

GC/ECNI-MSMS RESIDUE PATTERN OF HEXABROMINATED BIPHENYLS IN MARINE MAMMALS AND FISH

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Abstract

Seals, harbor porpoises and fish from the North Sea, the Baltic Sea, Iceland, and Canada were analyzed for residues of polybrominated biphenyls. Hexabromobiphenyls (hexaBBs) dominated in all samples. Eleven hexaBBs were detected in the different samples. PBB 155, PBB 154, as well as the characteristic fingerprint of the three neighbored peaks of PBB 133, PBB 132/PBB 146 and PBB 153 were prominent in harbour seals from the North and Baltic Seas. Most of these PBBs were determined for the first time in environmental samples. The major peak in technical hexabromobiphenyl, PBB 153, was only dominating in a few samples. By contrast, the other major hexaBBs in the seals must be metabolites of higher PBBs since they were not present in technical products. Blubber of harbour porpoises and also seal milk showed more peaks indicative of a lower ability to biotransform PBBs. A ringed seal from Canada showed a pattern different to the other seal samples. In this blubber sample, PBB 153 dominated and the residues almost exclusively originating from the previous use of technical hexabromobiphenyl. The samples from the North Sea most likely originate from (partly degraded) technical octabromobiphenyl or decabromobiphenyl.

Introduction

Technical mixtures of polybrominated biphenyls (PBBs) have been used as flame-retardants in textile, electronic equipment, and plastics¹. Three mixtures differing in their degree of bromination have been marketed, i. e. technical hexabromobiphenyl (THBB), technical octabromobiphenyl (TOBB), and technical decabromobiphenyl (TDBB) with bromine contents of 76, 81, and 85%, respectively². In 1974, production of THBB was ceased in the USA as a result of the so-called Michigan disaster³. After this incident, the remaining US-production volume of TOBB and TDBB was exported mainly to Europe. In addition, several companies in Europe produced TOBB and TDBB³. Despite reduced global production since 1973, PBBs continued to be detected in the different environmental samples. Usually PBBs account for ~1% of the pollution with polybrominated diphenyl ethers (PBDEs). Unfortunately, only a few of the 209 PBB congeners are commercially available. 2,2',4,4',5,5'-hexabromobiphenyl (PBB 153, **Figure 1**) remained the PBB congener most frequently reported in environmental samples. Consequently, PBB 153 is under discussion of becoming a new candidate for a global ban under the persistent organic pollutant (POP) mandate⁴. However, several PBB congeners detected in environmental samples could not be identified in technical PBB mixtures. Aim of this study was to investigate the PBB residue pattern in a range of biological samples.

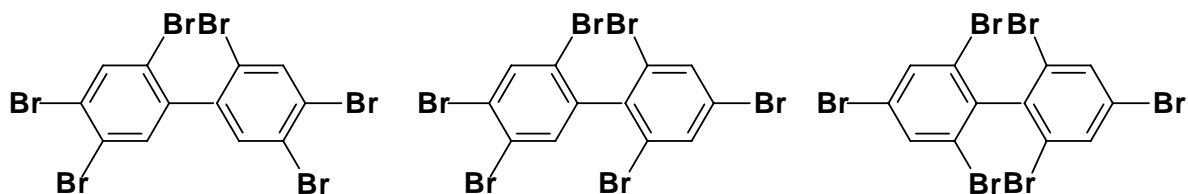


Figure 1: structures of PBB 153 (left), PBB 154 (center), and PBB 155 (right)

Material and Methods

Chemicals and standards. TOBB (lot number: NT01013) and THBB (lot number: NT01671) were obtained from ULTRA Scientific (North Kingstown, USA). Individual PBB standards were synthesized or isolated from TOBB or products of the photolysis of PBB 209. Structure elucidation will be reported elsewhere.⁵⁻⁶

Samples and sample preparation. Samples of marine mammals from the North Sea (German Wadden Sea) and the Baltic Sea (German coastline) were from animals which died during the seal plague or were found stranded in 1988. The grey seals (1990) were from Western Iceland and the Canadian ringed seal was sampled in 1998. The samples were prepared using methods previously described in detail⁷⁻⁸.

GC/ECNI-MSMS. Analyses were carried out with a 3800/1200 GC/MS system (Varian) according to von der Recke *et al.* with modifications⁹. A DB-5-like column (30 m x 0.25 mm i.d. x 0.25 μm d_f Factor Four[®] CP-Sil 8ms column, Varian) was used with the following GC oven program: after 2 min at 50 °C, the temperature was raised at 10 °C/min to 300 °C (hold time 38 min). The total run time was 65 min. Injections were performed in splitless mode (split opened after 2 min). He (purity 99.9990%, Sauerstoffwerke, Friedrichshafen, Germany) was used as carrier gas with a constant flow of 1.2 mL/min. Methane (purity 99.995%, pressure ~8.5 Torr; Air Liquide, Bopfingen, Germany) and argon (purity 99.995%, pressure 1.5 mTorr, Linde, Leuna, Germany) were used as reagent and collision gases, respectively. The electron energy was set at 70 eV, and the ion source temperature at 150 °C. The collision voltage was set at 12 V and the detector voltage at 1800 V. GC/ECNI-MSMS in the selected reaction monitoring mode was based on the fragmentation of the most abundant isotope peak of the respective molecular ion (*m/z* 233.0, 310.9, 390.8, 469.7, 548.6, 627.5 (hexaBBs), 706.5, 785.4 for PBBs and 515.7 for the internal standard 2'-MeO-BDE 68 (BC-2) with a peak width set to \pm 3.0 u) as precursor ions. The bromide isotope ions (*m/z* 80 with a peak width of \pm 1.5 u) were used as product ions.

Results and Discussion

PBBs were determined by GC/ECNI-MSMS in the selected reaction monitoring mode taking advantage of the high sensitivity of the transitions M^+ to Br^- .⁹ Although other degrees of bromination were screened as well, the major PBBs detected in all samples were hexabromo isomers. Therefore, we concentrated on the nine (plus two potential coeluting isomers) hexaBBs detected in the samples albeit at different abundance. To our surprise, PBB 153, the major congener in THBB was only dominating in three of the twelve samples presented (see **Figure 2**). Furthermore, only four of the thirteen hexaBBs were present in THBB and TOBB (PBB 153, PBB 132, PBB 149, and PBB 135).⁶ This produced evidence that the PBB residues in our samples did not reflect the composition of technical products but a modified pattern. In agreement with this, the remaining hexaBBs in the GC/ECNI-MSMS-SRM chromatograms of the marine samples were found to be those obtained from technical PBBs after exposure to sunlight.⁵ Thus, most of the hexaBBs found in the samples are the result of the abiotic (e.g. sunlight) and/or biological PBB transformation. The distinctly different pattern in samples from the same habitat but different trophic levels indicated that a significant part of the transformation of PBBs had already occurred before entering the marine food chain.

Samples from the North Sea. Two blubber samples of harbour seal (*Phoca vitulina*) from the North Sea were analyzed for studying potential variations in the residue pattern (**Figure 2a,b**). The samples showed the same hexaBBs and only slight variations in the patterns. For instance, the significant fingerprint of 133, 132/146, and 153 was found in both samples. On the other hand, PBB 153 was more abundant than PBB 154 (**Figure 1**) in seal 1 while this ratio was reversed in seal 2. Next to these four peaks, PBB 155 (**Figure 1**) was the fifth hexaBB of high relevance. A sample of seal milk (**Figure 2c**) also contained these five hexaBBs along with additional abundant hexaBBs, namely PBB 135 and PBB 149, as well as the peak consisting of PBB 136 and/or PBB 148. This difference reflects the higher power at metabolizing PBBs of seal blubber compared to seal milk. In addition, we analyzed a sample of harbour porpoise (*Phocoena phocoena*) (**Figure 2d**). Previous studies with PCBs and chloropesticides have shown that seals have a higher power at metabolizing organohalogens than cetaceans.¹⁰ This was also observed for hexaBBs (**Figure 2a,b**, and **d**). In fact, the hexaBB pattern in the harbour porpoise was similar to that determined in the seal milk except for the lower abundant peak of PBB 155. The harbour porpoise was found dead during the seal plague in the North Sea in 1988. This female carried a fetus which was also analyzed. As can be seen from **Figure 2d** and **1e**, the fingerprint of hexaBBs in fetus and mother animal showed a similar ratio for PBB 154 (major peak) and PBB 153. However, other hexaBBs such as PBB 149, PBB 132/146, and those eluting prior to PBB 154 were more abundant in the fetus. Finally, a sample of mackerel (*Scomber scombrus*) from the North Sea also showed a higher number of early-eluting hexaBBs. In this sample, PBB 155 was the dominating congener (**Figure 2f**).

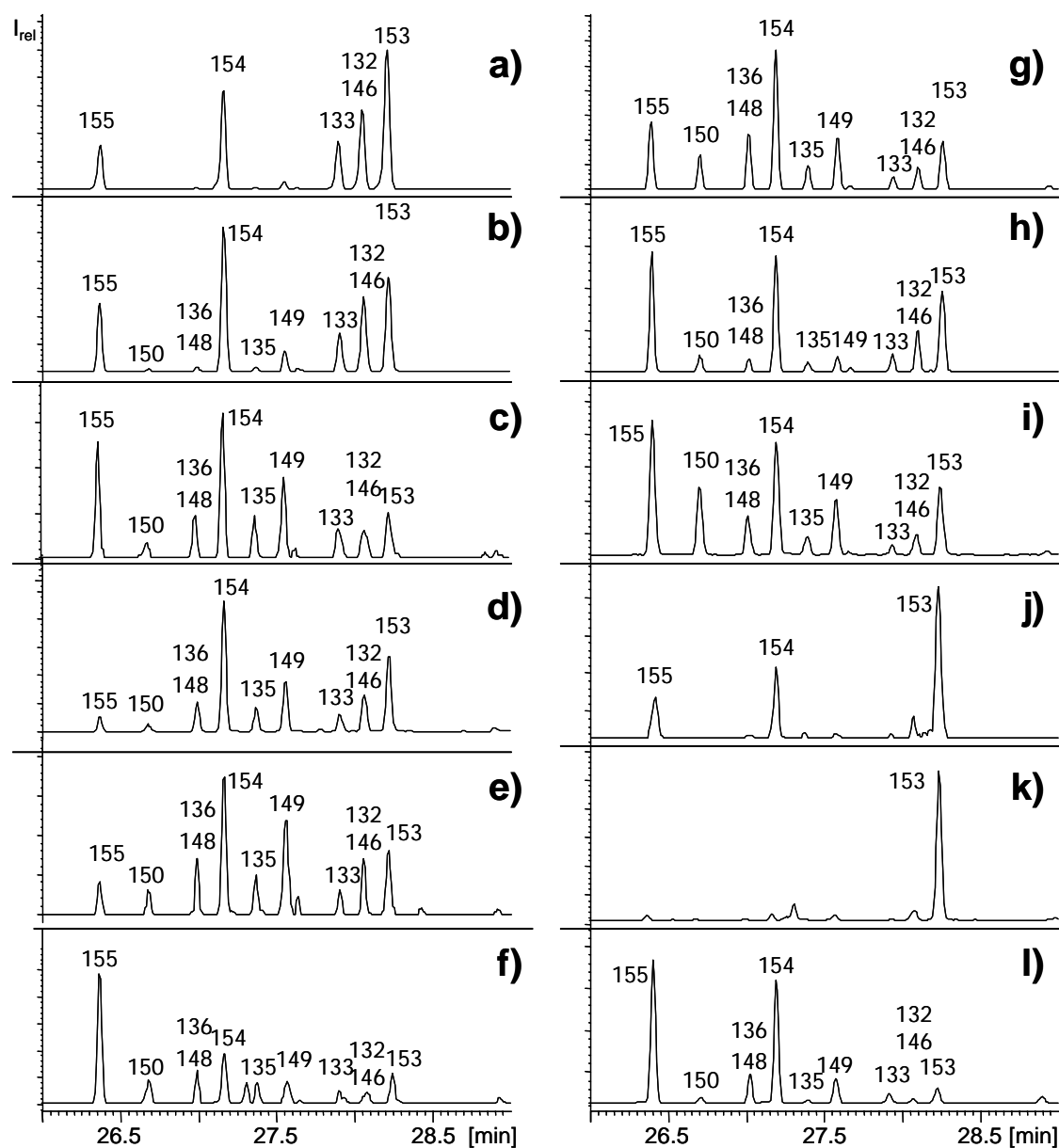


Figure 2: GC/ECNI-MSMS SRM ($627.5 \pm 3 \rightarrow 80 \pm 1.5$) chromatograms of the hexabromobiphenyl congeners in **a,b**) blubber of harbour seals (*Phoca vitulina*), **c**) harbour seal milk, **d,e**) harbour porpoise (*Phocoena phocoena*) and a fetus from this mother animal, and **f**) mackerel (*Scomber scombrus*) – all samples from the North Sea; **g,h**) harbour porpoise and harbour seal from the Baltic Sea, **i,j**) harbour porpoise and grey seal (*Halichoerus grypus*) from Iceland, **k**) ringed seal (*Phoca hispida*) from Canada, and **l**) cod liver (*Gadus morhua*) from the Baltic Sea

Samples from the Baltic Sea. The differences in the hexaBB pattern of harbor seal and harbour porpoises observed in the North Sea -- more hexaBB isomers in porpoises -- was also found in samples from the Baltic Sea. The harbour seal from the Baltic Sea contained the same hexaBBs as its relative from the North Sea but the peaks of PBB 155 and PBB 154 were more pronounced in relation to PBB 153 in the Baltic seal (**Figure 2h**). A higher proportion of PBB 155 was also determined in blubber of the harbor porpoise (compare **Figure 2g**). The

cod (*Gadus morhua*) liver oil from the Baltic Sea -- **Figure 2i** -- confirmed the information extracted from the North Sea mackerel. PBB 153 was only low abundant whereas PBB 155 was the dominating peak. In addition, the higher proportion of PBB 154 in the Baltic fish is worth mentioning. In general, the pattern in cod liver oil was more shifted towards earlier eluting hexaBBs.

Samples from the Iceland and Canada. The differences in the patterns of seals and cetaceans were also found in grey seals (*Halichoerus grypus*) and harbour porpoises from Iceland (**Figure 2i, j**). The hexaBB pattern in the porpoises was similar to that from the Baltic Sea (**Figure 2g,i**). In the grey seal sample, PBB 153 dominated followed by PBB 154 and PBB 155. The neighbour-peaks of PBB 153 -- PBB 133 and PBB 132/146 -- which provided a significant pattern in seals from the North and Baltic Seas were only very low abundant. Consequently, PBB 153 was more dominant than in samples from the German North and Baltic Sea coasts. The higher relative amount of PBB 153 indicated that the residues in Icelandic grey seals originated from THBB whereas the Baltic and North Sea samples were influenced by a higher proportions of the debromination of higher brominated technical products. Since Iceland is almost halfway between Europe and North America we also screened a ringed seal sample from Canada (**Figure 2k**). In fact, PBB 153 predominated in this sample whereas PBB 154 and PBB 155 were virtually absent. It is noteworthy that technical hexabromobiphenyl was the dominating product used in the USA whereas higher brominated mixtures were marketed in Europe.

Conclusions

In all samples, hexaBBs represented the dominating PBBs. However, depending on the region the PBB residue originated from previous use of THBB or higher-brominated products. In the latter case, a significant transformation of higher brominated congeners by reductive debromination must have occurred. It could not be established to what amount this happened in the marine organisms or if it was the consequence of previous abiotic processes. Irrespectively, the hexaBB residue pattern in seals from the same region was more thinned out than in cetaceans. From these comparisons it is evident that seals are at least partly able to metabolize lower brominated biphenyls.

The pattern in the samples from Iceland indicates that PBBs found in this habitat most likely originated from other sources than PBBs in samples from the German North Sea coast. In agreement with that, a ringed seal sample from Canada showed a PBB pattern dominated by PBB 153. Therefore, the pattern in Canada reflected the previous use of THBB whereas samples from the North and Baltic seals showed higher proportions of partly weathered higher brominated perproducts (i. e. TOBB and TDBB). The hexaBB pattern observed in the samples from Iceland appeared to be the mixture of both sources.

References

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