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RIVO report

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BROC (Biological Reference Materials for Organic Contaminants)

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Executive Summary

The feasibility of the production and certification of four new candidate certified reference materials (CRMs) has been investigated. The following candidate CRMs have been produced: a sterilised fish (flounder) material for organochlorine pesticides (OCPs); a sterilised fish (flounder) material for brominated flame retardants (BFRs); a dried sediment for BFRs and a sterilised shellfish (mussel) material for polycyclic aromatic hydrocarbons (PAHs). The (shell)fish materials have been produced as matrix type fresh sterilised materials in tins that physically resemble the samples analysed in every day routine. The sediment has been produced as a freeze-dried material in glass jars. The tins and glass jars can easily be stored and transported.
The between-lot homogeneity has been tested by analysing the target compounds in 15 lots from the complete batch. The within-lot homogeneity has been tested by 5 replicate analyses in one lot. The median relative standard deviation (RSD) for the inhomogeneity was 9.6% for the OCPs/BFRs in flounder material, 5.4% for the BFRs in sediment and 6.7% for the PAHs in mussel material. This variance is low compared with the variance normally observed in interlaboratory studies and therefore, these materials were considered to be suitable for the test certification.
The stability was tested using a slightly adopted isochronous approach. The samples were stored for 3 and 12 months at –20, 5, 20 and 45°C and nearly all samples were analysed after 12 months (except the 45°C, 3 months samples which were analysed earlier for a preliminary stability estimation). The data was statistically assessed using SoftCRM. There was no degradation detected of any of the target compounds in all samples (within the limits of analytical uncertainty), although low levels and analytical difficulties hampered a precise stability assessment for some compounds.
The interlaboratory study (test certification) showed that certification of all materials is very well feasible. The study of the OCPs showed that there is good coherence between the datasets of different laboratories for a considerable number of OCPs. This was also shown for BFRs in flounder and sediment and PAHs in mussel tissue. However, for some compounds, there was no overlap of the laboratories' results due to several reasons like interferences in the chromatogram with the target compound, limited availability of standards and lack of experience. The latter was in particular true for the BFR analysis, as this was the first time that a tentative certification was organised for these compounds. In some cases a high precision per laboratory of the BFR data resulted in a very low variance.. In combination with a low number of accepted datasets, this led to no overlap whereas the underlying data were of very good quality. Furthermore, several analytical recommendations could be made.
The analysis of BFRs receives a lot of international attention. European risk assessments are currently carried out, and hundreds of laboratories are currently installing methods for BFRs analysis. Almost every year Europe is shocked by marine oil pollution due to ship wrecking. The financial consequences are enormous, but reliable PAH analyses in shellfish are not possible due to the lack of reliable CRMs for PAHs in mussels. OCP levels are decreasing in Europe, but the analysis of the lower levels of OCPs is difficult and matrix-type CRMs for OCPs are not available. It is therefore recommended to start a project for the actual production of all materials as environmental and food control laboratories badly need CRMs to support the production of high quality data for research, monitoring and legislative purposes.

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1. Objectives of the project

The objective of this project was to study the feasibility of the production and certification of four candidate certified reference materials (CRMs): one for organochlorine pesticides (OCPs) in fish, one for brominated flame retardants (BFRs) in fish and one for BFRs in sediment, and one for polycyclic aromatic hydrocarbons (PAHs) in mussels. The objective consisted of:
- Preparation of the test solutions and candidate CRMs
- Testing the homogeneity and stability of the candidate CRMs
- Organisation of a mini-workshop for instruction of invited participants on analysis of OCPs, BFRs and PAHs in the candidate CRMs
- Completion of three interlaboratory studies (BFRs in fish and sediment, for OCPs in fish and for PAHs in mussels), which should be the basis for successful certification of the planned CRMs.
- Dissemination of the objectives and results of the project in order to make the information available to a wider audience in Europe and worldwide.

2. Results and discussion

In this chapter, the results are presented and discussed according to the work package in which they have been carried out.

2.1 Planning of work packages

Work package	Period
1. Sampling and preparation of candidate CRM flounder, sediment, mussel and test solutions	01-06-2001 – 31-08-2001
2. Homogeneity tests candidate CRMs	01-09-2001 – 30-11-2001
3. Stability tests candidate CRMs	01-09-2001 – 30-08-2002
4. Mini workshop	01-12-2002 – 28-02-2002
5. Interlaboratory study	01-03-2002 – 30-11-2002
6. Coordination	01-06-2002 – 30-05-2002

2.2 WP 1 Sampling and preparation of the candidate CRMs flounder, sediment, mussel, and test solutions

The selection and the preparation of the materials was coordinated by the Netherlands Institute for Fisheries Research (RIVO). The following materials were prepared as candidate CRMs:

Code	Material	Contaminant group
BROC-01	Flounder	Organochlorine Pesticides (OCPs) and Brominated Flame Retardants (BFRs)
BROC-02	Sediment	Brominated Flame Retardants (BFRs)
BROC-03	Mussels	Polycyclic Aromatic Hydrocarbons (PAHs)

2.2.1 Collection and pre-treatment of the flounder and mussel material

Hundred eleven kilograms of whole flounder, originating from the Western Scheldt (the Netherlands), were bought at the auction of Breskens (the Netherlands) and were transported to RIVO at 31-05-2001. After removal of the intestines, the fish was frozen at –25°C until further treatment. After defreezing, the fish was filleted at the Gebr. Zwanenburg BV company in IJmuiden (the Netherlands) on 11-07-2001.
The mussels for the candidate CRM were collected at 29-06-2001 by the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) near a former gas works site near Brighton, at the south coast of the England. The raw material was transported on 03-07-2001 to RIVO in cooled boxes (+4°C). After removing most of the waste material and the mussel seed, the mussels were cooked in 6 kg batches for 6 minutes. Subsequently, the shells were removed and the meat was collected. Remaining hard particles (small shells, sand etc.) were removed by leaving the mussel meat to float in a 6% salt-water bath. Hard particles were separated from the floating mussel meat by sinking to the bottom of the bath.

2.2.2 Preparation of the of the flounder and mussel material

The preparation of the flounder and mussel material was carried out by RIVO. The complete volume of meat from either the flounder or the mussels was minced using a mincer (Finis Machinefabriek, Ulft) in combination with a Fryma mill equipped with toothed rotary knives (Fryma Maschinen AG, Rheinfelden, Switzerland) to a final size of 3.5 mm^2. Subsequently, batches of ca. 25 kg sample were homogenised for three minutes, after adding 0.02% butylhydroxytoluene (BHT), in a Stephan cutter (Stephan Machines, Almelo, The Netherlands), type UMM/SK25 (made in 1979). Coated tins (Eurocan Food, Mechelen, Belgium, volume ca. 75 ml) were filled to the brim with mussel homogenate using a manual dosing machine (machinenfabrik Engler, Vienna, Switzerland). The flounder homogenate was filled in the coated cans using an icing bag. The tins were sealed by a Lanico TVM 335 sealing machine (Thomassen and Drijver, Deventer, The Netherlands). The tins were sterilised in a Muvero-Mat sterilizer (type 90E) for 45 minutes at 122 °C (pressure 1.4 bar, heating-time: 90 minutes, cooling time: 20 minutes). In total 290 tins of mussel and 305 tins of flounder were produced.

2.2.3 Collection and preparation of the sediment material

The collection of the sediment in the 'Nauw van Bath', a part of the Western Scheldt, was carried out by RIVO at 4 July 2001. The wet material (130 kg wet weight) was transported to RIVO and stored at 4°C. The wet material was transported at 25-07-2001 to the Wageningen Evaluating Programmes for Analytical Laboratories (WEPAL), at the Wageningen University, The Netherlands, for drying and breaking of the material. The material was dried in an oven by 40°C for 60 hours. Subsequently, the material was minimised by a breaker to < 2 mm particles. The sediment (75 kg dry weight) was put into tin drums of 30 litre for transportation to the Institute for Reference Materials and Measurements (IRMM), Geel, Belgium. The sediment was grinded by a Multi-Processing System (100 AFG Jet Mill/ Ultrafine Classification System, Alpine, Augsburg, Denmark). The total amount of 74 kg ground sediment powder (<125 µm) was produced at a mean rate of 4 kg/hour.
The homogenisation was carried out in a multipurpose cone mixer of 250 litre with semi-automatic filling equipment. Amber bottles of 100 ml were filled with 50 g of sediment and closed with a screw cap (with polyethylene insert). The mean production rate was about 60 bottles/h and 300 bottles were produced. The production date was 09-10-2001. During storage, preparation and bottling care was taken to avoid extended exposure to UV-radiation to prevent breakdown of BDE-209.
Microscopic examination showed a fine homogeneous powder and sieve analysis (Luftstrahl Sieb Analyser, Hosokawa-Alpine) of 3 bottles showed 53% of the particles is smaller than 32 µm, 97% is smaller than 45 µm and 53% is smaller than 63 µm. The mean moisture content is less than 1.1%.

2.3 WP 2 Homogeneity test of the candidate CRMs

The homogeneity tests and data analysis were carried out under the coordination of the Institute of Applied Environmental Research (ITM), Stockholm. The tables with the results are mentioned in Appendix 1.

2.3.1 Homogeneity test for flounder (BROC-01) for BFRs

The homogeneity test was carried out at ITM in Stockholm, Sweden. The samples were extracted according to a method described by Jensen et al (Jensen *et al.*, 1983) using a mixture of n-hexane/acetone, followed by n-hexane/diethyl ether. The lipids were removed by treatment with concentrated sulphuric acid. The final determination was carried out by gas chromatography/mass spectrometry with negative chemical ionisation (GC/ECNI-MS) measuring the m/z -79 and –81 ions. Ammonia was used as reaction gas. The capillary column used was a DB5 MS, 40 m x 0.18 mm x 0.18 µm.
The results of the homogeneity test are shown in tables 1a-4a. Table 1a shows the error made in the determination of the brominated substances, where a standard solution was injected repeatedly 10 times in one series.
The relative standard deviations (RSD) varied between 0.70 and 2.1 % for the polybrominated diphenyl ethers (PBDEs) and was 5.3 % for hexabromocyclododecane (HBCD). The between batch homogeneity of the material was tested by analysing the levels of the brominated substances once, in 15 different tins (table 3a). The within batch homogeneity was carried out by analysing the analytes five times from one, randomly selected, tin (table 2a).

2.3.2 Homogeneity test for flounder (BROC-01) for OCPs

The homogeneity test was carried out at the Marine Institute in Dublin, Ireland. The OCPs were determined after Smedes extraction (Smedes *et al.*, 1996) and clean-up by alumina column chromatography. The samples were fractionated on a silica gel column. The final determination was carried out by GC/ECD, using 50 m x 0.25 mm x 0.25 µm HT8 and CPSIL19CB capillary columns.
The results of the homogeneity test are shown in tables 1b-4b. Table 1b shows the error made in the GC determination of the pesticides, where a standard solution was injected 9 times in one series. The RSD varied between 1.1 and 4.0 %. The between batch homogeneity of the material was tested by analysing the levels of the pesticides once, in 15 different tins (table 3b). The within batch homogeneity was carried out by analysing the analytes five times from one, randomly selected, tin (table 2b).

2.3.3 Inhomogeneity of BROC-01 for OCPs and BFRs

The results of the inhomogeneity are given as $rsd_{inhomogeneity,}$ from the formula $rsd_{inhomogeneity}^2 = rsd_{between}^2 - rsd_{within}^2$ (table 4a and 4b).
The RSD values for the inhomogeneity vary between 2.6 and 19 % for the pesticides and 8.6 and 19% for the brominated analytes. If the highest value for the pesticides is excluded (?-heptachloroepoxide) and the two highest values for the brominated substances (BDE 28 and HBCD) are excluded due to analytical problems, the inhomogeneity for both pesticides and PBDEs shows a RSD between 2.6 to 14% with a median value of 9.6%.
Compared to previous homogeneity tests such as the CHRONO certification and the BSEF interlaboratory study this seems to indicate an inhomogeneity of the material. In the CHRONO study the inhomogeniety values for PCBs and non-ortho PCBs were between 1.9 and 6.9% (van Leeuwen *et al.*, 2002a; van Leeuwen *et al.*, 2002b). In the BSEF interlaboratory study the inhomogeneity RSD values for BDE 47 for sterilised canned eel samples and mussel samples were reported to be 5.3 and 5.5%, respectively (de Boer *et al.*, 2002). Most likely, slight alterations in the preparation procedure have caused this homogeneity problem. Also, flounder may be less suitable as a CRM, as it is more difficult to homogenise than other flat fish species.

However, a homogeneous flounder test materials (250 lots) has been produced before by RIVO for use in the QUASIMEME programme.
The inhomogeneity in this feasibility study is insignificant compared to the analytical error by a group of laboratories in a certification study, where the standard deviation for the mean values (from respectively laboratory) is typically 5-25%. However, a better homogeneity should be obtained for the final certification. The co-ordinator has discussed this result of the flounder sample with dr. S. Bøwadt of the European Commission and it was decided to continue with this sample. Given the relatively low OCP concentrations and these homogeneity problems, an alternative fish sample may be considered in the final certification.

2.3.4 Homogeneity test for sediment (BROC-02)

The homogeneity test was carried out at the ITM, Sweden. The samples were extracted according to a method described by Jensen et al (Jensen *et al.*, 1983) where n-hexane followed by n-hexane / acetone is used. As a clean-up step for removal of sulphur tetrabutylammonium sulphite was used. The extract was also treated with concentrated sulphuric acid. The final determination was carried out by GC/ECNI-MS measuring the m/z -79 and -81 ions. Ammonia was used as reaction gas. BDE 28, 47, 99, 100, 153, 154 and HBCD were analysed on a capillary column (DB5-MS, 40 m x 0.18 mm x 0.18 µm).
A 10 m x 0.18 mm x 0.18 µm DB5-MS column was used for BDE 209.
The results of the homogeneity test are shown in tables 1a, 2c-4c. Repeated analyses of standard solutions of brominated substances, included BDE 209, are shown in table 1a. The between batch homogeneity of the material was tested by analysing the levels of the brominated substances once, in 15 different glass jars (table 3c). The within batch homogeneity was carried out by analysing the analytes five times from one, randomly selected, glass jar (table 2c).
The results of the inhomogeneity are given as $rsd_{inhomogeneity,}$ from the formula $rsd_{inhomogeneity}^2 = rsd_{between}^2 - rsd_{within}^2$ (table 4c). The RSD values for the inhomogeneity vary between 2.3 and 14 % for the brominated analytes. If the three highest values for the brominated substances (BDE 28, 209 and HBCD) are excluded due to analytical problems, the inhomogeneity values are between 2.3 to 5.9% with a median value of 4.5%.
Compared to previous homogeneity tests such as the CHRONO certification and the BSEF interlaboratory study, as described under homogeneity test for flounder, the sediment shows an acceptable homogeneity and can be used for this study.

2.3.5 Homogeneity test for mussel (BROC-03)

The homogeneity test was carried out at the Centre for Fisheries and Aquaculture Sciences (CEFAS) Burnham on Crouch, United Kingdom. The method for the determination of PAH in biota involves alkaline saponification of wet tissues under reflux, extraction into pentane, clean-up on a short alumina column, and analysis by GC/MS in full scan EI mode on a bench top ion-trap instrument. A range of fully deuterated compounds are added as internal (surrogate) standards for quantification, and as a recovery standard (Kelly *et al.*, 2000).
The results of the homogeneity test are shown in Tables 1c, 2d-4d. Table 1c shows the error made in the GC determination of the PAHs, where a standard solution was injected 6 times in one series. The RSD values varied between 0.9 and 5.9%. The between-batch homogeneity of the material was tested by analysing the levels of the PAHs substances once, in 15 different glass jars (table 3d). The within-batch homogeneity was carried out by analysing the analytes five times from one, randomly selected, glass jar (table 2d).
The results of the inhomogeneity are given as $rsd_{inhomogeneity,}$ from the formula $rsd_{inhomogeneity}^2 = rsd_{between}^2 - rsd_{within}^2$ (table 4d).
The RSD values for the inhomogeneity vary between 0.92 and 21% for the PAHs. For the five higher mass PAHs the overall variation are increased (both within and between) due to analytical problems, and therefore the inhomogeneity values are relatively high. If those analytes and 1-C1-N (due to the high within-variance) are excluded, the inhomogeneity for the PAHs shows RSD values between 0.92 and 9.4%.

In a previous homogeneity test, the CHRONO certification (van Leeuwen *et al.*, 2002a; van Leeuwen *et al.*, 2002b), the inhomogeneity RSD values for PCBs and non-ortho PCBs were between 1.9 and 6.9 %. The inhomogeniety values in this study are slightly higher, but low enough to use the mussel sample in this study.

2.4 WP 3 Stability test

The Marine Institute, Dublin, Ireland, coordinated the stability tests. The stability test was carried out according to the isochronous approach with a slight modification (Lamberty *et al.*, 1998). The samples of all temperature/time combinations are analysed at the end (12 months) of the study, in five-fold. For BFRs in flounder, PAHs in mussel and OCPs in flounder, the 3 months-45°C samples were analysed after 3 months storage in order to have a preliminary view on the stability of these compounds at elevated temperature. For PAHs not all the t-T combinations have been analysed due to a misunderstanding. Therefore, there is no 3 months stability data (except at 45 °C). Five tins/jars of all materials (BROC-01 to -03) have been stored at -80°C in case any doubts would raise from the results of the –25°C reference temperature. However, it was not necessary to analyse these samples.
The data was assessed using the Soft-CRM software, which has been designed for this purpose (Bonas *et al.*). The data was entered in the software and the output was generated automatically. A separate report was produced for each candidate CRM (see Appendix 2). For the 0 months stability data at all temperatures the same data was used as the 12 months – 20°C reference temperature data as it is assumed that no degradation has taken place at the beginning of the study (directly after production of the CRM) and at the reference temperature. For some of the OCPs and BDEs, the results of the stability study showed a considerable variance as a result of the analytical variance of compounds that were determined close to the limit of quantification (LOQ). The higher the analytical variance, the more difficult an accurate stability determination becomes. Therefore, using this model (Soft-CRM) for stability determination, the aim should always be to keep analytical variability minimised. However, close to the LOQ the analytical variability will typically be 15-30% and therefore hamper the determination of the stability.

2.4.1 Stability of OCPs in BROC-01

The Soft-CRM generated report (Appendix 2a) on the analysis of all analytes determined shows that the slope of the linear regression for all compounds at each temperature does not differ significantly throughout the study period (at 3 and 12 months). Also the separate 3 months - 45°C study did not show significant degradation. The results of this stability study suggest that no difficulties will arise from a similar material with these compounds.
Analyses of three of the compounds throughout this study require some further comment. In the case of the analysis of dieldrin no differences were determined for the slope of the regression throughout the study. However, the levels of dieldrin detected in the samples are lower than those determined by the wider group of laboratories in the intercomparison exercises (see paragraph 2.6 WP 5 Interlaboratory study). This is also the case for the analysis of heptachlorepoxide where levels determined during this study were lower than observed in the intercomparison exercise. As in the case of dieldrin no significant slope was observed at either 95 or 99% confidence intervals for these data. The analysis of pentachlorobenzene (QCB) was also completed during this study. However levels observed fell below the LOQ of the method. This was further backed up in the intercomparison exercise, where similar levels were observed by participant laboratories.

2.4.2 Stability of BFRs in BROC-01

The stability report of the BFRs in flounder in Appendix 2b shows that there are no indications for degradation of any of the BFRs. This applied for all temperatures and throughout the entire study.
The levels of BDE 66, 153 and 154 in the flounder sample are close to the LOQ, which resulted in considerable variability of the stability results. However, within the limits of analytical variability, these compounds seem to be stable, which is also expected from the fact that these compounds are related to the other BDEs, which show good stability.
The determination of HBCD in this study using GC-MS is difficult due to instability of this compound at high temperatures (>180°C). As a consequence, the analytical variability of the results is high (57%) which hampers the accurate determination of stability. With GC/MS only a total HBCD concentration can be determined, whereas LC-MS enables an accurate separate determination of the stereoisomers ?-, ?- and ?-HBCD. Therefore, for a future certification, it is preferable to determine the stability of the HBCD isomers using LC-MS.

2.4.3 Stability of BFRs in BROC-02

The stability results of this candidate CRM show no significant degradation at nearly all temperature-time combinations, which shows that the BFRs in this freeze-dried sediment are stable over a period of 12 months (see Appendix 2c). Only the graph of BDE 85 showed a significant slope at 20°C (95 and 99% confidence intervals) and BDE 100 showed a significant slope at 45°C (95% confidence interval). This suggests instability of these compounds but the effect was not observed at other temperatures or for other, similar, BDEs. Therefore, it is assumed that BDE 85 and 100 are stable as well.
At 45°C, 12 months for nearly every compound (except BDE 66 and BDE 183) the 12 months ratio is 3-22% lower compared with the initial data (0 moths), which suggest a systematic effect. The reason for this is not completely understood but it might be caused by storage conditions at 45°C, which effect the matrix and possibly not the target compounds. This effect was not observed at other storage temperatures. Nevertheless, no statistical instability was detected for most of the BFRs.
From these data it is expected that the BFRs in sediment will be stable at different temperatures for 12 months and most likely also for longer period. The decreasing effect at 45°C needs further research, but no problems are expected with the production and storage of such a CRM as e.g. 20°C.

This stability study shows that the production of wet sterilised tinned flounder and mussel tissue is a very suitable approach for achieving no degradation of these OCPs, BFRs and PAHs in these materials for a period of 12 months. This also holds for the BFRs in the freeze-dried sediment material. Due to the persistent nature of the compounds, it is expected that the materials will be stable also for a longer period.

2.4.4 Stability of PAHs in BROC-03

The report on the PAH stability study in Appendix 2d shows that, within the limits of the analytical variability, there is no strong evidence for degradation of any of the PAHs, including the alkylated compounds and those with sulphur heteroatoms, during storage. This applies even to the short-term stability study at elevated temperature (45°C). Therefore, in future studies of this type no difficulties with specific compounds are foreseen.

2.5 WP 4 Mini-workshop

The mini-workshop was took place from 21-23 March 2002 in Stockholm, and was perfectly organised by ITM. After the introduction, presentations were given on available CRMs/SRMs and analytical aspects of analysing OCPs, BFRs and PAHs. The workshop concluded with the thorough discussion of the protocols and report forms of the interlaboratory study. The minutes of the workshop were included in the 1st progress report.

2.6 WP 5 Interlaboratory study

The interlaboratory study has started immediately after the mini-workshop (WP 4). The protocols and report forms were prepared and have been sent at 15 April 2002 together with the tins and/or jars and the unknown solutions to the participants of the study. An example of a protocol can be found in Appendix 3. The deadline for the interlaboratory study was 31 October 2002. However, several laboratories had problems respecting this deadline for several reasons like underestimation of the workload, no available capacity and instrumental problems. Therefore, the deadline for data submission was shifted slightly in order to obtain sufficient datasets for a valid statistical evaluation of the data. Draft reports were prepared for each candidate CRM and sent to all participants prior to the technical evaluation meeting which took place in Brussels at 27 to 29 January 2003. At this meeting the results were thoroughly discussed. Some datasets were withdrawn for several reasons like incomplete data, low recoveries, doubts on the technical quality of results and incomplete calibration (sample extract concentrations outside the calibration range). The final reports are prepared based on the adaptations as discussed in the technical meeting.
For (test) certification, several recommendations were made for the different candidate CRMs. They will be discussed below.

2.6.1 OCPs in flounder (BROC-01)

The data of twelve laboratories is included in the final report (Appendix 4a) A summary is mentioned in Table 1. Some compounds like dieldrin are difficult to analyse as it is easily degraded due to treatment of the extract with concentrated sulphuric acid or dirty liners in the GC injector. This negatively affects the quality of the data and the coherence of the individual datasets. Overlap of datasets is found for half the compounds included in this study. For ?-HCH and trans-chlordane there is no overlap due to one outlying dataset that could not be removed from the complete dataset, as there was no technical explanation for the deviation. Only a few laboratories could determine the very low levels for some compounds (e.g. ?-HCH and QCB). For production and certification of a CRM for OCPs, the material to be selected should preferably have higher levels of some OCPs in order to enable laboratories to analyse the compound that is above the LOQ. On the other hand, the levels in this sample should be realistic in order to reflect the current low levels found in fish.
The following analytical recommendations are made for certification:

- When ^{13}C labelled standards are used as internal standard to avoid recovery correction, then in principle for every native OCP a ^{13}C labelled standard should be applied because the OCPs are a very heterogeneous group of compounds with different properties. The use of one single ^{13}C labelled standard is insufficient.
- The sample intake for this lean sample should preferably correspond with 250 mg lipid or more in order to enhance detectability of low level OCPs in the sample.
- Some OCPs degrade under certain conditions. Dieldrin, endrin and heptachlorepoxide were found to decompose at acidified silica during clean-up.
- At very low concentrations of a compound in the final extract, close to the lower part of the calibration curve, the influence of the intercept can become considerable. This should be critically evaluated. Possibly only the lower calibration points should be used or bracketing can be applied. The lower part of the ECD detection range shows a different response and therefore measurements in this area should be avoided (or bracketing should be applied).

Table 1. Summary of the test certification of OCPs in BROC-01

Compound	No labs	Range (min..max) (µg/kg ww)	Mean of means (µg/kg ww)	Overlap (yes/no)
p,p'-DDT	3	0.018 – 0.053	0.027	Y
p,p'-DDE	9	1.31 – 2.50	1.86	Y
p,p'-DDD	10	0.363 – 1.09	0.717	Y
o,p'-DDT	3	0.013 – 0.226	0.078	N
Dieldrin	6	0.440-1.32	0.771	N*
Endrin	2	0.024-0.034	0.030	Y
?-HCH	3	0.004-0.226	0.046	N*
?-HCH	4	0.020-0.217	0.085	N
?-HCH	7	0.096-0.261	0.161	N
?-heptachloepoxide	7	0.120-0.320	0.205	N
HCB	9	0.110-0.320	0.222	Y
QCB	3	0.026-0.055	0.039	Y
Trans-nonachlor	6	0.066-0.190	0.094	N
Cis-chlordane	5	0.051-0.080	0.063	N
Trans-chlordane	5	0.041-0.064	0.051	Y
Oxychlordane	6	0.019-0.035	0.028	Y

* bimodal distribution

2.6.2 BFRs in flounder (BROC-01)

Observations in this dataset are similar to the OCPs in flounder. The most common BDE's 47, 99 and 100 were analysed by many laboratories with success due to the relatively high levels in this sample, whereas many laboratories had problems analysing the very low levels of e.g. BDE 183. BDE 153, on the other hand, could be successfully analysed by 7 laboratories although the levels were lower than 0.1 µg/kg ww. For BDE 49 only some laboratories have standards available, which is reflected in the low number of laboratories that have analysed this compound. BDE 154 showed no overlap due to one outlying dataset. Laboratories had problems analysing HBCD. The results seem to be method dependent as there was much difference between the 4 reported laboratories although their own variance was well below 10% (for 3 labs).

Table 2. Summary of the test certification of BFRs in BROC-01

Compound	No labs	Range (min..max) (µg/kg ww)	Mean of means (µg/kg ww)	Overlap (yes/no)
BDE 28	7	0.063-0.120	0.088	Y
BDE 47	10	2.39-4.48	3.33	Y
BDE 49	3	0.216-0.360	0.276	N
BDE 66	6	0.039-0.080	0.061	N*
BDE 99	8	0.185-0.370	0.273	Y
BDE 100	8	0.420-0.750	0.598	Y
BDE 153	7	0.060-0.106	0.088	Y
BDE 154	7	0.114-0.190	0.143	N
BDE 183	2	0.006-0.030	0.017	N
HBCD	4	0.525-1.37	0.900	N

* bimodal distribution

2.6.3 BFRs in sediment (BROC-02)

Surprisingly, only for 3 out of 12 compounds overlap exists between the individual datasets, although the levels are sufficiently high for accurate analysis. Often, the reason for no overlap is the low variance in datasets of the individual laboratories (e.g. BDE 49, 66, 85 and 99), which in fact means that their data is very precise. For some compounds the calibration shows a wide range due to some outliers, which means that calibration is not always under control. This can be caused by the fact that not all laboratories had purchased good quality standards (BDE 66), although this was only to a lower extent observed for BFRs in the flounder candidate CRM. The wide range of BDE 183 is caused by possible interferences in the chromatogram, for which should be checked by analysis using a GC column with different polarity of the stationary phase. Laboratories are less experienced in the analysis of BDE 183, which has been added to the set of important BDEs only recently. Improvement in this analysis is expected shortly. The dataset of BDE 209 seems to be bimodal although there is overlap caused by a wide variance of 1 lab. From table 3 it is clear that the accuracy of the BFR analysis requires more attention for a future certification exercise.

Table 3. Summary of the test certification of BFRs in BROC-02

Compound	No labs	Range (min..max) (µg/kg ww)	Mean of means (µg/kg ww)	Overlap (yes/no)
BDE 28	6	0.380-0.962	0.626	Y
BDE 47	7	7.99-14.6	10.14	N
BDE 49	4	2.11-3.38	2.75	N
BDE 66	5	0.220-0.346	0.289	N
BDE 85	7	0.430-0.890	0.656	N
BDE 99	7	11.9-17.3	14.2	N
BDE 100	9	2.20-3.98	3.04	N
BDE 153	8	1.50-2.40	1.93	N
BDE 154	8	1.24-2.12	1.71	Y
BDE 183	7	0.220-0.837	0.448	N
BDE 209	6	581-1381	1164	Y
HBCD	5	22.1-156	95.8	N

Analytical recommendations for BFRs in flounder (BROC-01) and sediment (BROC-02):

- Total-lipid determinations in flounder like the Bligh and Dyer or the Smedes lipid methods are preferred over non-selective extractable lipid determinations, as these also include the more polar phospholipids.
- The result of the total organic carbon (TOC) determination in sediment is method dependent.
- For BDE-66 no good standards are commercially available, which results in an inaccurate determination.
- The lot numbers or production dates of the (internal) standards should be provided by the participants to determine if any bias in results originates from specific manufacturers or lots.
- Most commercial standards for BFRs are available from Cambridge Isotope Laboratories and Wellington and have a poor accuracy of 10% (typically 50 +/- 5 µg/kg), according to their certificate. Although in practice the accuracies are often better, this undermines an accurate determination of the BFRs in a (candidate) CRM. Therefore, this problem will be discussed with the manufacturers (participants mentioned that the accuracies might be improved to 5%). Furthermore, it was mentioned that Accustandard supplies crystals of BDEs, which use is in favour over the ready-to use, but less accurate, standard solutions.
- BDEs 28 and 33 show co-elution on a DB-5 column. Accurate separation should be checked by addition of BDE-33 to the sample.
- BDE 85 should be carefully checked for interferences.
- BDE 154 does not show any interference with BB 153.
- Different interferences have effects on the BDE 183 result. Therefore, the reported result should be confirmed by analysis on a second GC column with different stationary phase.

- BDE 209 requires a lot of attention for an accurate determination (de Boer *et al.*, 2001). BDE 209 breaks down at GC-oven temperatures > 320°C. Therefore, analysis time should be reduced as much as possible e.g by using a short GC column (15 m). Furthermore, samples, standards and extracts should be protected from UV light as this causes degradation of BDE 209. Also, evaporation to dryness should be avoided, and the control of blanks is extremely important.
- HBCD consists of three individual stereoisomers, which should preferably be separated and determined by HPLC-MS without thermal rearrangements taking place as with GC, resulting in a more accurate and more precise determination. HBCD is an important BFR. Its certification is highly desirable.

2.6.4 PAHs in mussel (BROC-03)

The data of nine laboratories were accepted after a thorough technical discussion. A summary of the data is shown in Table 4. Naphthalene is based on only a limited number of datasets as due to the volatility of naphthalene the compound is easily lost during the analysis. In a number of cases, there was no overlap of data for reasons like outliers that could not be removed for technical reasons (e.g. fluoranthene, phenanthrene and benzo[e]pyrene). Furthermore, the quality of the data was sometimes so good that individual datasets showed a low variance (resulting in no overlap), whereas the averages of the datasets were close together (e.g. benz [a] anthracene). Also the occasionally low number of accepted datasets resulted in less overlap. Therefore, the minimum number of acceptable datasets should preferably be 6 or higher. Generally, outliers could not be explained from problems with calibration.

Table 4. Summary of the test certification of PAHs in BROC-03.

Compound	No labs	Range (min-max) (µg/kg ww)	Mean of means (µg/kg ww)	Overlap (yes/no)
naphthalene	3	1.79-4.00	3.20	Y
1-methyl naphthalene	4	1.85-7.04	4.06	Y
Fluorene	8	7.20-25.00	14.09	N
Dibenzothiophene	7	8.50-17.40	13.85	Y
Phenanthrene	5	119.0-152.4	140.4	N*
Anthracene	6	22.79-31.05	26.21	N
2-methyl anthracene	5	14.00-26.88	21.46	N
Fluoranthene	7	111.0-207.7	163.3	N
Pyrene	7	131.3-257.0	186.3	N
1-Methyl pyrene	4	18.00-37.13	25.14	N
Benz [a] anthracene	6	73.92-96.64	84.13	Y
Chrysene	6	45.10-80.00	61.99	N**
Benzo [b] naphtho [2,1-d] thiophene	6	10.08-19.00	14.82	N
Benzo [e] pyrene	7	20.28-49.00	28.67	N*
Benzo [a] pyrene	8	10.42-22.00	14.65	N*
Indeno [1,2,3-c,d] pyrene	6	3.200-5.300	4.258	Y
Benzo [ghi] perylene	6	5.326-7.500	6.414	Y
Dibenz [a,h] anthracene	6	0.610-2.100	1.201	N**
1-methylphenanthrene	5	36.80-73.98	57.55	N
Benzo [k] fluoranthene	9	6.240-14.00	8.857	N*

* one outlier

** bimodal distribution

Analytical recommendations for PAHs in mussel (BROC-03):

- Losses of naphthalene easily occur during extraction and clean-up due is a very volatile nature. Losses can be avoided by using pentane and to evaporate gently at low (ca 28°C) temperatures (Kelly *et al.*, 2000).
- Some PAHs are sensitive for breakdown in light. Therefore, exposure should be avoided as much as possible.
- For the PAH standards, three categories are available:
  - certified BCR or NIST standards ✍ best available quality, should preferably be used
  - certificate with some purity test by the manufacturer (typically >98%) ✍ OK
  - no certificate and no test results (typically 95% +/- 5%) ✍ should not be used

  If no suitable standards are available, than the option for synthesising should be considered in a future certification study.
- The PAH profile in the unknown solution for calibration check should resemble the PAHs profile in the sample.
- A few % (m/m) of dichloromethane in the unknown solution can cause problems for the high MW PAHs in an on-column injection system. Preferably stocks should be made in ethylacetate and the dilutions in toluene. Also hexane and iso-octane are suitable.
- LC results show generally lower variance compared with GC results, most likely because instrumentation of LC is less complicated with less possible error sources. However, GC-MS is complementary to LC as the range of compounds is different for both techniques. Therefore, it is recommended to base the final certification on both techniques.

General analytical recommendations

- The amount of ^{13}C-labeled internal standard should be in the same order of magnitude compared with the target compound in the sample. This is to prevent that isotope ratio's in the sample are different from the ratio's in the standards.
- When using a ^{13}C labelled or deuterated compound for recovery correction, also a lower limit should be applied for accepting or rejecting results.
- A maximum for a blank value (or percentage of the sample value) should be determined.
- The extraction efficiency is tested by re-extraction of the sample using a different (more polar) solvent system. The 1st extraction should show an efficiency of over 95% (the amount of residual contaminant (determined by the re-extraction) should be less than 5% of the first extraction).
- The recovery is determined by spiking at four different levels and determination of recovery from the regression curve. As this is not every day practice, some laboratories ran into problems using this procedure. As a result, their recovery data was outside the detectable range and therefore was withdrawn from the final dataset. In this way, some good datasets were lost for the certification.
- The injected amount of compound in the final extract should be within the injected range of the calibration curve. Several datasets were withdrawn for being outside the calibration range. More attention should be paid to this during a certification exercise.

3. Coordination and dissemination (WP-6)

The overall project coordination and dissemination is carried out by the Netherlands Institute for Fisheries Research. The coordination has been going smoothly, due to the relatively simple structure of the project and the experience the coordinator obtained from previously coordinated S, M&T projects (CERMUS, CHRONO). Also the small number of partners minimised the complexity of the project. No significant coordination problems have risen during the project.
The following meetings with the project consortium took place:

- 18-19 June 2001, Marine Institute, Dublin, Ireland, BROC kick-off meeting
- 21-24 March 2002, ITM, Stockholm University, Stockholm, Sweden, BROC workshop and progress meeting
- 6 September 2002, EC, Brussels, Belgium, Mid-term meeting
- 27-29 January 2003, EC, Brussels, Belgium, Technical discussion of the results of the test certification.

3.1 Deliverables table

The deliverables table stating all deliverables and their current status is added to this report in Appendix 5. The work as mentioned in the original work plan (description of the work) has all been carried out as is clear from the deliverables table. Only the PAH stability study has not been carried out completely by the sub-contractor due to miscommunication. However, the stability of the candidate reference materials could well be estimated from the data that was available.

3.2 Dissemination

The BROC project was presented at the following meetings:

- S.P.J. van Leeuwen (13 September 2001) Preparation of a Certified Reference Material (CRM) for PAH in mussels, QUASIMEME PAH Workshop, FRS Marine Laboratory, Aberdeen, Scotland, oral presentation
- J. de Boer, S. van Leeuwen and M. Kotterman (21-26 October 2002), Certified Reference Materials for contaminants in biological matrices, QUASIMEME 10th anniversary conference, Barcelona, Spain, oral and poster presentation
- S.P.J. van Leeuwen (14-15 June 2001) Certified Reference Material for Biological Matrices Environment, health, safety – a challenge for measurements, Paris, France, poster presentation
- P. Korytar, P.E.G. Leonards, J. de Boer, U.A.Th. Brinkman (27-31 August 2001). 11th International Symposium, Advances and Applications of Chromatography in Industry, Bratislava, Slovak Republic, oral presentation
- J. de Boer (June 2002), Certified reference materials for persistent contaminants in biological matrices, International conference on Measurements and Testing in Europe, Warsaw, Poland, poster presentation

Furthermore, the project is disseminated at various other (national and international) meetings and occasions, for example, the annual meeting of the Marine Chemistry Working Group of the International Council for Exploration of the Sea (ICES, Copenhagen, Denmark), the Dutch Working group on Dioxins in Food, UNEP meeting on a global POP monitoring program, Geneva, Switzerland, March 2003, the annual meeting of the QUASIMEME scientific assessment group, Aberdeen, Scotland. The BROC results have been published at the web site of Netherlands Institute for Fisheries Research and that will be maintained for the coming years.

Further presentations are being planned, for example at the Dioxin 2004 symposium in Berlin. Also, at least one, but possibly two or three scientific papers are planned to be published.

3.3 Technological Implementation Plan (TIP) and exploitation plans

A draft TIP was prepared prior to the mid-term meeting in Brussels at a meeting of 6 September 2002 in Brussels. The TIP was discussed with the former scientific officer dr. Søren Bøwadt and he agreed with the outline of the draft-TIP. The final TIP is added to this final report in Appendix 6. The results will be presented to different parties (e.g. IRMM or NIST) in order to interest them for the actual production of these materials.

4. Conclusions

The results of this project show that:

- The production of wet, sterilised flounder and mussel sample in tins results in high quality materials that can easily be stored and transported. The tins are easily opened in the laboratory prior to analysis. The same conclusion accounts for the sediment material in glass jars.
- The homogeneity test showed that the materials were homogeneous within one lot and between several lots. Only the flounder material showed a slight inhomogeneity (median 9.6%) that is possibly related to alterations in the production of the flounder muscle material. However, all materials were suitable for this test certification. Another fish material than flounder, with somewhat higher contaminant concentrations may be considered for a future certification study on OCPs and BFRs.
- The stability of all compounds in the three candidate CRMs during a 12 months period was good (within the limits of uncertainty). No degradation was observed. The isochronous approach is very useful for testing stability as it can reduce the variance of the results. Reduction of analytical variance is beneficial to the stability estimation of the compounds in the candidate CRMs.
- The test-certification of the OCPs in flounder showed that the certification of flounder material is feasible, although the levels in the sample were low and sometimes close to the LOQ.
- The test-certification of the BFRs in flounder showed that it is feasible to certify a fish material for BFRs. Some analytical difficulties should be overcome in a future certification study but it is expected that participating laboratories will further improve their analysis in the near future. HBCD should preferably be analysed by LC-MS instead of GC.
- The test-certification of the BFRs in sediment showed that the it is feasible to certify this material for BFRs. As mentioned for the flounder, certification would improve by further improvement of the analytical techniques. The sample with lower, and more realistic level of BDE 209 would be preferable.
- Test certification of PAHs in mussels showed that it is possible to certify these contaminants in this matrix.
- The coordination of the project went smoothly due to highly motivated partners and the experience of the coordinator obtained earlier in similar EU M&T projects.

5. Recommendations

There is an increasing awareness of the presence of BFRs in food and the environment. European risk assessments are going on for several classes of BFRs. The analysis, occurrence and toxicity of BFRs receive a lot of international attention, for example at the International Dioxin2003 conference in Boston, USA, August 2003 (www.dioxin2003.com) and an international workshop on BFRs in Toronto, Canada, June 2004. Hundreds of laboratories are currently installing methods for BFR analysis in their labs. It is therefore highly recommended to start a project on the actual production of CRMs for BFRs.
Almost every year Europe is confronted with oil pollution from shipwrecking. These calamities cause have enormous financial consequences. Reliable measurements of PAHs in shellfish are therefore of utmost importance. No matrix-type PAH CRM with relevant PAH levels and including alkylated PAHs exist at this moment. It is therefore also highly recommended to produce such a CRM.
OCPs have been analysed by, many European laboratories since the early 1970s. Generally OCP levels in the environment are decreasing. However, it is expected that the OCPs measurements will be maintained in several European monitoring programmes for the environment and food for at least the coming decade. The analysis of OCPs at lower levels is difficult. The complexity of the analysis, and the different analytical behaviour of individual OCPs requires a high quality matrix-type CRM with realistic and accurate OCP levels. Such a CRM is currently not available, and it is therefore recommended to prepare and certify such a CRM.

6. Acknowledgements

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8. Appendices

Appendix

1	Results of the homogeneity study of BROC-01, -02 and –03.
2a	Stability study of OCPs in flounder (BROC-01)
2b	Stability study of BFRs in flounder (BROC-01)
2c	Stability study of BFRs in sediment (BROC-02)
2d	Stability study of PAHs in mussel (BROC-03)
3	Example of a protocol used for the interlaboratory study.
4a	Final report on the feasibility study of certification of OCPs in flounder
4b	Final report on the feasibility study of certification of BFRs in flounder
4c	Final report on the feasibility study of certification of BFRs in sediment
4d	Final report on the feasibility study of certification of PAHs in mussel
5	List of deliverables
6	Technology implementation plan

BROC (BIOLOGICAL REFERENCE MATERIALS FOR ORGANIC CONTAMINANTS)

Final Report

Partners

Project acronym:	BROC	
Project full title:	Biological Reference Materials for Organic Contaminants	
Proposal number:	GRD2-2000-30019	
Contract number:	G6RD-CT-2001-00518	
Starting date:	1 June 2001	
End date:	31 May 2003	
Duration:	24 months	
Project co-ordinator:	Netherlands Institute for Fisheries Research	NL
Contractors:	Marine Institute	IRL
	Stockholm University, Institute of Applied Environmental Research	S
Sub-contractor	Centre for Fisheries and Aquaculture Sciences	UK

Appendix 1. Results of the homogeneity study of BROC-01, -02 and –03.

Table 1a Error in the GC-MS determination of PBDEs and HBCD

	BDE28 µg/kg	**BDE47** µg/kg	**BDE100** µg/kg	**BDE99** µg/kg	**BDE154** µg/kg	**BDE153** µg/kg	**HBCD** µg/kg	**BDE209** µg/kg
	7.2	7.0	7.0	6.9	7.3	7.1	37	0.16
	7.4	7.3	7.2	7.1	7.5	7.1	35	0.16
	7.3	7.2	7.2	7.2	7.6	7.2	35	0.16
	7.2	7.1	7.1	7.1	7.6	7.1	36	0.16
	7.5	7.4	7.3	7.3	7.6	7.1	38	0.16
	7.0	7.0	7.0	7.0	7.4	7.1	34	0.16
	7.1	7.1	7.1	7.0	7.5	7.1	33	0.16
	7.1	7.0	7.0	7.0	7.4	7.0	33	0.16
	7.2	7.1	7.0	7.0	7.5	7.0	34	0.17
	7.1	7.0	7.0	7.0	7.4	7.0	37	0.16
mean	**7.2**	**7.1**	**7.1**	**7.1**	**7.5**	**7.1**	**35**	**0.16**
sd	0.15	0.127	0.12	0.11	0.11	0.049	1.9	0.003
rsd(%)	**2.0**	**1.8**	**1.7**	**1.5**	**1.5**	**0.70**	**5.3**	**2.1**

Table 1b Error in the GC-ECD determination of OCPs

	pentachloro-benzene µg/kg	**hexachloro-benzene** µg/kg	**a-HCH** µg/kg	**?-HCH** µg/kg	**ß-HCH** µg/kg	**ß-heptachloro-epoxide** µg/kg	**trans-chlordane** µg/kg	**cis-chlordane** µg/kg	**trans-nonachlor** µg/kg	**p,p'-DDE** µg/kg	**p,p'-DDD** µg/kg	**p,p'-DDT** µg/kg	**o,p'-DDT** µg/kg	**dieldrin** µg/kg	**endrin** µg/kg
	0.53	1.25	0.85	2.08	1.00	0.34	0.94	0.90	0.81	0.61	0.60	0.42	0.28	0.59	0.48
	0.50	1.28	0.86	2.15	1.03	0.35	0.94	0.90	0.82	0.61	0.61	0.43	0.28	0.60	0.49
	0.49	1.24	0.85	2.08	1.02	0.34	0.93	0.88	0.80	0.60	0.60	0.42	0.27	0.60	0.47
	0.49	1.25	0.85	2.09	1.02	0.34	0.94	0.88	0.81	0.60	0.61	0.43	0.28	0.61	0.48
	0.48	1.28	0.86	2.15	1.03	0.35	0.95	0.89	0.82	0.60	0.62	0.46	0.29	0.61	0.50
	0.48	1.28	0.88	2.19	1.05	0.36	0.96	0.90	0.83	0.60	0.63	0.48	0.30	0.62	0.50
	0.49	1.25	0.86	2.10	1.03	0.35	0.94	0.88	0.82	0.61	0.61	0.43	0.27	0.61	0.47
	0.48	1.24	0.86	2.06	1.02	0.34	0.94	0.87	0.80	0.59	0.59	0.43	0.28	0.60	0.47
	0.48	1.26	0.85	2.07	1.03	0.35	0.93	0.88	0.82	0.59	0.59	0.43	0.28	0.59	0.48
mean	**0.49**	**1.3**	**0.86**	**2.1**	**1.0**	**0.35**	**0.94**	**0.89**	**0.81**	**0.60**	**0.61**	**0.44**	**0.28**	**0.60**	**0.48**
sd	0.017	0.018	0.011	0.046	0.012	0.005	0.011	0.011	0.010	0.006	0.013	0.018	0.009	0.008	0.011
rsd(%)	**3.4**	**1.4**	**1.3**	**2.2**	**1.2**	**1.3**	**1.1**	**1.2**	**1.2**	**1.0**	**2.1**	**4.0**	**3.1**	**1.3**	**2.3**

Appendix 1 (continued). Results of the homogeneity study of BROC-01, -02 and –03.

Table 1c Error in the GC-MS determination of PAHs

	N	**1-C1-N**	**Fluorene**	**DBThio**	**P**	**A**	**2-C1-A**	**Fl**	**Py**	**C1-Py**	**BaA**	**Chrysene**	**BNThio**	**BeP**	**BaP**	**I123-cdP**	**BghiP**	**DahA**
	pg/ul	pg/ul	pg/ul	pg/ul	pg/ul	pg/ul	pg/ul	pg/ul	pg/ul	pg/ul	pg/ul	pg/ul	pg/ul	pg/ul	pg/ul	pg/ul	pg/ul	pg/ul
	485	514	504	547	519	513	542	555	542	545	558	587	592	553	496	543	527	553
	498	532	489	562	526	520	557	555	541	566	580	610	594	546	492	569	544	572
	493	522	497	558	518	516	554	552	538	554	558	593	587	542	497	573	552	560
	492	520	506	549	516	511	555	622	569	558	577	640	596	561	503	594	555	559
	494	520	515	553	495	492	548	595	559	545	571	640	601	555	505	573	527	562
	489	524	508	550	514	509	571	626	555	547	579	611	580	544	504	569	527	566
mean	**492**	**522**	**503**	**553**	**515**	**510**	**554**	**584**	**551**	**553**	**570**	**614**	**592**	**550**	**500**	**570**	**539**	**562**
sd	4.3	5.9	9.1	5.8	10	10	10	35	12	8.5	10	22	7.3	7.3	5.3	16	13	6.8
rsd(%)	**0.86**	**1.1**	**1.8**	**1.0**	**2.0**	**1.9**	**1.8**	**5.9**	**2.2**	**1.5**	**1.8**	**3.6**	**1.2**	**1.3**	**1.1**	**2.9**	**2.5**	**1.2**

N	=naphthalene
1-C1-N	=1-methyl naphthalene
Fluorene	=fluorene
DBThio	=dibenzothiophene
P	=phenanhrene
A	=anthracene
2-C1-A	=2-methyl anthracene
Fl	=fluoranthene
Py	=pyrene
C1-Py	=1-methyl pyrene
BaA	=benz [a] anthracene
Chrysene	=chrysene
BNThio	=benzo [b] naphtho [2,1-d] thiophene
BeP	=benzo [e] pyrene
BaP	=benzo [a] pyrene
I123-cdP	=indeno [1,2,3-c,d] pyrene
BghiP	=benzo [ghi] perylene
DahA	=dibenz [a,h] anthracene

Appendix 1 (continued). Results of the homogeneity study of BROC-01, -02 and –03.

Table 2a Within-batch variance in flounder of PBDEs

Code	BDE28 µg/kg ww	BDE47 µg/kg ww	BDE100 µg/kg ww	BDE99 µg/kg ww	BDE154 µg/kg ww	BDE153 µg/kg ww	HBCD µg/kg ww
BROC01 155:1	0.080	3.5	0.70	0.35	0.16	0.13	1.0
BROC01 155:2	0.078	3.6	0.70	0.35	0.16	0.13	1.0
BROC01 155:3	0.079	3.7	0.72	0.37	0.16	0.13	1.1
BROC01 155:4	0.11	4.0	0.75	0.36	0.16	0.13	0.92
BROC01 155.5	0.11	3.8	0.73	0.36	0.16	0.13	0.94
mean	**0.091**	**3.7**	**0.72**	**0.36**	**0.16**	**0.13**	**1.0**
sd	0.017	0.19	0.020	0.008	0.002	0.001	0.087
rsd(%)	**19**	**5.1**	**2.8**	**2.2**	**1.4**	**1.0**	**8.6**

Table 2b Within-batch variance in flounder of OCPs

Code	hexachloro-benzene µg/kg ww	?-HCH µg/kg ww	ß-heptachloro-epoxide µg/kg ww	trans-chlordane µg/kg ww	p,p'-DDE µg/kg ww	p,p'-DDD µg/kg ww
BROC01 156:1	0.19	0.45	0.11	0.029	2.4	0.39
BROC01 156:2	0.19	0.44	0.15	0.027	1.8	0.42
BROC01 156:3	0.19	0.42	0.11	0.029	1.9	0.41
BROC01 156:4	0.21	0.34	0.13	0.024	1.9	0.34
BROC01 156:5	0.19	0.37	0.14	0.024	1.8	0.40
mean	**0.19**	**0.40**	**0.13**	**0.027**	**2.0**	**0.39**
sd	0.008	0.047	0.016	0.003	0.25	0.03
rsd(%)	**4.2**	**12**	**13**	**9.6**	**13**	**7.5**

Pentachlorobenzene, a-HCH, ß-HCH, cis-chlordane, trans-nonachlor, p,p'-DDT, o,p'-DDT, dieldrin and endrin were analysed but below the limit of quantification, which were 0.04, 0.09, 0.12, 0.02, 0.10, 0.05, 0.06 and 0.14 ng/g respectively. Oxy-chlordane was not analysed.

Table 2c Within-batch variance in sediment of PBDEs

Code	BDE28 µg/kg dw	BDE47 µg/kg dw	BDE100 µg/kg dw	BDE99 µg/kg dw	BDE154 µg/kg dw	BDE153 µg/kg dw	HBCD µg/kg dw	BDE209 µg/kg dw
BROC01 155:1	0.72	12	3.5	19	1.8	2.7	139	804
BROC01 155:2	0.72	12	3.5	19	1.7	2.5	152	805
BROC01 155:3	0.66	11	3.4	18	1.8	2.5	134	570
BROC01 155:4	0.73	12	3.5	19	1.7	2.6	137	671
BROC01 155:5	0.75	12	3.6	19	1.8	2.5	131	749
mean	**0.72**	**12**	**3.5**	**19**	**1.8**	**2.5**	**139**	**720**
sd	0.034	0.24	0.044	0.21	0.043	0.11	8.0	100
rsd(%)	**4.8**	**2.0**	**1.2**	**1.1**	**2.5**	**4.2**	**5.8**	**14**

Appendix 1 (continued). Results of the homogeneity study of BROC-01, -02 and –03.

Table 2d Within-batch variance in mussel of PAHs

Code	**N**	**1-C1-N**	**Fluorene**	**DBThio**	**P**	**A**	**2-C1-A**	**Fl**	**Py**	**C1-Py**	**BaA**	**Chrysene**	**BNThio**	**BeP**	**BaP**	**I123-cdP**	**BghiP**	**DahA**
	µg/kg ww	µg/kg ww	µg/kg ww	µg/kg ww	µg/kg ww	µg/kg ww	µg/kg ww	µg/kg ww	µg/kg ww	µg/kg ww	µg/kg ww	µg/kg ww	µg/kg ww	µg/kg ww	µg/kg ww	µg/kg ww	µg/kg ww	µg/kg
XPOT1#1	2.7	5.9	18	16	120	26	17	166	152	26	74	64	16	38	20	4.9	8.5	2.8
XPOT1#2	2.7	5.9	17	16	118	27	19	169	150	26	72	64	17	36	17	5.0	9.8	2.5
XPOT2#3	2.5	5.8	18	14	117	25	16	175	145	25	64	62	17	30	15	4.4	8.2	2.4
XPOT2#4	2.7	5.4	17	14	117	26	17	178	144	27	72	59	17	33	17	4.6	7.8	3.0
XPOT2#5	3.2	8.0	17	14	124	28	16	175	165	26	74	59	16	38	20	4.0	5.3	2.0
mean	**2.8**	**6.2**	**17**	**15**	**119**	**26**	**17**	**173**	**151**	**26**	**71**	**62**	**17**	**35**	**18**	**4.6**	**7.9**	**2.5**
sd	0.26	1.0	0.55	1.1	2.9	1.1	1.0	4.9	8.4	0.71	4.2	2.5	0.55	3.6	2.3	0.40	1.6	0.3
rsd(%)	**9.4**	**17**	**3.1**	**7.4**	**2.5**	**4.3**	**6.2**	**2.9**	**5.6**	**2.7**	**5.8**	**4.1**	**3.3**	**10**	**13**	**8.8**	**21**	**15**

N	=naphthalene
1-C1-N	=1-methyl naphthalene
Fluorene	=fluorene
DBThio	=dibenzothiophene
P	=phenanhrene
A	=anthracene
2-C1-A	=2-methyl anthracene
Fl	=fluoranthene
Py	=pyrene
C1-Py	=1-methyl pyrene
BaA	=benz [a] anthracene
Chrysene	=chrysene
BNThio	=benzo [b] naphtho [2,1-d] thiophene
BeP	=benzo [e] pyrene
BaP	=benzo [a] pyrene
I123-cdP	=indeno [1,2,3-c,d] pyrene
BghiP	=benzo [ghi] perylene
DahA	=dibenz [a,h] anthracene

Appendix 1 (continued). Results of the homogeneity study of BROC-01, -02 and –03.

Table 3a Between-batch variance in flounder of PBDEs

Code	BDE28 µg/kg ww	BDE47 µg/kg ww	BDE100 µg/kg ww	BDE99 µg/kg ww	BDE154 µg/kg ww	BDE153 µg/kg ww	HBCD µg/kg ww
BROC01 005	0.086	3.5	0.67	0.37	0.15	0.13	1.03
BROC01 025	0.075	3.8	0.73	0.36	0.16	0.12	1.13
BROC01 045	0.074	3.4	0.68	0.33	0.16	0.13	0.96
BROC01 065	0.074	3.0	0.60	0.29	0.14	0.12	0.92
BROC01 085	0.108	3.2	0.62	0.29	0.14	0.11	0.78
BROC01 105	0.101	3.1	0.59	0.29	0.14	0.11	0.91
BROC01 125	0.103	3.1	0.60	0.28	0.14	0.11	0.73
BROC01 145	0.102	3.1	0.60	0.29	0.14	0.11	0.74
BROC01 155	0.080	3.5	0.70	0.35	0.16	0.13	1.03
BROC01 165	0.125	4.0	0.74	0.36	0.17	0.14	0.86
BROC01 185	0.101	3.2	0.61	0.30	0.14	0.11	0.83
BROC01 205	0.117	3.3	0.63	0.34	0.15	0.12	1.20
BROC01 225	0.131	4.1	0.80	0.40	0.20	0.15	1.02
BROC01 245	0.099	3.3	0.62	0.30	0.14	0.11	0.73
BROC01 265	0.107	3.6	0.69	0.35	0.16	0.13	0.82
mean	**0.10**	**3.4**	**0.66**	**0.33**	**0.15**	**0.12**	**0.91**
sd	0.018	0.34	0.063	0.039	0.017	0.013	0.15
rsd(%)	**18**	**10**	**10**	**12**	**11**	**10**	**16**

Table 3b Between-batch variance in flounder of OCPs

Code	hexachloro-benzene µg/kg ww	?-HCH µg/kg ww	ß-heptachloro-epoxide µg/kg ww	trans-chlordane µg/kg ww	p,p'-DDE µg/kg ww	p,p'-DDD µg/kg ww
BROC01 006	0.15	0.34	0.15	0.027	1.8	0.47
BROC01 026	0.15	0.30	0.15	0.021	1.9	0.40
BROC01 046	0.17	0.31	0.15	0.026	2.1	0.48
BROC01 066	0.16	0.30	0.16	0.023	1.9	0.43
BROC01 086	0.14	0.33	0.15	0.027	1.7	0.41
BROC01 106	0.14	0.31	0.14	0.021	1.9	0.41
BROC01 126	0.16	0.32	0.10	0.026	2.1	0.42
BROC01 146	0.17	0.35	0.12	0.025	2.0	0.44
BROC01 156	0.19	0.37	0.14	0.024	1.8	0.40
BROC01 166	0.16	0.29	0.14	0.025	2.4	0.42
BROC01 186	0.15	0.34	0.14	0.020	2.0	0.40
BROC01 206	0.15	0.31	0.10	0.027	2.2	0.37
BROC01 226	0.15	0.34	0.095	0.026	1.9	0.34
BROC01 246	0.14	0.27	0.094	0.025	1.7	0.34
BROC01 266	0.15	0.33	0.064	0.020	2.0	0.37
mean	**0.16**	**0.32**	**0.13**	**0.024**	**2.0**	**0.41**
sd	0.014	0.026	0.029	0.002	0.19	0.040
rsd(%)	**9.3**	**8.0**	**24**	**9.9**	**9.6**	**9.9**

Pentachlorobenzene, a-HCH, ß-HCH, cis-chlordane, trans-nonachlor, p,p'-DDT, o,p'-DDT, dieldrin and endrin were analysed but below the limit of quantification, which were 0.04, 0.09, 0.12, 0.02, 0.10, 0.05, 0.06 and 0.14 ng/g respectively. Oxy-chlordane was not analysed.

Appendix 1 (continued). Results of the homogeneity study of BROC-01, -02 and –03.

Table 3c Between-batch variance in sediment of PBDEs

Code	**BDE28** µg/kg dw	**BDE47** µg/kg dw	**BDE100** µg/kg dw	**BDE99** µg/kg dw	**BDE154** µg/kg dw	**BDE153** µg/kg dw	**HBCD** µg/kg dw	**BDE209** µg/kg dw
No0005	0.68	11.0	3.46	18.5	1.78	2.58	156	761
No0025	0.75	11.9	3.63	19.2	1.81	2.51	145	560
No0045	0.73	11.7	3.58	19.0	1.84	2.68	141	573
No0065	0.63	10.3	3.23	17.2	1.67	2.45	133	769
No0085	0.77	11.9	3.54	19.0	1.77	2.57	139	543
No0105	0.62	10.4	3.26	17.4	1.65	2.55	134	736
No0125	0.72	11.6	3.66	19.3	1.83	2.59	152	778
No0145	0.76	12.0	3.71	19.6	1.89	2.90	139	593
No0155	0.72	11.6	3.52	18.9	1.82	2.72	139	804
No0165	0.65	10.7	3.33	18.0	1.70	2.43	136	673
No0185	0.66	10.7	3.32	17.8	1.69	2.71	125	794
No0205	0.68	11.1	3.44	18.4	1.75	2.58	136	844
No0225	0.77	12.3	3.69	19.7	1.85	2.74	146	809
No0245	0.72	11.5	3.56	19.2	1.82	2.62	147	784
No0265	0.85	12.9	3.87	20.4	1.92	2.76	136	757
No0285	0.72	11.6	3.63	19.4	1.86	2.76	146	724
mean	**0.71**	**11**	**3.5**	**19**	**1.8**	**2.6**	**141**	**719**
sd	0.059	0.72	0.18	0.87	0.079	0.13	7.8	99
rsd(%)	**8.3**	**6.3**	**5.0**	**4.6**	**4.4**	**4.8**	**5.6**	**14**

Appendix 1 (continued). Results of the homogeneity study of BROC-01, -02 and –03.

Table 3d Between-batch variance in mussels of PAHs (µg/kg ww)

Code	N	1-C1-N	Fluorene	DBThio	P	A	2-C1-A	Fl	Py	C1-Py	BaA	Chrysene	BNThio	BeP	BaP	I123-cdP	BghiP	DahA
BROC Tin 5	3.3	8.6	19	14	127	28	27	169	164	28	74	60	17	35	18	4.4	3.3	1.3
BROC Tin 25	3.2	7.4	19	15	122	28	27	170	163	28	72	62	17	35	23	5.4	5.4	1.3
BROC Tin 45	3.2	7.8	20	15	123	29	25	175	165	28	74	65	18	34	23	5.4	3.6	1.8
BROC Tin 65	3.4	8.1	20	14	125	28	25	179	165	28	73	64	19	32	17	4	3.6	1.8
BROC Tin 85	3.3	7.5	19	16	129	27	25	176	168	27	76	57	14	30	19	4	3.9	1.1
BROC Tin 105	2.9	8.1	19	15	125	28	25	181	169	26	72	64	16	34	18	3	5.7	2.1
BROC Tin 125	3.0	7.3	20	15	124	27	26	183	168	26	72	62	18	35	18	5.2	4.9	1.4
BROC Tin 145	2.8	7.0	20	15	126	26	26	186	169	27	73	68	17	39	18	3.5	3.9	1.6
BROC Tin 155	2.9	7.5	19	15	129	26	25	181	165	26	76	66	15	36	18	4.1	5.7	1.6
BROC Tin 165	2.7	7.2	19	14	129	26	26	180	168	24	72	64	18	37	18	3.7	4.9	2.4
BROC Tin 185	2.9	6.8	19	14	125	27	25	175	162	24	73	63	16	35	20	3.7	5.3	2.1
BROC Tin 205	3.0	8.1	18	15	121	29	27	181	166	26	77	68	17	36	20	4.2	5.4	2.2
BROC Tin 225	3.0	7.7	19	13	127	29	25	175	164	26	74	66	16	33	18	4.7	5.3	1.1
BROC Tin 245	3.5	8.0	21	16	121	31	23	188	162	25	75	70	19	43	23	3.5	4	2.1
BROC Tin 265	2.8	7.9	21	16	126	31	24	178	162	26	79	66	16	41	23	4.6	3.9	1.2
mean	**3.1**	**7.7**	**19**	**15**	**125**	**28**	**26**	**178**	**165**	**26**	**74**	**64**	**17**	**36**	**20**	**4.2**	**4.6**	**1.7**
sd	0.24	0.48	0.83	0.85	2.8	1.5	1.2	5.4	2.4	1.3	2.0	3.2	1.3	3.3	2.2	0.72	0.86	0.43
rsd(%)	**7.9**	**6.3**	**4.3**	**5.8**	**2.2**	**5.5**	**4.8**	**3.0**	**1.4**	**4.9**	**2.7**	**5.0**	**7.8**	**9.4**	**11**	**17**	**19**	**26**

N =naphthalene
1-C1-N =1-methyl naphthalene
Fluorene =fluorene
DBThio =dibenzothiophene
P =phenanhrene
A =anthracene
2-C1-A =2-methyl anthracene
Fl =fluoranthene
Py =pyrene
C1-Py =1-methyl pyrene
BaA =benz [a] anthracene
Chrysene =chrysene
BNThio =benzo [b] naphtho [2,1-d] thiophene
BeP =benzo [e] pyrene
BaP =benzo [a] pyrene
I123-cdP =indeno [1,2,3-c,d] pyrene
BghiP =benzo [ghi] perylene
DahA =dibenz [a,h] anthracene

Appendix 1 (continued). Results of the homogeneity study of BROC-01, -02 and –03.

Table 4a Overview of relative standard deviations of PBDEs in flounder (%)

	BDE28	BDE47	BDE100	BDE99	BDE154	BDE153	HBCD
Rsd_{st}	2.0	1.8	1.7	1.5	1.5	0.7	5.3
Rsd_{within}	19	5.1	2.8	2.2	1.4	1.0	8.6
$Rsd_{between}$	18	10	10	12	11	10	16
$Rsd_{inhomogeneity}$	**19***	**8.6**	**9.2**	**12**	**11**	**10**	**14**

Table 4b Overview of relative standard deviations of OCPs in flounder (%)

	hexachloro-benzene	?-HCH	ß-heptachloro-epoxide	trans-chlordane	p,p'-DDE	p,p'-DDD
Rsd_{st}	1.4	2.2	1.3	1.1	1.1	2.1
Rsd_{within}	4.2	12	13	9.6	13	7.5
$Rsd_{between}$	9.3	8.0	24	9.9	9.6	9.9
$Rsd_{inhomogeneity}$	**8.3**	**12***	**20**	**2.6**	**13***	**6.5**

Pentachlorobenzene, a-HCH, ß-HCH, cis-chlordane, trans-nonachlor, p,p'-DDT, o,p'-DDT, dieldrin and endrin were analysed but below the limit of quantification. Oxy-chlordane was not analysed.

Table 4c Overview of relative standard deviations of PBDEs in sediment (%)

	BDE28	BDE47	BDE100	BDE99	BDE154	BDE153	HBCD	BDE209
Rsd_{st}	2.0	1.8	1.7	1.5	1.5	0.7	5.3	2.1
Rsd_{within}	4.8	2.0	1.2	1.1	2.5	4.2	5.8	14
$Rsd_{between}$	8.3	6.3	5.0	4.6	4.4	4.8	5.6	14
$Rsd_{inhomogeneity}$	**6.8**	**5.9**	**4.9**	**4.5**	**3.7**	**2.3**	**5.8***	**14***

* theoretically no inhomogeneity can be calculated and therefore the highest inhomogeneity value (rsd_{within}) is applied here

Appendix 1 (continued). Results of the homogeneity study of BROC-01, -02 and –03.

Table 4d. Overview of relative standard deviation of PAHs in mussel (%)

	N	1-C1-N	Fluorene	DBThio	P	A	2-C1-A	Fl	Py	C1-Py	BaA	Chrysene	BNThio	BeP	BaP	I123-cdP	BghiP	DahA
Rsd_{st}	0.86	1.1	1.8	1.0	2.0	1.9	1.8	5.9	2.2	1.5	1.8	3.6		1.3	1.1	2.9	2.5	1.2
Rsd_{within}	9.4	17	3.1	7.4	2.5	4.3	6.2	2.9	5.6	2.7	5.8	4.1	3.3	10	13	8.8	21	15
$Rsd_{between}$	7.9	6.3	4.3	5.8	2.2	5.5	4.8	3.0	1.4	4.9	2.7	5.0	7.8	9.4	11	17	19	26
$Rsd_{inhomogeneity}$	**9.4***	**17***	**2.9**	**7.4***	**2.5***	**3.4**	**6.2***	**0.92**	**5.6***	**4.1**	**5.8***	**2.9**	**7.1**	**10***	**13***	**15**	**21***	**21**

N =naphthalene
1-C1-N =1-methyl naphthalene
Fluorene =fluorene
DBThio =dibenzothiophene
P =phenanhrene
A =anthracene
2-C1-A =2-methyl anthracene
Fl =fluoranthene
Py =pyrene
C1-Py =1-methyl pyrene
BaA =benz [a] anthracene
Chrysene =chrysene
BNThio =benzo [b] naphtho [2,1-d] thiophene
BeP =benzo [e] pyrene
BaP =benzo [a] pyrene
I123-cdP =indeno [1,2,3-c,d] pyrene
BghiP =benzo [ghi] perylene
DahA =dibenz [a,h] anthracene

* theoretically no inhomogeneity can be calculated and therefore the highest inhomogeneity value (rsd_{within}) is applied here

Appendix 4. Example of a protocol used for the interlaboratory study.

Protocol for the certification of organochlorine pesticides (OCPs) in a candidate reference material flounder **(BROC)**

1. The organochlorine pesticides (OCPs) to be analysed are p,p'-DDT, p,p'-DDE, p,p'-DDD, o,p'-DDT, dieldrin, endrin, ?-HCH (hexachlorocyclohexane), ?-HCH, ?-HCH, ?-heptachloroepoxide, HCB (hexachlorobenzene), QCB (pentachlorobenzene), trans-nonachlor, cis-chlordane, trans-chlordane and oxychlordane.
2. The flounder has been sampled at the Western-Scheldt estuary (The Netherlands). The fish has been filleted, homogenised and tinned (ca. 70 ml). Ca. 300 lots of the tinned flounder have been sterilised at 120°C.
3. Six individual, fully independent analyses have to be carried out, at at least two different days, and from the available tins, using two independent calibration curves. The lipid content is approximately 15 g/kg and the moisture content is approximately 78%. The minimum sample intake should be at least 250 mg lipid or 30 g flounder homogenate. A test analysis should be carried out prior to the actual six-fold analysis of the earlier mentioned tins.
   In case of HR-MS only, one calibration curve may be used for both series. However, in that case the stability of the calibration curves should be checked by the response of the compounds in one of the dilutions in the central area of the curve. If that response has changed by more than 5%, then a new calibration curve should be made and used. Two procedure blanks should be carried out, one in each series.
   In no case a selection should be made of the six best results out of a higher number of analyses. On the other hand, no doubtful results should be submitted.
4. One second, exhaustive extraction should be carried out after the first extraction. This extraction should be carried out with a different solvent or solvent combination, which contains at least a medium-polar solvent. Internal standards can be added prior to the second extraction in order to enable quantification of the target compounds. An extraction efficiency should be calculated.
5. The tins should be opened carefully, without causing losses of the contents (some separation of liquid and lipids from the tissue might have taken place). This can be done by holding them upside down and opening the bottom. Pre-cooling may also help to decrease the eventual over-pressure. Immediately before sub-sampling the complete contents of the tins should be thoroughly re-homogenised .
6. Three replicates of a total lipid determination have to be carried out. The sub-sampling for the total lipid determination shall take place at the same moment of the sub-sampling for the OCP determination. The method used should preferably be a Bligh and Dyer method or a Smedes method. Methods which are not comparable to these methods should not be used. For participants who also participate for the BFRs in flounder, they can report the same results both for OCPs and BFRs.
7. The recovery of the method should be determined. One of the below mentioned methods should be used.
   Method 1 using ^{13}C-labeled compounds as internal standard (isotope-dilution technique):
   Preferably for each compounds an ^{13}C-labeled compound should be used but as a minimum one ^{13}C-labeled congener should be used per homologue group. The flounder homogenate should be spiked with the ^{13}C-labeled congeners before extraction. The incubation time should be at least 16 hours. Acetone or a similar semi-polar solvent should be used to enable good solubility of the spike solution in the matrix. An estimate of recovery should be given (the results should not be corrected for these recoveries).
   Method 2 using standard addition of the native compounds: For all congeners, recovery should be determined by means of standard addition. This method includes singular spiking at four different levels (ca. 50, 100, 150 and 200%) and linear. The method for the calculation of the

recovery is given below. The incubation time should be at least 16 hours. Acetone or a similar semi-polar solvent should be used to enable a good solubility of the spike solution in the flounder matrix. The mean value of each OCP of the six OCP determinations (see 3.) should be used for the 0-spike level. The pattern of the spiking solution should be adapted to the OCP pattern of the flounder sample. When a recovery <100% is found, the final result should be corrected for this recovery unless the recovery value plus the uncertainty (one relative standard deviation (RSD)) overlap with 100% (e.g. 99 ± 2%). In case the value of the recovery is >100%, a correction should not be applied. Recovery percentages >120% and < 60% will not be accepted. Recoveries based on a calculation showing an RSD of >20% will not be accepted.

8. Standards used for calibration should be of a known and sufficiently high purity (>98%) and of a traceable origin.
9. For a check on the calibration, standard solutions of OCP in unknown concentrations will be supplied and should be analysed in six-fold, with two independent calibration curves, on two different days. The labels and concentrations of the OCPs are mentioned below.

Label	Compound	Concentration range (ng/g)	Solvent mixture
OCP-A	p,p'-DDT, p,p'-DDE, p,p'-DDD, HCB (hexachlorobenzene), QCB (pentachlorobenzene), trans-nonachlor and oxychlordane.	100-1500	100% iso-octane
OCP-B	o,p'-DDT, dieldrin, ?-HCH (hexachlorocyclohexane), ?-HCH, ?-HCH, ?-heptachloroepoxide, cis-chlordane and oxychlordane.	100-1500	100% iso-octane
OCP-C	Endrin	100-1500	110% iso-octane

Participants can voluntarily submit the results of the unknown solutions prior to the start of the analysis of the six tins. The co-ordinator will report the participant as soon as possible (but at least within a few working days) on the results of the unknown solution.

10. Electron capture detection or mass spectrometric detection should be applied. Multi-level calibration curves should be made for each OCP and submitted. The concentrations of the OCPs analysed in the flounder extract should be between the lowest and the highest concentration of the calibration curve. The expected concentration range of the OCPs in the flounder is ca. < 1.0 $ng.g^{-1}$ ww. for p,p'-DDT, , p,p'-DDD, o,p'-DDT, ?-heptachloroepoxide, endrin, ?-HCH (hexachlorocyclohexane), ?-HCH, ?-HCH, HCB (hexachlorobenzene), QCB (pentachlorobenzene), trans-nonachlor, cis-chlordane, trans-chlordane and oxychlordane and 1-10 $ng.g^{-1}$ ww. p,p'-DDE and dieldrin. It should be emphasized that a number of OCPs present in the flounder may be in the lower part of these ranges, which may imply that one or two points of the calibration curve related to much higher concentrations may better not be used in such cases. However, at least four points of the calibration curves should be used for the determination. When not using isotope-dilution, the use of at least one syringe standard is mandatory to correct for injection variations and to correct for the final concentration step prior to injection.
11. At least two columns of different polarity should be used. For each compound, the results obtained from only one column, i.e., the one that is considered the best for that compound, should be submitted. The minimum column length should be 50m and the maximum internal diameter 0.25 mm. When using MS, one of the columns used may be shorter than 50m. Also, in case of using MDGC, column lengths may be different from the specifications mentioned above.

When using HR-MS detection the analysis on one column is sufficient and when using LR-EI-MS participants should convince themselves from sufficient selectivity of their system for all compounds.

12. Chromatograms of all six measurements on both columns have to be submitted, plus chromatograms of the unknown solutions, a blank, a standard and a sample extract without internal standards added of both columns. The chromatograms should give a clear impression of the separation. One copy of a chromatogram of the flounder sample at each column and one copy of a chromatogram of the unknown solutions at each column should be presented at the evaluation meeting. All OCPs to be determined should be indicated in these chromatograms by their name and the chromatograms should be clearly marked with your laboratory code, the column and the analysed matrix or solution.
13. The report forms can be emailed to s.p.j.vanleeuwen@rivo.wag-ur.nl. Furthermore, all data on the method and the results (hard-copy of the report forms), and the chromatograms should be received at RIVO, IJmuiden, NL at or before 31 October 2002.

14. The participant should be represented at the evaluative meeting for which a tentative date is set at 27-29 January 2003 in Brussels by the responsible for the work or by the actual performer of the work.
15. Method for calculation of the recovery when using standard addition.
Excel (Windows) or (MacIntosh)

Y = ax + b

Recovery = 100% . a

	0%	50%	100%	150%	200%
theoretical value	...	...	...	...	...
measured value	...	...	...	...	...

Under Tools, go to Data analysis (if necessary: install through Add-Inns and ToolPak), select Regression

Input Y range: range of the measured values
Input X range: range of the theoretical values

Recovery = 100 . X variable 1
st dev = 100 . standard error
rel st dev = st dev/recovery . 100%