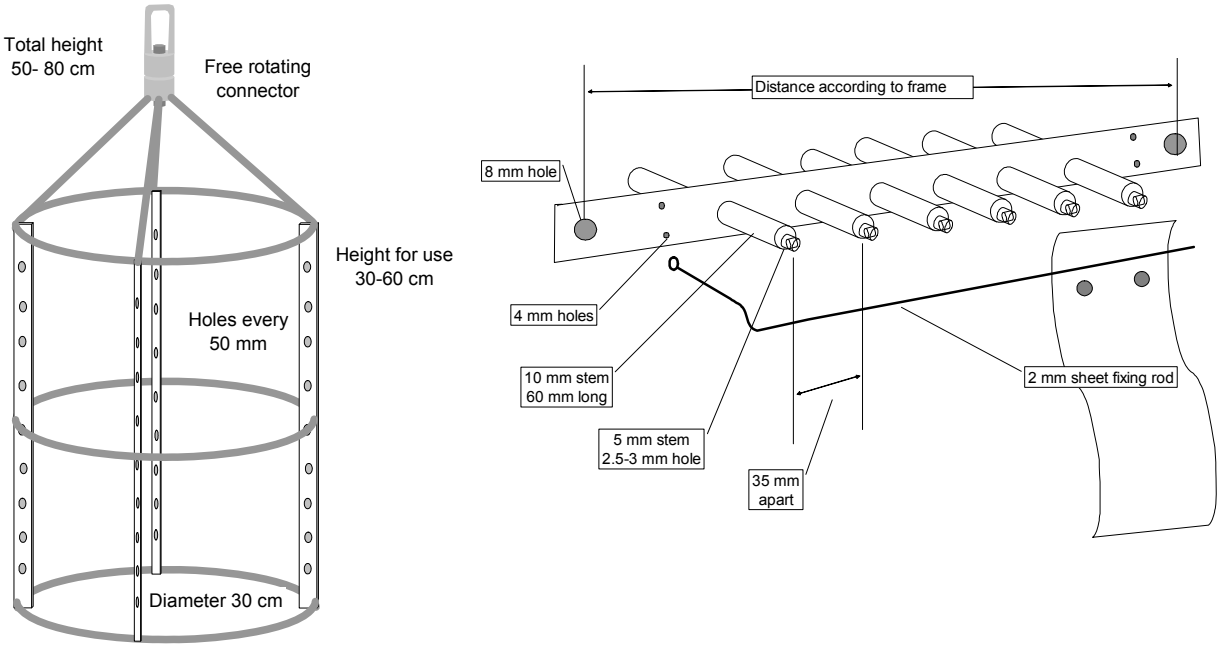

Figure 1. Schematic drawing of frame and sampler holder used in ICES passive sampling trial survey.

2.4 Deployment

Sampling positions are selected depending on the aim of the programme, but also taking into account of practical issues such as availability of suitable moorings, such as quays, jetties or buoys. If using navigation buoys, be aware that antifouling may have been applied and make sure that your deployments should not interfere with the cleaning schedule. Long ropes will wind up around nearby objects. Floating objects may collide with the sampler frame.

Typically, a sampling depth of 2 metres below the surface is appropriate for coastal waters. If the local water depth is less than 3 metres, then half depth can be chosen. In tidal areas the shallowest depth, i.e. low tide, should be considered to prevent exposure to air during lowest low tide. The risk of vandalism or theft should be considered, and appropriate measures should be taken to minimise this risk (e.g. by hiding, camouflaging, using secure areas or using locks)

Exposure of the samplers to the air should be minimised as much as possible. Therefore, sampler holders and fixing ropes are installed prior to mounting the samplers. Samplers are mounted on the holders using two sets of non-sharp tweezers. Use a clean working surface. This working surface can be a stainless steel board, a glass sheet, a glass chopping board or a large glass Petri dish. Sampler sheets may stick to these surfaces. Sticking is minimised by wetting the surfaces with water collected from the site or ultra pure water. A convenient alternative is a stainless steel wire mesh (1 cm) for sorting the sheets. Make sure all material is cleaned.

If the fixing rod is best stored in the holder by a cable strap. At deployment cut the cable strap that keeps fixing rod in place and pull it out. Take all the sheets from the jar and separate them using tweezers. Then, mount the sheets on the holder with the short side upwards. Feed the fixing rod through the holes on the stem and fix it tight with the cable strap. Mount the sheets on all positions and deploy the samplers immediately. Appropriate exposure time depends on the properties of target compounds, their concentration in the water body, the purpose of monitoring and local hydrodynamic conditions. For 600 cm^2 sampler surface in turbulent marine coastal waters sampling rates of 30-60 L day^{-1} are observed and in quiescent open sea areas sampling rates decrease to 4-10 L day^{-1}. From this information and the required limits of detection the optimal exposure period can be derived.

The following information should be recorded:

- Position
- Date and time of deployment
- Salinity
- Water and air temperature.

On sites where air exposure is most critical, field controls can be used to check for contamination during sampler deployment/retrieval operations. To this end, the field control samplers are removed from their jars during the process of deployment and place back after the sampler is exposed in the water. Control samplers are spread out on a stainless steel wire mesh >5 cm above the working surface.

2.5 Recovery after deployment

During recovery, the same parameters are recorded as at the time of deployment. Depending on the season and place of deployment, the recovered sampler can be clean or totally overgrown with organisms. It is suggested to document the degree of biofouling by taking a picture of the recovered sheets. A clean working surface is used for handling the samplers, and local exposure water or ultra pure water is used for rinsing. Sheets that are almost clean are first wiped with a wet tissue and subsequently patted dry with tissue. If significant fouling has occurred, the biofouling is scraped off the sampler as completely as possible. Remaining residues can be removed using a scourer that has been immersed in local water. A nylon type kitchen scourer without sponge, rinsed with methanol and water, is appropriate. Only use gloves if local water is so contaminated that skin contact needs to be avoided. Other-

wise, properly washed and rinsed hands pose a lower contamination risk than gloves. The cleaning should be done in the shortest time possible, e.g. less than 5 minutes. It is not necessary to aim at a sheet as clean as an unexposed sampler. Documenting the appearance of the cleaned samplers using a camera may be useful. If a particular location was selected for exposing a field control, then this control should also be exposed to air during the entire cleaning procedure. However, these controls should not be cleaned with local exposure water.

The recovered samplers are placed back in their storage jar with lids that are lined with aluminium foil and stored in the dark, in a coolbox, and are as soon as possible transferred to a freezer (−20 °C), until analysis or dispatch to the laboratory.

If samplers were not cleaned in the field, it may be more critical to immediately freeze the sampler as the fouling may contain organisms that degrade compounds on the sampler. Cleaning should then be done immediately after taking the sampler out of the freezer. If the fouling is substantial, it sometimes can be broken off, as the fouling layer will be frozen hard, while the sheets remain flexible.

3 Analysis

3.1 Extraction

Many schemes for extracting the samplers are possible, and those which have been successfully applied to the extraction of exposed silicon sheets are summarised below. It is important to have the sheets as dry as possible before extraction. Recovery standards can be dripped on the sheets or added to the sheets in the soxhlet thimble or extraction flask before extraction starts. Preparation controls and field controls are treated like samples.

The simplest and safest way to extract the 4–6 sheets that make up one sampler is to put them loosely together in a soxhlet thimble and extract with methanol/acetonitrile (1:2 v/v) for 8 hours. Alternatively pure methanol can be used. If all sheets do not fit in one soxhlet apparatus, extractions can be done in portions by replacing the extracted sheets after 8 hours and continuing with the next portion using the same portion of solvent. A procedural blank and a procedure recovery are done in the same way, but without sheets.

Another method to extract sheets is cold extraction procedure. Sheets are transferred to a 300 or 500 mL Erlenmeyer flask with glass stopper. 150 mL methanol is added and the flask is shaken gently overnight. Subsequently, the extraction is repeated with fresh solvent for 8 hours, after which the two extracts are combined.

A procedural blank and recovery is done in the same way, but without sheets.

Although the approximate weight of the 6 sheets in one sampler is known, the exact weight must be determined **after extraction**. The total dry weight of the 6 sheets is considered as the sampler mass (m).

A mixture of methanol/acetonitrile (1:2 v/v) is the preferred solvent to extract the samplers for various reasons:

- it has little ability to extract oligomers.
- Acetonitrile boils at 85°C. Addition of 20% methanol decreases the boiling point to 64°C; lower than methanol
- Acetonitrile forms an azeotrope with water so the extract is dried during Kuderna Danish concentration

- Kuderna Danish concentration ends in methanol, which is suitable for cleanup with C18-bonded silica.

Extraction with non-polar solvents is possible but causes considerable swelling of the sheets (ethylacetate up to 200%, and hexane up to 400%) and may extract any oligomers that have not been removed during pre-extraction. Oligomers in the extracts may cause considerable problems, for example by blocking HPLC and by coating GC liners.

3.2 Optional cleanup for the removal of silicone oligomer traces

Although oligomers should be quantitatively removed from the sheets during pre-extraction, traces of these oligomers possibly show up as interferences in some applications. The analytical systems can be protected by an additional clean-up of the extract with C18-bonded silica cartridges. This ensures the removal of oligomer traces, but also removes other highly hydrophobic compounds. Concentrate the extract obtained in 4.1 to <2 mL. Pre-rinse a glass column containing 300-500 mg of C18-bonded silica cartridges with 6-10 mL methanol/acetonitrile (1+2). Transfer the extract to the column and elute with 6–10 mL methanol/acetonitrile. This will elute HCB, all PCBs, and all PAHs up to Coronene. The elution volume for other compounds needs to be separately tested.

3.3 Solvent transfer to non-polar solvents

During all concentration steps, extreme care should be taken to prevent evaporation of the sample to or close to dryness, because the matrix content of the sample is too low to act as a keeper. Using azeotropes for solvent transfer, extracts do not need to be reduced to small volumes for solvent transfer.

Solvent transfer of methanol/acetonitrile to hexane can be done by concentrating the extract to 2 mL and then adding 10 mL hexane for each mL of methanol/acetonitrile. Add boiling stones and boil the (two phase) mixture down to <2 mL on a water bath. Repeat the procedure if two phases are still present. If the two phases remain, the lower phase is probably not methanol, but water. If this is the case, add 20 mL hexane, vortex 1 minute, remove the water phase with a capillary pipette, and concentrate the hexane phase to 2 mL. (Note that this azeotropic solvent exchange does not work when nitrogen blow-down is used for concentrating the extract).

A less convenient method to exchange the solvent is solvent extraction in a separation funnel. Concentrate the methanol to <50 mL, transfer the extract to a separation funnel and add ultra pure water until the methanol-water phase contains less than 20% methanol. Extract the aqueous phase twice with 100 mL of *n*-hexane. Emulsion can be broken, after removal of the separated water phase, by dropping some methanol on it. Evaporation will end in a hexane extract

3.4 Cleanup and analysis

Clean-up of the extracts and instrumental analysis can be carried out according to standard laboratory methods. Extracts in non-polar solvents are suitable for direct use in common cleanup and analytical methods as applied for water, biota or sediment extracts.

4 Usage of blanks and control samples

For the first stage of the data processing, it is important to distinguish between procedural blanks, preparation controls, and field controls. The preparation controls are primarily analysed to give information on the spiked amounts of performance reference compounds (N_0). Secondly the preparation control shows what target compounds have been taken up during preparation and storage of the samplers. The field controls in addition give information on sampler handling during deployment and retrieval procedures.

The amounts detected in the procedural blanks, can be subtracted from the amounts detected in analysis of exposed samplers, preparation controls, and field controls.

Amounts of target compounds in the preparation controls should preferably be similar to those in the procedural blanks. Results of the preparation controls are used to estimate LOD/LOQ. If those amounts in the preparation controls are much higher than the procedural blank, the preparation procedure should be critically assessed to identify and eliminate the causes of these elevated levels (and subsequent higher LOD/LOQ). Correction of the amount in exposed samplers using preparation blanks is a not simple subtraction. For compounds that reach equilibrium, the amount of target compounds after exposure will not be influenced by the preparation blank while for very hydrophobic compounds the amount after exposure is the sum of preparation blank and uptake during exposure. As a rule of thumb amounts of target compounds in preparation controls less than one tenth of those in the exposed samplers are acceptable and no correction of the amounts in the exposed samples is needed. A possible correction scheme for exposed samplers is described in Appendix A.

Field controls may show elevated contaminant levels (compared with the preparation controls) in the case of PAHs, where sampler deployment/retrieval operations are conducted near factories, highways, or on board of ships or when the working area is in the plume of engine exhaust. Since elevated levels in field controls can be highly site-specific, it is not recommended to use these controls for determining average blank levels, detection limits and quantification limits. Instead, these controls may be used to assess contamination from the atmosphere during transport and deployment/retrieval in a qualitative manner. Elevated levels in the field controls may, for example, indicate that revision of transport and deployment/retrieval operations is necessary.

5 Calculation of aqueous concentrations

Various models exist for estimating aqueous concentrations (C_w) from the amounts in passive samplers. Recent summaries of these models can be found in the literature (Huckins *et al*., 2006; Booij *et al*., 2007). It can be expected that improved models and calculation schemes will become available in the near future. Below, a relatively simple calculation scheme is given in which the small (~20%) decrease in sampling rates with increasing size of the contaminants is neglected. The accuracy of the C_w estimates obtained is of the order of 0.3 log units, and the precision about 30 %.

5.1 Assessment of PRC amounts

The PRC data are screened prior to calculating sampling rates. Detection limits for the PRCs may depend on the amounts of other compounds present in the extract. Therefore, all PRC responses should be carefully inspected for possible interferences from other analytes, particularly if the concentration in the extract is low. In principle, each

PRC peak that shows no interference from other compounds and that is well above the instrumental detection limit is acceptable for further processing. In addition, the amounts of PRCs retained should be less than 70% of the amount in the preparation controls. These PRCs are used to calculate the sampling rate: R_S.

5. 2 Sampling rate estimation

The sampling rate can be estimated from the release of the PRCs that were spiked on the sampler before exposure. The release of compounds from the passive sampler (PS) follows:

$$N_t = N_0 e^{-k_e t} \tag{1}$$

Where N_0 is the mass of PRC measured in preparation control samplers N_t is the mass of PRC remaining in the sampler after deployment, k_e (day^{-1}) is the first order dissipation constant that rules the release process, and t the sampling time (day). After transposing the formula, k_e is calculated from:

$$k_e = -\frac{\ln(N_t/N_0)}{t} \tag{2}$$

The sampling rate R_S (L day^{-1}) is calculated through the following equation:

$$R_S = k_e\ m\ K_{SW} \tag{3}$$

where m is the mass of the sampler (kg) and K_{sw} is the sampler-water partition coefficient (concentration in the sampler/concentration in the water, L kg^{-1}) which can be obtained from Smedes *et al*., 2009.

The R_S values are calculated for all the PRCs that pass the criteria in 6.1. The median of all R_s estimates is adopted for further calculation of aqueous phase concentrations, because this statistical parameter is insensitive to outliers.

5. 3 Estimation of aqueous concentrations

Aqueous concentrations (C_w) are calculated with the following formula:

$$C_w = \frac{N}{K_{sw} m \left[1 - \exp\left(-\frac{R_s t}{K_{sw} m}\right)\right]} \tag{4}$$

where N is N_t-N_0.

It is important to use consistent units in Eq. 4. The group $R_s t/(K_{sw}$ m) should be dimensionless. This can be achieved by expressing R_s in L day^{-1}, t in day, K_{sw} in L kg^{-1} and m in kg. If, in addition, N is given in ng, then Eq. 4 gives C_w in ng L^{-1}. Care also should be taken to use the value of K_{sw} rather logK_{sw} in Eq. 4. It may be useful to have

the calculations independently checked by a second person, because mistakes in the calculations are easily made.

5.4 Reporting

Report of the data should include:

- Geographical position
- Object used for sampler frame attachment
- Exposure period\Date and time of deployment and retrieval
- Salinity at deployment and retrieval
- Water and air temperature at deployment and retrieval
- Sampling rate
- Calculated concentrations in the water phase.
- Estimated LODs in the water phase.

Additionally it is relevant to indicate source of sampler material and calibration data (K_{sw})

Because C_w estimation methods and calibration data are regularly improved, it is important to keep records of the amounts of target and PRC compounds detected in the samplers as well as the estimated C_w values. This includes:

- Discription of degree of biofouling after exposure
- PRC/analyte amounts in solvent blanks
- Analytical recovery
- Sampler mass
- PRC/analyte amounts in preparation control, filed control and exposed samplers
- PRC/analyte amounts in exposed samplers
- PRC/analyte amounts in field control samplers (if applicable)
- N_t/N_0 for possible recalculation of R_s
- R_s estimate for each PRC
- Obtained estimates of C_w.

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Acknowledgements: This annex was prepared by Foppe Smedes and Kees Booij

Appendix A:

Further comments on amounts detected in the preparation controls

The amounts in the preparation controls may be used to determine blank levels and detection/quantification limits, but caution is needed to interpret these blank levels correctly. It has been observed that the amounts of compounds that quickly equilibrate with the samplers can be higher in the preparation controls than in the exposed samplers (Ref Durell MPB paper on Norwegian drilling sites). The reason for this observation is that compounds are released from the sampler if their initial concentration in the sampler is higher than their equilibrium concentration, and that their equilibration time is similar to, or shorter then, the deployment time of the sampler. Sophisticated calculation schemes have been suggested to account for (partial) release of analytes with nonzero concentrations in the exposure water ((Vrana *et al*., 2005), Booij *et al*., 2007). The evolution of analyte amounts (N) in the sampler is governed by Booij *et al*.(2007)

$$N = N_0 \exp\left(-\frac{R_s t}{K_{sw} m}\right) + C_w K_{sw} m \left[1 - \exp\left(-\frac{R_s t}{K_{sw} m}\right)\right] \quad \text{(A1)}$$

The first term on the right hand side of Eq. A1 represents the dissipation of the initial amount during exposure. This term can be interpreted as a (time dependent) blank level that can be subtracted from the amount detected in the exposed samplers. For quickly equilibrating compounds ,this term goes to zero, and for slowly equilibrating compounds this term is more or less constant. The blank-corrected amounts can subsequently be used to estimate C_w, using Eq. 4 from the main text. It should be stressed, however, that this method has not been critically assessed and accepted by the passive sampling community. Eq. A1 should only be used as a last resort. The necessity to apply Eq. A1 indicates that sampler preparation procedures need to be improved to reduce the analyte levels in the preparation controls.

Annex 16: Performance of passive samplers for monitoring priority compounds

The established or expected/potential performance of passive samplers of compounds that are listed under WFD, OSPAR, HELCOM, AMAP, BSC, UNEP POP is summarised in the table below.

The following considerations apply:

potential of nonpolar samplers (SPMD, LDPE, silicone, Chemcatcher):

+ = $\log K_{ow} > 4$

? = $3 < \log K_{ow} < 4$ (but a + is listed when experimental evidence shows that the compound accumulates in one or more samplers)

- = $\log K_{ow} < 3$

potential of hydrophilic samplers (POCIS, and possibly the hydrophilic version of Chemcatcher)

+ = $\log K_{ow} < 3$; ? = $3 < \log K_{ow} < 4$; - = $\log K_{ow} > 4$

Stage of development: d = performance has been demonstrated in the laboratory and/or in the field;

p = performance likely is good, but experimental evidence is not available.

- = sampler is not likely to perform well

LOD for nonpolar samplers: expected limit of detection in ng L^{-1} units based on the model equation (for SPMDs, $K_{sw}m$ is replaced with $K_{sw}V_s$)

$$C_{w,\,LOD} = \frac{N}{K_{sw} m \left[1 - \exp\left(-\frac{R_s t}{K_{sw} m}\right)\right]}$$

The following assumptions were made:

- R_s/A = 0.025 L cm^{-2} d^{-1}

- detection limit in amount units: N = 1 ng per sample

- exposure time = 28 d

- sampler water partition coefficient (K_{sw}) is equal to the octanol-water partition coefficient (K_{ow}).

LOD for polar samplers: expected limit of detection in ng L^{-1} units for a 28-d exposure, based on an R_s of 70 mL d^{-1}, assuming that the sampler acts as an infinite sink.

t_{int} : time-window over which the sampling is time-integrative ($t_{int} = K_{sw} V_s / R_s$).

Example 1: the t_{int} for naphthalene in silicone samplers is 3 d. This means that the amount of naphthalene in the sampler reflects the time-weighted average C_w of the 3 days prior to sampling.

Example 2: the t_{int} for mirex in silicone samplers is 20000 d. This means that the amount of mirex in the sampler reflects the time-weighted average C_w up to 20000 d.

	LogKow	Potential of nonpolar samplers	Potential of polar samplers	SPMD 460cm2, 1 mL triolein, 85 µm LDPE			LDPE 85 um, 460x2.5 cm			Hydrophobic Chemcatcher 46 mm diameter, 450 µL octanol			Silicone strip sampler 600 cm² 0.5 mm			POCIS 47 mm diameter	
				stage	*LOD (ng/L)*	t_{int} *(d)*	*stage*	*LOD (ng/L)*	t_{int} *(d)*	*stage*	*LOD (ng/L)*	t_{int} *(d)*	*stage*	*LOD (ng/L)*	t_{int} *(d)*	*stage*	*LOD (ng/L)*
Classical organochlorines																	
Aldrin	6.5?	+	-	p	0.003	1000	p	0.003	500	p	0.08	4000	p	0.002	4000	-	-
Chlordane	6.1	+	-	d	0.003	500	p	0.003	200	p	0.08	2000	p	0.002	2000	-	-
DDT	6.0	+	-	d	0.003	400	p	0.003	200	p	0.08	1000	p	0.002	1000	-	-
DDE	5.7	+	-	d	0.003	200	p	0.004	90	p	0.09	700	d	0.002	600	-	-
Dieldrin	5.4	+	-	d	0.004	100	p	0.004	40	p	0.09	400	p	0.002	300	-	-
Endosulphan	3.7	+	-	-	0.04	2	-	0.10	1	-	0.3	7	d	0.01	6	p	0.5
Endrin	5.2	+	-	d	0.004	70	p	0.005	30	p	0.09	200	p	0.003	200	-	-
Heptachlor	5.3-6.0	+	-	d	0.003	200	p	0.004	90	p	0.09	700	p	0.002	600	-	-
Hexabromobiphenyl	6.4	+	-	p	0.003	200	p	0.004	90	p	0.09	700	p	0.002	600	-	-
Hexachlorobenzene	5.5	+	-	d	0.003	100	p	0.004	50	p	0.09	400	d	0.002	400	-	-
Hexachlorocyclohexane isomers (HCH)	3.8	+	-	d	0.03	3	p	0.08	1	p	0.3	9	d	0.009	8	p	0.5
Mirex	7.2	+	-	d	0.003	7000	p	0.003	3000	p	0.08	20000	p	0.002	20000	-	-
PCBs dichloro	5.1	+	-	d	0.004	50	d	0.006	20	d	0.09	200	d	0.003	200	-	-
PCBs trichloro	5.7	+	-	d	0.003	200	d	0.004	90	d	0.09	700	d	0.002	600	-	-
PCBs tetrachloro	6.0	+	-	d	0.003	400	d	0.003	200	d	0.08	1000	d	0.002	1000	-	-
PCBs pentachloro	6.3	+	-	d	0.003	900	d	0.003	400	d	0.08	3000	d	0.002	2000	-	-
PCBs hexachloro and higher	>7	+	-	d	0.003	4000	d	0.003	2000	d	0.08	10000	d	0.002	10000	-	-
PCDDs mono	4.9	+	-	d	0.005	30	p	0.007	10	p	0.09	100	p	0.003	100	-	-
PCDDs di	5.7	+	-	d	0.003	200	p	0.004	90	p	0.09	700	p	0.002	600	-	-
PCDDs tri	6.3	+	-	d	0.003	900	p	0.003	300	p	0.08	3000	p	0.002	2000	-	-
PCDDs tetra	6.9	+	-	d	0.003	3000	p	0.003	1000	p	0.08	10000	p	0.002	10000	-	-
PCDDs penta	7.4	+	-	d	0.003	10000	p	0.003	4000	p	0.08	40000	p	0.002	30000	-	-

PCDDs hexa and higher	>7.8	+	-	d	0.003	30000	p	0.003	10000	p	0.08	90000	p	0.002	80000	-	-
PCDFs mono	5	+	-	d	0.004	40	p	0.006	20	p	0.09	100	p	0.003	100	-	-
PCDFs di	5.4	+	-	d	0.004	100	p	0.004	40	p	0.09	400	p	0.002	300	-	-
PCDFs tri	5.7	+	-	d	0.003	200	p	0.004	90	p	0.09	700	p	0.002	600	-	-
PCDFs tetra	6.1	+	-	d	0.003	500	p	0.003	200	p	0.08	2000	p	0.002	2000	-	-
PCDFs penta	6.5	+	-	d	0.003	1000	p	0.003	500	p	0.08	4000	p	0.002	4000	-	-
PCDFs hexa and higher	>7	+	-	d	0.003	4000	p	0.003	2000	p	0.08	10000	p	0.002	10000	-	-
PCNs mono	4.0	+	-	p	0.02	4	p	0.05	2	p	0.2	10	p	0.006	10	-	-
PCNs di	4.6	+	-	p	0.006	20	p	0.01	7	p	0.1	60	p	0.003	50	-	-
PCNs tri	5.2	+	-	p	0.004	70	p	0.005	30	p	0.09	200	p	0.003	200	-	-
PCNs tetra	5.8	+	-	p	0.003	300	p	0.004	100	p	0.09	900	p	0.002	800	-	-
	logKow	potential of non-polar samplers	Poten-tial of polar samplers	SPMD 460cm², 1 mL triolein, 85 µm LDPE			LDPE 85 um, 460x2.5 cm			hydrophobic Chemcatcher 46 mm diameter, 450 µL octanol			silicone strip sampler 600 cm² 0.5 mm			POCIS 47 mm diameter	
				stage	LOD (ng/L)	tint (d)	stage	LOD (ng/L)	tint (d)	stage	LOD (ng/L)	tint (d)	stage	LOD (ng/L)	tint (d)	stage	LOD (ng/L)
PCNs penta	6.5	+	-	p	0.003	1000	p	0.003	500	p	0.08	4000	p	0.002	4000	-	-
PCNs hexa and higher	>7.1	+	-	p	0.003	5000	p	0.003	2000	p	0.08	20000	p	0.002	20000	-	-
Polychlorinated terphenyls	8.7	+	-	p	0.003	200000	p	0.003	90000	p	0.08	700000	p	0.002	600000	-	-
Toxaphene (OSPAR: heptachloronorbornene)	4.8-6.6	+	-	p	0.003	200	p	0.004	90	p	0.09	700	p	0.002	600	-	-
More recent pesticides																	
Acrylonitrile	0.3	-	+	-	-	-	-	-	-	-	-	-	-	-	-	p	0.5
Alachlor	2.6-3.5	?	?	-	0.2	-	-	0.5	-	-	2	-	-	0.06	-	p	0.5
Aramite	3.2	?	?	-	0.1	-	-	0.3	-	-	1	-	-	0.04	-	p	0.5
Atrazine	2.6	-	+	-	-	-	-	-	-	-	-	-	-	-	-	d	0.5
Chlordecone	4.5-6.0	+	-	p	0.004	70	p	0.005	30	p	0.09	200	p	0.003	200	-	-
Chlordimeform	1.8	-	+	-	-	-	-	-	-	-	-	-	-	-	-	p	0.5
Chlorfenvinphos	3.8	?	?	-	0.03	-	-	0.08	-	-	0.3	-	-	0.009	-	p	0.5
Chlorpyrifos	5	+	-	d	0.004	40	p	0.006	20	p	0.09	100	d	0.003	100	-	-
Dicofol	4-5	+	-	p	0.007	10	p	0.02	5	p	0.1	40	p	0.003	40	-	-

Diuron	2.7	-	+	-	-	-	-	-	-	-	-	-	-	-	-	d	0.5
Ethyl O-(p-nitrophenyl) phenyl phosphonothionate (EPN)	3.9	?	?	-	0.03	-	-	0.06	-	-	0.2	-	-	0.007	-	p	0.5
Flucythrinate	6.6	+	-	p	0.003	2000	p	0.003	700	p	0.08	6000	p	0.002	5000	-	-
Fluoroacetic acid and derivatives	-0.1	-	+	-	-	-	-	-	-	-	-	-	-	-	-	p	0.5
Isobenzane	4.5	+	-	p	0.007	10	p	0.02	5	p	0.1	40	p	0.003	40	-	-
Isodrin	6.7	+	-	p	0.003	2000	p	0.003	900	p	0.08	7000	p	0.002	6000	-	-
Isoproturon	2.9	-	+	-	-	-	-	-	-	-	-	-	-	-	-	d	0.5
Kelevan	4.5-6.0	+	-	p	0.004	70	p	0.005	30	p	0.09	200	p	0.003	200	-	-
Methoxychlor	4.9	+	-	p	0.005	30	p	0.007	10	p	0.09	100	p	0.003	100	-	-
Morfamquat	<2?	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5
Nitrophen	4.6	+	-	p	0.006	20	p	0.01	7	p	0.1	60	p	0.003	50	-	-
Pentachlorophenol (PCP)	5.1	+	-	p	0.004	50	p	0.006	20	p	0.09	200	p	0.003	200	-	-
Quintozene	5.1	+	-	p	0.004	50	p	0.006	20	p	0.09	200	p	0.003	200	-	-
Simazine	2.2	-	+	-	-	-	-	-	-	-	-	-	-	-	-	d	0.5
2,4,5-T	3.3	?	?	-	0.1	-	-	0.3	-	-	0.8	-	-	0.03	-	p	0.5
Tetrasul	6.9	+	-	p	0.003	3000	p	0.003	1000	p	0.08	10000	p	0.002	9000	-	-
Trifluralin	5.3	+	-	p	0.004	90	p	0.005	30	p	0.09	300	d	0.003	200	-	-

	LogKow	Potential of nonpolar samplers	Potential of polar samplers	SPMD 460cm², 1 mL triolein, 85 µm LDPE			LDPE 85 µm, 460x2.5 cm			Hydrophobic Chemcatcher 47 mm diameter, 450 µL octanol			Silicone strip sampler 600 cm² , 0.5 mm			POCIS 47 mm diameter	
				stage	LOD (ng/L)	tint (d)	stage	LOD (ng/L)	tint (d)	stage	LOD (ng/L)	tint (d)	stage	LOD (ng/L)	tint (d)	stage	LOD (ng/L)
Volatile organic compounds																	
1,2,3-Trichlorobenzene	4.1	+	-	p	0.02	5	p	0.04	2	p	0.2	20	p	0.005	20	-	-
1,2,4-Trichlorobenzene	4.1	+	-	p	0.02	5	p	0.04	2	p	0.2	20	p	0.005	20	-	-
1,2-Dibromomethane	2.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1,2-Dichloroethane	1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
1,3,5-Trichlorobenzene	4.1	+	-	p	0.02	5	p	0.04	2	p	0.2	20	p	0.005	20	-	-
Benzene	2.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dichloromethane	1.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trichloromethane	2.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PAHs																	
PAHs	>3.4	+	-	d	0.007	10	d	0.02	5	d	0.1	40	d	0.003	40	-	-
Anthracene	4.5	+	-	d	0.007	10	d	0.02	5	d	0.1	40	d	0.003	40	-	-
Fluoranthene	5.2	+	-	d	0.004	70	d	0.005	30	d	0.09	200	d	0.003	200	-	-
Naphthalene	3.4	+	?	d	0.08	1	d	0.2	0	d	0.7	4	d	0.02	3	p	0.5
Polyaromatic hydrocarbons	>3.4	+	-	d	0.007	10	d	0.02	5	d	0.1	40	d	0.003	40	-	-
Recent organohalogens																	
Brominated flame retardants (WFD: PBDEs only)	>5	+	-	d	0.004	40	p	0.006	20	p	0.09	100	d	0.003	100	-	-
1,3,5-tribromo-2-(2,3-dibromo-2-methyl propoxy)-benzene	>5?	+	-	p	0.004	40	p	0.006	20	p	0.09	100	p	0.003	100	-	-
Hexabromocyclo dodecane	5.6	+	-	p	0.003	200	p	0.004	70	p	0.09	600	p	0.002	500	-	-
Hexachlorobutadiene	4.9	+	-	p	0.005	30	p	0.007	10	p	0.09	100	p	0.003	100	-	-

Hexachlorocyclopenta-diene (HCCP)	5	+	-	p	0.004	40	p	0.006	20	p	0.09	100	p	0.003	100	-	-
Pentabromoethylbenzene	7.5	+	-	p	0.003	10000	p	0.003	5000	p	0.08	40000	p	0.002	40000	-	-
Pentachloroanisole	5.3	+	-	p	0.004	90	p	0.005	30	p	0.09	300	p	0.003	200	-	-
Pentachlorobenzene	5	+	-	p	0.004	40	p	0.006	20	p	0.09	100	p	0.003	100	-	-
2-Propenoic acid, (pentabromo)methyl ester	6.9	+	-	p	0.003	3000	p	0.003	1000	p	0.08	10000	p	0.002	9000	-	-

	LogKow	Potential of nonpolar samplers	Potential of polar samplers	SPMD 460cm², 1 mL triolein, 85 µm LDPE			LDPE 85 um, 460x2.5 cm			Hydrophobic Chemcatcher 46 mm diameter, 450 µL octanol			Silicone strip sampler 600 cm² 0.5 mm			POCIS 47 mm diameter	
				stage	LOD (ng/L)	tint (d)	stage	LOD (ng/L)	tint (d)	stage	LOD (ng/L)	tint (d)	stage	LOD (ng/L)	tint (d)	stage	LOD (ng/L)
Short-chain chlorinated paraffins (SCCPs)	5.1-8.1	+	-	p	0.003	2000	p	0.003	700	p	0.08	6000	p	0.002	5000	-	-
Tetrabromobisphenol A (TBBP-A)	5.9	+	-	p	0.003	300	p	0.003	100	p	0.09	1000	p	0.002	1000	-	-
Endocrine disruptors																	
Nonylphenol	4.5	+	-	p	0.007	10	p	0.02	5	p	0.1	40	p	0.003	40	-	-
Nonylphenol ethoxylates (NPEOs) and related substances	4.2	+	-	p	0.01	7	p	0.03	3	p	0.1	20	p	0.005	20	-	-
Octylphenol	4	+	-	p	0.02	4	p	0.05	2	p	0.2	10	p	0.006	10	-	-
dibutylphthalate,	4.6	+	-	p	0.006	20	p	0.01	7	p	0.1	60	p	0.003	50	-	-
diethylhexylphthalate (DEHP)	7.6	+	-	p	0.003	20000	p	0.003	7000	p	0.08	60000	p	0.002	50000	-	-
Other organic chemicals																	
1,5,9-Cyclododecatriene	5.0	+	-	p	0.004	40	p	0.007	20	p	0.09	100	p	0.003	100	-	-
2,4,6-Tri-tert-butylphenol	6.1	+	-	p	0.003	500	p	0.003	200	p	0.08	2000	p	0.002	1000	-	-
3,3'-(ureylene dimethylene) bis(3,5,5-trimethylcyclo-hexyl)-Diisocyanate	7.3	+	-	p	0.003	9000	p	0.003	4000	p	0.08	30000	p	0.002	20000	-	-
4-(dimethylbutylamino)-Diphenylamin (6PPD)	4.7	+	-	p	0.006	20	p	0.01	8	p	0.1	70	p	0.003	60	-	-
4-tert-Butyltoluene	4.3	+	-	p	0.01	9	p	0.03	3	p	0.1	30	p	0.004	20	-	-
Clotrimazole	4.1	+	-	p	0.02	5	p	0.04	2	p	0.2	20	p	0.005	20	-	-

Cyclododecane	6.7	+	-	p	0.003	2000	p	0.003	900	p	0.08	7000	p	0.002	6000	-	-
Diosgenin	6.3	+	-	p	0.003	1000	p	0.003	400	p	0.08	3000	p	0.002	3000	-	-
Hexamethyldisiloxane (HMDS)	4.2	+	-	p	0.01	7	p	0.03	3	p	0.1	20	p	0.005	20	-	-
Musk xylene	4.9	+	-	p	0.005	30	p	0.007	10	p	0.09	100	p	0.003	100	-	-
Neodecanoic acid, ethenyl ester	4.9	+	-	p	0.005	30	p	0.007	10	p	0.09	100	p	0.003	100	-	-
Perfluorooctanol sulphonic acid and its salts (PFOS)	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	-	-
Triphenyl phosphine	5.7	+	-	p	0.003	200	p	0.004	90	p	0.09	700	p	0.002	600	-	-

	LogKow	Potential of nonpolar samplers	Potential of polar samplers	SPMD 460cm², 1 mL triolein, 85 µm LDPE			LDPE 85 um, 460x2.5 cm			Hydrophobic Chemcatcher 46 mm diameter, 450 µL octanol			Silicone strip sampler 600 cm² 0.5 mm			POCIS 47 mm diameter	
				stage	LOD (ng/L)	tint (d)	stage	LOD (ng/L)	tint (d)	stage	LOD (ng/L)	tint (d)	stage	LOD (ng/L)	tint (d)	stage	LOD (ng/L)
Metals and related compounds																	
Cadmium	na	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lead	na	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic lead compounds	?	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mercury	na	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic mercury compounds	?	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nickel and its compounds	na	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Organic tin compounds	2.2-4.4	?	?	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Selenium and its compounds	na	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Annex 17: Technical minutes of the JAMP Review Group related to MCWG 2009 report

Review group:

Jarle Klungsøyr (Chair), Lars Edler, Jose Fumega, Carlos Vale, Ian Davies, Francis O'Beirn

Expert group's involved:

WGMS, MCWG, WGHABD, BEWG, SGIMC

4/2008.Tools for coordinated monitoring of dioxins, planar CBs and PFOS

To prepare the following tools to support the coordinated monitoring of dioxins, planar CBs and PFOS under the OSPAR CEMP:

a. technical annexes to the JAMP Guidelines for monitoring Contaminants in Sediments (OSPAR agreement 2002-16) and JAMP Guidelines for monitoring Contaminants in Biota (OSPAR agreement 1992-2) according to the structure of the existing technical annexes covering the following:

   (i) monitoring of dioxins in biota (in) and sediments, taking into account advice from SIME 2007 that monitoring of dioxins in sediments should only be carried out in specific areas (such as sedimentation areas or estuaries) because of time lag (10 – 12 years) in deposition of quantities required for sampling;

   (ii) monitoring of PFOS in sediments, biota and water;

b. to review the existing technical annexes on monitoring of chlorinated biphenyls in biota and sediment and propose revisions so that they are adequate for monitoring of planar CBs in these compartments, taking into account advice from SIME that monitoring in sediments should be undertaken only if levels of marker PCBs are e.g. 100 times higher than the BACs and that for biota monitoring of concentrations in seabird eggs could provide an alternative matrix;

c. to develop background concentrations for dioxins.

Comments by RG

In response to OSPAR work requests to ICES to prepare *technical annexes* for analysis of certain groups of organic contaminants for inclusion in the *JAMP guidelines for monitoring contaminants in marine biota and sediments* MCWG2009 delivered several products to support coordinated monitoring activities.

MCWG 2009, Annex 7 – Planar CBs in biota.

An updated version on the analysis of planar CBs in biota has been finished and is put into the Revised Organic Contaminants in biota Technical Annex. MCWG suggests that the existing OSPAR guidelines for monitoring contaminants in biota could benefit from some restructuring. The technical annex on organic contaminants in biota could preferably be split into a part that deals with sampling and sample handling common to all types of organic contaminant analysis under JAMP, and the analysis of CBs could become a separate technical annexes linked to the first. Other organic contaminant groups could then be added as separate annexes. The RG basically support that this is a good suggestion.

The technical annex on "Determination of chlorobiphenyls in biota – analytical method" answers the request from OSPAR for an updated guideline including also planar CBs. The text covers relevant topics and is fit for purpose. If considered for publication it suggested that the text should go through technical editing. This is a general remark to all the prepared technical annexes of MCWG 2009.

MCWG 2009, Annex 8 - Planar CBs in sediments

Analysis of planar CBs has now been included in "Revised technical annex on analysis of PCBs in sediments". The quality of the revised text is appropriate and should be fit for purpose.

MCWG 2009, Annex 9 - Dioxins/furans in marine biota.

One separate annex has been prepared "Technical Annex on dioxins/furans and dioxin-like PCBs in biota". The quality of the revised text is appropriate and should be fit for purpose.

MCWG 2009, Annex 10 - Perfluorinated compounds (PFCs) in sediments.

The content of the technical annex on PFCs, including methods for analysis of PFOS and a number of other perfluorinated compounds in sediment, is appropriate and should be fit for purpose.

MCWG 2009, Annex 11 Perfluorinated compounds (PFCs) in seawater.

The technical annex on PFCs in seawater is appropriate and should be fit for purpose. The technical annex is partly based on the new ISO25101 guideline but is adapted for water samples containing suspended particle matter (SPM) and broadened to cover the analysis of other PFCs besides perfluorooctanesulfonate (PFOS) and perfluorooctanoic acid (PFOA).

Annex on analyses of dioxines/furans in marine sediments has not been prepared but will be completed 2010 as a joint effort for MCWG and WGMS. At the WGMS 2009 a first draft was prepared which provides some advice on the main steps of the analytical procedure to determine PCDDs and PCDFs in marine sediments. It is mainly based on EPA Method 1613 B (US EPA, Method 1613 Revision B, 1994) that seems to be the most generally accepted method by laboratories involved with dioxin analysis. This document will be completed intersessionally.

MCWG 2009 developed a document on background concentrations for dioxins in marine biota. The approach first taken for developing background concentrations for contaminants in biota was to consider a percentile of contaminant data from areas that could be considered "remote" or "pristine" as a basis for recommending *low concentrations.* However, very few data were available in remote locations for the preferred species/matrix combinations. Most of the information available was from sampling in coastal areas, and data from coastal areas were therefore used. The medians of minimum values as indicative of low concentrations suggested were: 0.15 pg WHO-TEQ PCDD/F g^{-1} wet weight for marine fish muscle, and 0.06 pg WHO-TEQ PCDD/F g^{-1} wet weight for bivalve molluscs. It was realized that these are crude estimates based on very limited data. MCWG 2009 did not foresee any substantial additional information being available in the near future. RW took note of this conclusion and support this information be sent to OSPAR.

WGMS 2009 concluded that the data collected so far for dioxines/furans in marine sediments were not sufficient to allow a reliable expression of background conditions and recommends that work be undertaken to collect other data and their appropriate

cofactors. RG took note of this conclusion and recommends this message be forwarded to OSPAR.

6/2009. Review and update of JAMP Eutrophication monitoring guidelines

Given that the JAMP Monitoring Eutrophication Monitoring Guidelines for nutrients, oxygen, benthos, phytoplankton and chlorophyll are now over 10 years old, there is a need to review, and where required update, the guidelines to reflect technical developments, best practice and to ensure that the guidance remains fit-for-purpose. The purpose is to support the monitoring of these parameters for the assessment of eutrophication under the Comprehensive Procedure and, more generally, for WFD and MSFD monitoring. The request to ICES has two aims:

a) add more specifications in the current guidelines which includes not only listing different possibilities on analysis but also expressing the most commonly used method if it comes to a choice between different methods and prioritise recommended methods and illustrating best practice so it should be more clear which option to go for as a priority.

b) add standards and protocols to be used for developing techniques that have not been used as a standard parameter but have recognised added value to support assessments from a more general validation perspective to complement ship-borne measurements.

For each parameter further clarification in the guidelines is needed on the aspects set out below:

Inorganic/organic nitrogen

a) advice on the period and frequency of sampling to have an accurate idea on winter nutrient concentrations

b) a more detailed explanation of contamination risks during sampling and analysis and appropriate temperatures and duration of preservation

c) standards and protocols for moored instrumentation

d) standards and protocols for satellite assessments to complement ship-based measurements

Biomass of phytoplankton: chlorophyll a

a) advice on the kind of analysis to be performed on chlorophyll a (advantages and disadvantages of acidification procedure)

b) advice on the type of chlorophyll a most suitable to report on (total, active, Phaeophytin)

c) required frequency of sampling for accurate estimate of mean and 90^{th} percentiles during growing season (study of Sweden)

Oxygen

d) a more detailed explanation of contamination risks during sampling and analysis and appropriate temperatures and duration of preservation needs to be included

e) advantages of developing sampling methodology and analysis needs to be included

f) recommendations on accurate analysis of trends (decreased concentration, increased frequency of low O_2 concentration, increased consumption rate)

Benthic community structure

a) advice on monitoring of sufficient surface should be included (advantages of sampling with different devices: Van Veen grab, Reineck boxcore, others)

b) advice on fixation should be added: different fixation mechanisms are in place, like fixation before and after sieving the samples, including advice on staining

c) monitoring of zoobenthos should be done in accordance to ISO 16665 at accredited laboratories or laboratories that can show to perform on this basis

d) advice on calculating biomass of benthos.

Inorganic/organic nitrogen

a) advice on the period and frequency of sampling to have an accurate idea on winter nutrient concentrations

b) a more detailed explanation of contamination risks during sampling and analysis and appropriate temperatures and duration of preservation

c) standards and protocols for moored instrumentation

d) standards and protocols for satellite assessments to complement ship-based measurements

Comments by RG:

The MCWG 2009, Annex 13 is an updated "Revised guideline for monitoring nutrients/JAMP Eutrophication Monitoring Guidelines: Nutrients". It covers in a general way the advice on the period and frequency of sampling to have an accurate idea on winter nutrient concentrations. This may be as far as one can go when not having detailed station positions and the detailed hydrographic knowledge. Sampling frequency is not specified and this is probably because it has to do with the region surveyed. Moreover, there may be changes from one year to another, e.g. winter blooms of phytoplankton may occur at a time when it is normally a phytoplankton minimum. However, it should in principle be possible to define reasonable well sampling periods and frequencies for different parts of the Convention area for the measurement of winter nutrient concentrations. No discussion has been made by MCWG of how "accurate idea of winter nutrient concentrations" could be interpreted, and what reliability might be placed on the results.

The new guideline text giving explanation of contamination risks during sampling and analysis and appropriate temperatures and duration of preservation has been expanded and developed from the previous text.

The text on standards and protocols for moored instrumentation needs to be developed further but the revised guideline does not answer this question. There is nothing about standards and little that could be described as a protocol. The text frequently refers to ferrybox technology, but the question refers to moored instruments. There is no text about standards and protocols for satellite assessments to complement ship-based measurements satellites.

WGHABD 2009 provides some new text on analysis of biomass of phytoplankton and analysis of chlorophyll a and/or other phytoplankton pigments. The report states that "OSPAR needs to decide the overall purpose of monitoring chlorophyll." The requested advice on what kind of analysis to be performed on chlorophyll a (advan-

tages and disadvantages of acidification procedure have not been provided. Further on they have not given clear advice on what form of chlorophyll should be reported.

WGHABD 2009 has suggested changes of the guidelines for Chlorophyll monitoring. All suggestions are appropriate and useful. However, there is a need for a clear statement on what kind of Chlorophyll that should be measured and reported. It is advisable that all contracting parties use the same. This may on the other hand pose a problem for the evaluation of existing long time series.

The new guideline informs about the fluorescence method only and nothing about spectrophotometric methods, which a number of laboratories still use. It is suggested that filters together with extraction solvent can be stored deep frozen. Obviously filters – before extraction – should not be stored deep frozen. This statement is unclear and needs an explanation.

It is stated that the sampling frequency is difficult to give detailed advice on. Considering the short generation time of phytoplankton a very high sampling frequency is needed to cover the succession and development of the phytoplankton communities. Knowing the area you work in will help optimize the sampling frequency.

The section dealing with additional microscopical quantitative and qualitative analysis of phytoplankton is very important and should be more highlighted. The methods used by HELCOM with biovolume estimation can be consulted.

The statistical 90 percentile method is probably a good suggestion, but it should be more elaborated.

A more detailed explanation of contamination risks during sampling and analysis for oxygen and appropriate temperatures and duration of preservation have been provided and the text is adequate.

The question on advantages of developing sampling methodology and analysis is not very clear, but it may be covered by the comments regarding oxygen probes. This needs clarification.

OSPAR asked for recommendations on accurate analysis of trends (decreased concentration, increased frequency of low O_2 concentration, increased consumption rate). This question has only partly been addressed. It is a mixed question on data structures and statistical analysis methods for detection of trends. The text makes no reference to sampling schemes to detect changes in the frequency of low dissolved oxygen events. It makes limited reference to oxygen consumption rates, but RG is not sure that the question refers to dissolved oxygen consumption measurements in the field, or to BOD measurements. RG concludes that the text is improved, and should be offered to OSPAR, but that it should be accompanied by questions seeking clarification on whether the WG has correctly interpreted and adequately answered points b) and c).

BEWG 2008 and 2009 has reviewed and amended new JAMP monitoring guidelines on benthos.

Technical Annex 1 (hard bottom zoo and phytobenthos etc) gives comprehensive descriptions of sampling strategies and methods given along with many relevant references. In Technical Annex 2 on soft bottom macrozoobenthos comprehensive details on sampler use is not given. However, there are many references to published work dealing with the advantages of using specific samplers.

Good advice is presented on phytobenthic fixation and preservation. Again, references only for fixing zoobenthos but these papers contain all adequate information on this topic.

ISO 16665 is mentioned in the Guideline introduction. Further detail on general QA is given in Item 8. The text does not stipulate that monitoring of zoobenthos should be done in accordance to ISO 16665 at accredited laboratories or laboratories that can show to perform on this basis, as indicated in the task from OSPAR.

References to calculating soft bottom benthic biomass are very brief and not informative. They amount to "Procedures for the sorting and biomass determination of soft-bottom macrozoobenthos samples are at sections 3.4 and 3.5 of Rumohr (2009)." in the Analytical procedures section of Technical Annex 2. In view of the direct question from OSPAR, it would have been better to be more explanatory.

Overall, the revised Guidelines answer the questions asked by OSPAR, with the possible exception of the soft bottom biomass calculation methods. However, the whole document depends very heavily on the readers having access to quite a wide range of literature. If it is intended that the document should stand alone and be complete in itself, it fails this requirement.