Results of the ICES/IOC/OSPARCOM interlaboratory study on the determination of chlorobiphenyls in seal, marine sediment and fish

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Abstract

An interlaboratory study on the determination of chlorobiphenyls (CBs) in seal, marine sediment and fish was organised by the International Council for Exploration of the Sea, the Intergovernmental Oceanographic Commission and the Oslo and Paris Commissions. Seventy-five laboratories from 18 countries participated in this study which was carried out according to a stepwise design, each step dealing with one or two essential parts of the CB analysis: optimisation of the GC, calibration, gas chromatographic separation, long term variation, clean-up and extraction. Before and after each step extensive advice was given to the participants. In contrast with earlier organised interlaboratory studies on the determination of CBs, significant improvement in the performance of laboratories was observed. Finally, standard errors for the reproducibility of around 15% were obtained for CBs 138, 153 and 180 in seal blubber oil and CBs 118, 138 and 153 in dried marine sediment. The determination of CBs in wet lean fish tissue resulted in much larger standard errors, and should, therefore, be further improved.

1. Introduction

While for more than 20 years the contamination of the environment by polychlorinated biphenyls (PCBs) has been a major source of concern, at the same time there have been problems with the comparability of PCB determinations [1,2]. After a model designed by the Community Bureau of Reference (BCR) of the European Community, with which an improvement in the comparability of results of PCB determinations within a relatively small group of laboratories was obtained [3,4], the International Council for Exploration of the Sea (ICES), the Intergovernmental Oceanographic Commission (IOC) and the Oslo and Paris Commission (OSPARCOM) decided to organise a stepwise designed interlaboratory study on the determination of individual chlorobiphenyls (CBs) in different samples from the marine environment for laboratories regularly reporting to these organisations and related laboratories carrying out CB analyses for marine environmental monitoring purposes. The objectives of the study were defined in the following way: to determine the variation in the results of the CB determination among the participating laboratories, to identify the sources that cause this variation, and to reduce the variation in the results by means of a learning process through a step-by-step approach. The different steps of the study were arranged in the following way:

(1) analysis of standard solutions - study on the optimisation of the GC,

(2) analysis of cleaned sediment and seal blubber extracts - study on the calibration and the chromatographic separation,
(3a) repeated analysis of certified reference material (CRM) - study on the long term variance,
(3b) analysis of uncleaned sediment and seal blubber extracts - study on the clean-up,
(4) analysis of dried sediment, seal blubber oil and wet fish tissue - study on the extraction.
The results of the first and second step have been reported before [5,6]. This paper will, therefore, focus on the results of the last three steps of the study.

2. Experimental

The following 10 CBs were selected for this study: CB28 (2,4,4'-trichlorobiphenyl), CB31 (2,5,4'-tri CB), CB52 (2,5,2', 5'-tetra CB), CB101 (2,4,5,2',5'-penta CB), CB 105 (2,3,4,3',4'-penta CB), CB118 (2,4,5,3',4'-penta CB), CB 138 (2,3,4,2',4',5'-hexa CB), CB153 (2,4,5,2',4',5'-hexa CB), CB156 (2,3,4,5,3',4'-hexa CB) and CB180 (2,3,4,5,2',4',5'-hepta CB). Because CB138 co-elutes with CB163 and, presumably, CB160 on most commonly used GC columns [7,8], in this exercise participants, were requested to report the sum of the CBs 138, 163 and 160 as CB 138. For step 3a participants were requested to determine CBs 52, 153, 156 in BCR-CRM 349 - cod liver oil in six independent analyses with intervals of at least one week. For step 3b participants were requested to determine the 10 CBs in a standard solution in iso-octane - as was already done in step 2 of the study [6], and to determine the 10 CBs in cleaned-up and uncleaned extracts of seal blubber and marine sediment. In step 4 the determination of the CBs in a standard solution was repeated again in order to keep a continuous control on the calibration of the participants. Furthermore, it was requested to determine the 10 CBs in a seal blubber oil, a dried marine sediment and a wet cod tissue. For each step of this study participants were given a maximum time-span of 4 months.
The standard solutions with the 10 CBs in undisclosed concentrations were each time freshly prepared from crystalline CBs with a minimum purity of 98% for CBs 31 and 156 and 99% for the other CBs. The solid standards were weighed with ultimate precision and dissolved in iso-octane. Dilution and ampouling were carried out strictly according to existing guidelines [9]. Each ampoule contained ca. 5 ml. standard solution. In step 3b and 4 a blank of 5 ml iso-octane (nanograde quality (Promochem, Wesel, Germany)) was also made available. The uncleaned seal blubber extract was prepared from a juvenile seal from the Dutch Wadden Sea by extraction in dichloromethane/pentane (1:1 v/v) and replacing these solvents by iso-octane. Cleaned-up extracts were obtained after clean-up over alumina and fractionation over silica columns. The seal blubber oil was prepared from an adult male grey seal from Sable Island (east of New Foundland, Canada). The marine sediment sample originated from the Dutch Wadden Sea and the sediment extract was prepared from the same sample by extraction with acetone/hexane (3:2 v/v). Cleaned-up extracts were obtained after concentration (removal of acetone), desulphurizing, elution over silica columns and replacing the hexane by iso-octane. The cod muscle tissue was obtained from southern North Sea cod. After homogenizing the samples were canned and sterilised.
For all standard solutions, extracts and samples homogeneity tests were carried out by analysing the 10 CBs in 5 out of ca. 50 samples of each material. No significant differences between the respective samples were found and a total homogeneity may be assumed. For each step participants received guidelines for the exercise with extensive advice. All determinations had to be carried out on two different GC columns with minimum lengths of 50 m and maximum internal diameters of 0.25 mm. The use of an internal standard was mandatory, but the selection of it was left to the participants.

Hydrogen was recommended as a carrier gas, but helium was accepted as an alternative. A fixed injection volume ≤ 1 µl and the use of iso-octane as a solvent was recommended. Participants were allowed to use GC/ECD or GC/MS, with splitless or on-column injection. Optimisation was, however, essential. In case of GC/ECD a multilevel calibration was strongly recommended to overcome difficulties from the limited linear range of the ECD [5].

3. Statistics

Because the error in this type of study shows a relative character, different from the ISO 5725 standard, a model with a multiplicative error structure was used. After log-transformation and back transformation the model provided a repeatability value r and a reproducibility value R which must be applied as a factor instead of using them as coefficients of variation. The standard deviations s_r and s_R should be applied in the same way. For a small s_r and s_R the values s_r -1 and s_R -1 may roughly be compared with the values of the coefficients of variation used in ISO 5725.
In order to examine the correlation structure between the different CBs, a principal component analysis (PCA) was performed on the correlation matrix of the logarithms of each CB concentration. The results of these PCAs are shown in the form of a biplot [10]. Such a figure also shows the correlation ("loading") between each CB and the first two components by means of a vector. The orthogonal projection of each observation on such a vector approximates the value of the accompanying CB. The length of the vector indicates the reliability of the approximation. In this way the overall performance of a laboratory is clearly made visible in one picture. For the cleaned-up and uncleaned samples the paired sums (equivalent to the means) and paired differences have been used for the PCA[11].

4. Results and discussion

With 75 participating laboratories this study is the largest intercomparison on CB analysis ever organised. Of all participants, 39 have participated in all five steps of the study. A number of improvements in the performance of the participating laboratories was observed. In step 4 all laboratories used column dimensions as recommended after the first two steps with minimum lengths of 50 m and maximum internal diameters of 0.25 mm. A further improvement could be obtained when more laboratories would reduce the internal diameter of their column down to 0.20 or 0.15 mm. In step 1 35% of the participants used hydrogen as a carrier gas, while this was more than 50% in the last step. Nitrogen, which was incidentally used in the beginning, is not used anymore now. In general a distinct improvement in the quality of the chromatograms was observed, reflecting optimised GC conditions. Most participants used GC/ECD for the serial determination. In the final step only three laboratories used GC/MS. Their results generally showed higher deviations from the mean values, which is possibly partly due to the use of manual injection instead of autosamplers. Splitless injection was used by 80% and on-column injection by 11% of the participants.
The study on the long term variation (step 3a) resulted in an s_R of 19% for CBs 52 and 153 and 78% for CB 156 (the multuplicative model was not used in this part of the study). The s_r was: 8.5% for CB 52, 7.0% for CB 153 and 25% for CB 156. The s_r/s_R ratio of CB 153 could be compared with that obtained in step 2 of the study and was almost 50% higher. This showed the effect of the long term variation. A duplicate analysis always gives a too optimistic idea of the variation of the determined CB concentration.

Table 1. Comparison of s_Rs of the determination of CBs in the unknown solution, the seal blubber oil and the sediment with s_Rs in (extracts of) similar samples from previous steps of this study and s_Rs in cod.

Step	Unknown solution			Seal			sediment			Cod
CB	2	3b	4	2[1)]	3b[2)]	4	2[1)]	3b[2)]	4	4
28	1.19	1.22	1.17	-	3.39	2.28	-	1.39	1.23	1.93
31	1.33	1.17	1.16	-	5.30	4.81	-	1.31	1.21	3.84
52	1.19	1.11	1.12	1.36	1.15	1.39	1.36	1.72	1.23	2.15
101	1.16	1.12	1.14	1.14	1.13	1.20	1.24	1.39	1.30	1.62
105	1.30	1.15	1.19	1.38	1.32	1.32	1.38	1.45	1.25	1.95
118	1.26	1.12	1.14	1.24	1.17	1.27	1.24	1.29	1.16	1.57
138	1.17	1.13	1.13	1.30	1.15	1.12	1.30	1.32	1.15	1.58
153	1.17	1.11	1.13	1.34	1.09	1.16	1.34	1.20	1.17	1.51
156	1.27	1.15	1.14	1.62	1.30	1.65	1.62	1.65	1.33	2.10
180	1.18	1.11	1.15	1.40	1.11	1.13	1.40	1.17	1.22	1.68
mean	1.22	1.14	1.14	1.36[3)]	1.18[3)]	1.28[3)]	1.36[3)]	1.39	1.23	1.99

[1)] cleaned extract; [2)] uncleaned extract; [3)] without CBs 28 and 31.

The high values of s_r and s_R found for CB 156 may be explained in two ways: (1) the determination of CB 156 is more difficult than that of the other two CBs due to co-elution on several GC columns - for a selective group of laboratories which had determined CB 156 free from co-eluting peaks, an s_R of 19% was found - and/or (2) the concentrations of CB 52 and CB 153 were certified and known to the participants, whereas this was not the case for CB 156. This advance knowledge may unperceived have influenced the final result.

The progress made for the CB determinations in the various matrices is shown in Table 1. A major improvement was obtained for the determination of CBs in the unknown solution after step 2. The mean s_R decreased from 1.22 to 1.14 and stayed at this level (1.15) in step 4. After step 4 extensive advice was given on column dimensions and preparation of calibration solutions. At that moment many participants installed new columns and prepared new calibration solutions. The mean s_R for the determination of CBs in seal also improved after step 2 but increased again in step 4, although not to the same extent. The increase may be due to differences in the two seal samples used in 3b and 4, with more co-eluting peaks present and a higher CB level in the latter one. Nevertheless the s_Rs for CBs 138, 153 and 180 obtained in step 4 (1.12=1.16) are acceptable and correspond with an R of 1.37-1.52. Due to metabolism in seals, the concentrations of CB 28 and 31 are extremely low compared to the other CBs which makes a proper determination almost impossible.

Most progress was obtained for the determination of CBs in marine sediment. The mean s_R in step 2, 1.35, and 1.39 in step 3b decreased to 1.23 in step 4. However, only for CBs 118, 138 and 1.53, with an s_R of 1.15-1.17, an R of around 1.5 is obtained.

The poor performance of the laboratories for the determination of CBs in a wet fish tissue, which was only analysed in step 4 (Table 1), is most likely due to difficulties in extraction. Whereas intercomparison studies organised before always used fish oil, here, for the first time, a wet fish tissue was used. Insufficient time between chemical drying and extraction, extraction only with apolar solvents and insufficient sample intake may have been reasons for the poor results. The use of saponification under controlled condition is recommended. A repeat of this exercise, eventually in combination with a more fatty tissue and with advice on details of extraction techniques, is therefore recommended.

Figs. 1 and 2 show examples of PCA biplots of results of the determination of CBs in the unknown solution and in seal blubber oil in step 4, respectively. The concentration of

laboratories in the centre of Fig. 1 shows the relatively low variance present in the results of the unknown solution. The position of some outliers is clearly identified: lab. no. 33 has low values for all CBs, lab. 34 has low results especially for CB 52, lab. 63 has extremely low results for CB 52, but relatively high results for the other CBs and lab. no. 37 has high results for all CBs. In Fig. 2 a more scattered picture is obtained. The position of the laboratories refers again directly to their problems: lab. no. 63 has e.g. very high values for CBs 28 and 31, very low values for CBs 138, 153 and 180 and acceptable results for the other CBs. Lab. no. 7 has low results for CBs 138, 153 and 180, but high results for the other CBs. Together with information from the chromatograms these biplots are extremely helpful in the identification of the type of error which might have occurred at a certain laboratory.The multitude of information collected in this type of study can, in this way, very efficiently be evaluated and specific advice in order to improve the situation is made possible.

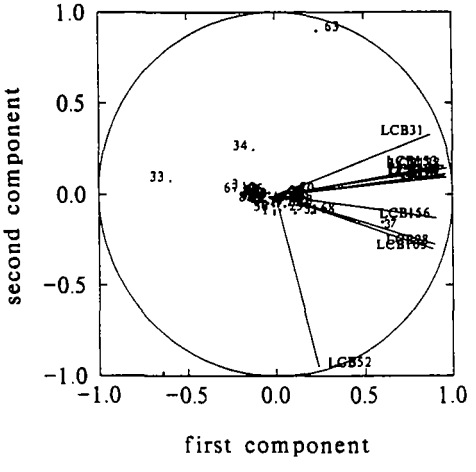

Figure 1. Biplot of a principal component analysis of results of the unknown CB solution in step 4 (LCB = log CB vector).

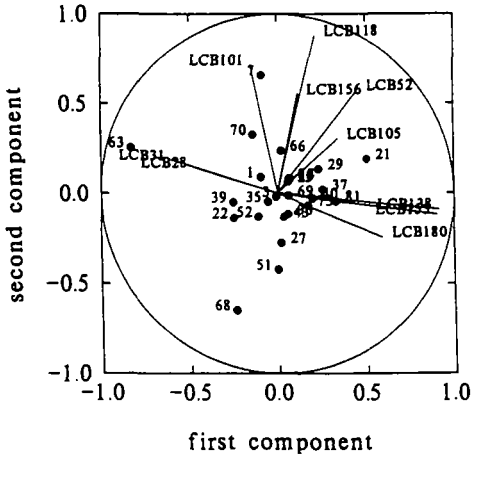

Figure 2. Biplot of a principal component analysis of results of the seal blubber oil (step 4).

5. Conclusions

The stepwise design of the interlaboratory study on the determination of CBs together with extensive advice on analytical methods have led to a considerable improvement in the performance of the participants.

PCA is a powerful tool in the evaluation of such studies and enables a specific advice to laboratories in order to improve their performance.

A duplicate CB determination gives a too optimistic impression of the variance of the analytical method. The long term variation should be recorded, and, if possible, improved by analysing internal laboratory reference materials.

A major part of the international community of marine laboratories now agrees on the determination of CBs 138, 153 and 180 in seal blubber oil and 118, 138 and 153 in marine sediment with a reproducibility of around 1.5.

Further study on the determination of CBs in lean fish tissue is required in order to improve the comparability of the data.

6. References

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