THE THIRD INTERNATIONAL INTERLABORATORY STUDY ON BROMINATED FLAME RETARDANTS

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Introduction

Polybrominated diphenyl ethers (PBDEs) have been produced as brominated flame retardants (BFRs) since the early 1970s and have been found in the aquatic environment since the late 1970s[1,2]. However, as a result of their detection in sperm whales from deeper Atlantic waters[3] and in human milk[4], many laboratories are now measuring PBDEs in environmental samples. A first international interlaboratory study (ILS) on the analysis of PBDEs, organised by the Bromine Science and Environmental Forum (BSEF), Brussels, Belgium, in collaboration with the Netherlands Institute for Fisheries Research (RIVO) was conducted in 1999-2000[5]. The results showed that the 18 participating laboratories produced comparable results for BDE 47 in various matrices but had analytical difficulties for other BDEs, in particular for the BDEs 99 and 209. A second study was organised in 2001-2002 by BSEF, QUASIMEME and RIVO[6]. That study showed improvement in comparability of the participating laboratories for BDE99 and some other BDEs. However, there was no improvement for BDE209. Hexabromocyclododecane (HBCD), tetrabromobisphenol-A (TBBP-A) and the dimethyl derivative of TBBP-A (dimethyl TBBP-A) were included in the second study. However, it appeared that only two or three laboratories were able to analyse these determinands. Others laboratories were still in the development phase with their methods for these BFRs.

This third study was organised as a development exercise by QUASIMEME, in collaboration with RIVO between September and December 2003. The BFRs selected were the same as in the second study. Two biota test materials, a harbor sediment, a sewage sludge, and two standard solutions were dispatched to the participants.

Materials and methods

Two biota test materials, a harbor sediment and a sewage sludge were prepared for this study. One of the biota materials was a herring fillet from the southern North Sea (QBC004BT), which was homogenised and canned by RIVO[7]. The other biota test material was a refined capelin oil (QBC005BT). The sediment (QBC003MS), sampled from Barcelona harbour and the industrial sewage sludge (QBC004MS) were dried, homogenised and sub-sampled into glass jars. The harbor sediment and sewage sludge were prepared by MAT Control, University of Barcelona, Spain. In addition, two standard solutions, one (QBC003SS) containing the BDEs 28 (2,4,4'-tri BDE), 47 (2,4,2',4'-tetra BDE), 99 (2,4,5,2',4'-penta BDE), 100 (2,4,6,2',4'-penta BDE), 153 (2,4,5,2',4',5'-hexa BDE), 154 (2,4,5,2',4',6'-hexa BDE), and 183 (2,3,4,6,2',4',5'-heptaBDE) in iso-octane and one (QBC004SS) containing BDE209 (decaBDE), HBCD, TBBP-A, and dimethyl TBBP-A in toluene in undisclosed concentrations were prepared and ampouled. The standard solutions were a gift from Cambridge Isotope Laboratories, Andover, MA, USA. All homogeneity test results showed that the sediments, biota and standard solutions were fit for use in this study.

All standards were derived from 99.9% pure crystals, but were provided as solutions with an uncertainty of ± 10%. The emphasis of the study was to examine the between-laboratory agreement. Therefore, one result per determinand per test material was requested. The participants used their own analytical method. However, advice was provided on how to avoid specific errors during the determination. The statistical evaluation of this study was carried out using the Cofino model[5,6,8,9]. An underlying normal distribution of the data was assumed and a proportional error of 12.5% and constant errors of 0.1µg/kg for the standard solutions and sediment test materials, and 0.01µg/kg for the biota test materials was used for the analysis of all determinands[10]. This was comparable to what was done in the evaluation of the second BFR interlaboratory study[6]. Z-scores were calculated for all determinand/matrix combinations.

Results and Discussion

Results of 23 laboratories from 10 different countries were received. A summary of the results of this study is given in Table 1. A number of datasets were incomplete not only for HBCD, TBBP-A and dimethyl TBBP-A, but also for a number of PBDEs. Some participants only analysed the harbor sediment (QBC003MS) and the sewage sludge (QBC004MS), while others only analysed the biota test materials (QBC004BT and QBC005BT).

BDE28

The CV values obtained for the harbor sediment (QBC003MS) and sewage sludge (QBC004MS), and the biota test materials (QBC004BT and QBC005BT), 6-17%, are acceptable (Table 1). The overall performance of the laboratories was better compared to the previous study with CV values between 28 and 51% for biota and sediment test materials and BDE28 concentrations in the same range (0.08 - 0.54µg/kg)[6]. It should be noted that the number of data sets taken into account was smaller than in the previous study, ca. 8-10 (and 16 for the herring) vs. 18-19, respectively. The result for the standard solution (CV of 15%) was comparable to that of the previous round (16%), and therefore less of an improvement compared with the other test materials. This phenomenon of relatively poor results in standard solutions in interlaboratory studies has been discussed previously[6]. Dilution and calculation errors are often the cause of this problem.

BDE47

The BDE47 concentrations in the test materials of this study were considerably lower (0.2-9.3µg/kg) than those in the previous round (1.6-9.1µg/kg). This is also reflected in the results, which range from 9.0-40% vs. 15-25% in the previous round[6]. Concentrations close to the detection limits always show higher CV values. The CV value of 22% in the sewage sludge (QBC004MS) is better than that of the capelin oil (QBC005BT) (40%). This is caused by the high fat content of 100% of the capelin oil putting a restriction on the intake mass which is limited by the capacity of clean-up systems (alumina, silica and GPC columns). A larger intake mass of the dried sewage sludge creates fewer difficulties for the clean-up. The CV value of the standard solution is 2.9%, which is extremely good. Twenty-three percent of the BDE47 results had a Z-score of $2<Z<3$, 9% were $3<Z<6$ and 9% were $>Z=6$. Again, for a simple analysis of a standard solution, the spread of Z-scores is very wide.

BDE99

The results of BDE99 were slightly better than those obtained in the previous round. The CV values ranged from 15-42% at concentrations between 0.2 and 3.8µg/kg, compared to 22-35% in the previous round at concentrations between 1 and 20µg/kg. The BDE99 result in the herring (QBC004BT) is particularly good with a CV of 15% at a concentration of 3.8µg/kg, based on 19 observations (Table 1). It is encouraging to see that the analysis of this BDE is now under control,

Table 1. Summary Statistics

Matrix/ Determinand	Assigned Value µg/kg	NObs	Model CV%	% Data in Model	Assigned Value µg/kg	NObs	Model CV%	% Data in Model
QBC003/4SS					*QBC003MS*			
BDE028	136,392	18	14,56	68,91	0,128	8	13,15	64,54
BDE047	549,676	22	2,88	36,86	0,959	12	16,00	43,22
BDE099	388,483	22	11,55	56,73	1,053	12	18,30	60,62
BDE100	417,984	22	18,15	62,57	0,116	11	27,07	47,58
BDE153	137,676	18	5,48	37,51	0,211	11	13,41	48,73
BDE154	99,795	21	5,44	46,27	0,107	9	25,06	52,91
BDE183	62,993	17	15,49	59,03	0,140	9	30,00	53,06
BDE209	761,685	11	9,52	45,11	45,155	8	22,50	65,84
QBC004MS					*QBC004BT*			
BDE028	0,069	6	6,27	51,41	0,666	16	12,92	52,38
BDE047	0,196	9	22,05	43,37	9,336	19	8,97	45,03
BDE099	0,331	11	42,51	59,59	3,799	19	15,21	66,06
BDE100	0,075	7	77,65	60,36	1,263	19	15,76	48,32
BDE153	0,212	8	19,50	56,11	0,329	16	12,62	59,67
BDE154	0,072	7	4,06	39,14	0,306	18	13,30	59,25
BDE183	0,146	6	22,08	57,43	*0,038*	*7*	*62,27*	*54,36*
BDE209	103,416	8	5,87	44,39				
QBC005BT								
BDE028	0,045	10	17,54	38,45				
BDE047	*0,887*	*14*	*40,43*	*54,12*				
BDE099	0,221	13	15,11	44,03				
BDE100	0,132	11	29,95	65,66				
BDE153	0,033	8	53,64	76,47				
BDE154	0,034	9	59,49	58,54				
BDE183	*0,013*	*6*	*76,76*	*44,59*				

Entries in italics are given as indicative values only;
NObs = Total number of datasets from each laboratories.

since this was one of the more difficult BDEs to analyse in the first interlaboratory study[5]. The sewage sludge (QBC004MS) created more difficulties for the laboratories with a CV value of 42%, probably due to the presence of more interfering compounds. The analysis of BDE99 in the capelin oil (QBC005BT) resulted in a smaller CV (15%). The concentration was only 0.22µg/kg.

BDE100

The BDE concentrations in the four test materials were rather low: 0.07-1.3µg/kg. This has resulted in a set of relatively high CV values, from 16-77% (Table 1). The analysis of the herring (QBC004BT) resulted in a CV value of 16% at a concentration of 1.3µg/kg. The BDE100 results of the previous round show CV values of 24-37% related to a concentration range of 0.4-4.3µg/kg.

BDE153

Very low concentrations of BDE153 were observed in all four test materials, ranging from 0.03 to 0.32µg/kg (Table 1). This has resulted in CV values between 12 and 54%, which is relatively good. However, these CV values are based on a small set of observations (Table 1). The standard solution showed a good CV value of 5.5%. The overall results are considerably better than those of the previous round with CV values of 31-55% at higher concentrations (0.06-2.8µg/kg).

BDE154

The results of BDE154 show many similarities with those of BDE153, as was the case in previous rounds[5,6]. The standard solution analysis shows the same low CV value of 5.4% (Table 1). The CV values for the other test materials vary between 4.1 and 59%. Only the 4.1%, in the sewage sludge (QBC004MS), is lower, but is based on a small data set. The BDE154 concentrations were also low: 0.03-0.31µg/kg, with three of the four very close to or under the detection limits of many laboratories. The previous round showed CV values of 32-56% at concentrations of 0.06-2.7µg/kg. Therefore, here also an improvement in the performance of the laboratories is observed.

BDE183

The results of BDE183 also show an improvement compared to the previous round. The CV values for the sewage sludge (QBC004MS) and the harbor sediment (QBC003MS) are 30 and 22%, at concentrations of 0.14 and 0.15µg/kg, respectively. The BDE183 concentrations in the biota test materials (QBC004BT and QBC005BT) were extremely low: 0.04 and 0.01µg/kg, which resulted in CV values of 62 and 77%. Improvement is necessary here. It may be concluded that the majority of participating laboratories are now able to analyse BDE183 in

sediments and sewage sludge, and presumably also in biota test materials, provided the concentrations will be > 0.1µg/kg.

BDE209

Until now no satisfactory result has been obtained in interlaboratory studies[5,6] for BDE209. Even in the standard solutions CV values were around 40% or more. These poor results were due to a number of different possible errors: on one hand the gas chromatographic (GC) analysis can cause erratic results when BDE209 is exposed for long periods at elevated temperatures in the injector and detector. On the other hand high blank values can be a serious barrier in obtaining results in good agreement, as this compound is present in small dust particles in laboratories, causing high background values. The GC problems were highlighted in the advice on the first study. Although this advice may have helped a number of laboratories, it appeared from the results of that study that more problems were expected. The second study included a sediment extract in addition to a dried sediment sample. That study showed that part of the analytical problems were caused by the extraction and clean-up procedure: the cleaned extract analysis resulted in a considerably lower CV value of 27%, compared to a CV of 65% for the dried sediment[7]. Prior to this study, advice was given on extraction, clean-up and the blank determination. Due to the low solubility of BDE209 in some organic solvents, BDE209 may go out of solution during evaporation. Re-dissolving may take place but only rather slowly. A blank determination that is carried out by shaking a piece of glassware with an amount of solvent for only a few minutes may reflect only a part of the present BDE209 quantity, while the rest remains at the glass wall. A sample extract in a flask may re-dissolve a larger amount of (background) BDE209 because this extract remains there for a longer period. In that way blanks suggest a relatively low background, while more BDE209 is present as contamination of the glassware.

The results of this study show that at least a number of laboratories have succeeded to obtain comparable results, possibly by spending more attention to this blank problem. The harbor sediment (QBC003MS) showed a CV of 22.5% (Table 1), which is much better than BDE209 CV values from the two previous rounds (48-78%)[5,6]. The BDE209 CV value of the sewage sludge (QBC004MS) is even better with a value of 5.8%. Also the standard solution has resulted this time in a low CV value of 9.5%. It should be noted that the number of laboratories that have achieved these results is relatively small (n=11 for the standard solution, n=8 for the harbor sediment and the sewage sludge). Interestingly, 75 and 63% of the Z-scores are <2 for the harbour sediment and the sewage sludge respectively, whereas no Z-scores between 2 and 3 were found. The percentages of Z-scores >3

were 25% and 37% for the harbor sediment and the sewage sludge respectively. This result seems to confirm the hypothesis of the blank problem, discussed above. It is also in line with several reports in the literature that report a series of BDE209 results with most concentrations below the detection limits, but a few substantially higher BDE209 values. Such values should be checked thoroughly because serious doubt exists with regard to the influence of the blank. Also there were no Z-scores between 2 and 3 for the standard solution. However, this corresponds with earlier results for standard solution analyses in which the results are either good or very poor due to dilution or calculation errors. In this case the majority of the Z-scores are >6, which is not the case for the harbor sediment and sewage sludge results.

Only a few laboratories reported results for BDE209 in biota. The data set is too small to provide an assigned value. It looks as if this small number of laboratories [5 for the herring (QBC004BT) and 4 for the capelin oil (QBC005BT)] agree on the absence of BDE209 in these biota samples, although a few very low concentrations have been reported as well (0.004, 0.03 and 0.2 µg/kg in the herring and 0.8 µg/kg in the capelin oil). One value of 9.5µg/kg in the herring seems to be a typical outlier. This is possibly also true for one value of 0.8µg/kg for the capelin oil. It seems as if for the time being the only conclusion that can be drawn is that at the current level of sensitivity, BDE209 cannot be detected in most biota test materials. The detection of BDE209 in biota might be possible at levels of <0.1µg/kg but only when more sensitive methods will become available.

HBCD, TBBP-A and dimethyl TBBP-A

The number of data returned for HBCD, TBBP-A and dimethyl TBBP-A was 4 for the standard solution and 1 or 2 for the other test materials. The four observations for HBCD were not far from the target value of 354.8µg/kg (58.8µg/kg for α-HBCD, 58.8µg/kg for β-HBCD and 237.3µg/kg for γ-HBCD). The two values reported for TBBP-A and the one for dimethyl TBBP-A were also not too far from the target values of 328.8µg/kg and 461.7µg/kg, respectively.

HBCD in particular is a bioaccumulating compound, which is found in many organisms. Reliable interlaboratory studies are needed to evaluate the analytical methods of the laboratories and this can only be established by applying good statistical methods based on a sufficiently high number of data sets. All standards, including ^{13}C-labeled compounds, are now commercially available. Sensitivity will be an important factor for the diastereomer-specific LC-MS analysis of HBCD[11].

Conclusions

This interlaboratory study has shown a considerable improvement in the performance of the participating laboratories for most of the PBDEs studied. The improvement of the BDE209 results in the harbor sediment and the sewage sludge is in particular encouraging, as many factors can interfere with this analysis and none of the previous interlaboratory studies showed acceptable results. Advice with regard to specific analytical difficulties, such as blank problems with BDE209 has apparently helped a number of laboratories. Fewer laboratories delivered results for BDE209 in biota, but those who did, agreed on the absence of BDE209 in the herring and the capelin oil at a level of ca. <0.1µg/kg. Other BDE congeners for which improvements were observed in the majority of the test materials were BDE28, BDE99, BDE 153, BDE154, and BDE183.

The majority of laboratories did not report results for HBCD, TBBP-A and dimethyl TBBP-A. However, these compounds will be included again in the next study. The laboratories should be encouraged to continue to improve their methods, in particular at the relatively low levels at which BFRs occur in the marine environment.

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