POLYHALOGENATED COMPOUNDS (PCBs, CHLORDANES, HCB AND BFRs) IN POLAR BEARS *(URSUS MARITIMUS)* THAT SWAM TO AND ENDED UP IN ICELAND

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Introduction

A range of POPs has been detected in arctic flora and fauna due to atmospheric long range from industrialized and agricultural areas.[1] Bioaccumulation in the arctic food web has led to a high pollution of polar bears (*Ursus maritimus*), i.e. the top predator of the marine food web. Polar bears are not sedentary in Iceland, which is located between two major industrial and agricultural zones, i.e. North America and Europe. In years with pack ice drifts polar bears sometimes reach the shores of Iceland (at least 500 polar bears during the last millennium) but in recent years (2008 to 2011) four polar bears swam to Iceland when the ice edge was 70 to 110 miles away from the island.[2] Here we analyse their adipose tissue, liver, kidney and muscle tissue on POPs in order to verify whether these individuals belonged to the East Greenland (major impact North America) or Spitsbergen (major impact Europe) population. Since these animals most likely starved prior to their arrival in Iceland, the residue pattern was thought to reflect the one of their origin(s).

Materials and methods

Sample cleanup procedure

Samples were lyophilized and aliquots were supplemented with the internal standard α-PDHCH. It followed open-vessel microwave-assisted extraction of the lipids according to Vetter *et al*. (1998).[3] Cyclohexane/ethyl acetate (46:54, w/w) was used for extraction. The samples were filtered, condensed by rotary evaporation (30 °C, 170 mbar) and adjusted to 5 mL. The bulk (4.5 mL) of the sample solution was subjected to gel-permeation chromatography with 5 mL/min of cyclohexane/ethyl acetate (46:54, w/w). Then, the solvent was changed to *iso*-octane. The sample was further purified by adsorption chromatography (1 cm i.d. column) with 3 g silica deactivated with 30% water (w/w) and topped with Na_2SO_4.[3] The sample was eluted with 60 mL *n*-hexane. Then, the volume was reduced and made up to exactly 5 mL. Aliquots were analyzed by GC/ECD and GC/MS.

Gas chromatography with electron capture detection (GC/ECD)

GC/ECD measurements were performed with a Hewlett-Packard (HP) 5890 series II plus system equipped with a GC-PAL auto sampler (CTC Analytics, Zwingen, Switzerland). Helium (5.0) was used as the carrier gas. One microliter was splitless injected (injector temperature 280 °C) onto a 30 m length x 0.25 mm i.d., film thickness 0.25 µm BGB-1 capillary column (100% dimethyl polysiloxane, BGB-Analytik, Boeckten, Switzerland). The GC oven program started at 80 °C (2 min), then at 10 °C/min to 300 °C (hold time: 20 min). The detector temperature was set to 300 °C and nitrogen 5.0 was used as the makeup gas.

Gas chromatography with electron-capture negative ion mass spectrometry (GC/ECNI-MS)

A 7890A GC with 7693A auto sampler and a 5975C mass spectrometer (Agilent Technologies, Waldbronn, Germany) was used for the measurements. Helium (5.0) was used as the carrier gas at a flow rate of 1.2 mL/min. Injections (1 µL) were made in splitlos mode with a pressure pulse of 25 psi/min. Methane (5.5) was used as the moderation gas at a flow of 2.0 mL/min. The transfer line, ion source and quadrupole temperatures were set at 300 °C, 150 °C and 150 °C. A DB-5MS column (95% methyl, 5% phenyl polysiloxane; Agilent Technologies, Folsom/USA) of 30 m length, 0.25 mm i.d. and 0.25 µm film thickness was installed in the GC oven. The solvent delay was 8 min. In the full scan mode (*m/z* 50-800), the GC oven program was the following: 50 °C (1 min), then at 10 °C/min to 300 °C (14 min). In the selected ion monitoring (SIM) mode, the GC oven program was 60 °C (2 min), at 10 °C/min to 300 °C (14 min). Different SIM methods were used for quantification and screening purposes.

Results and discussion

PCBs. The GC/ECNI-MS full scan chromatograms of the different samples looked very similar, and a large portion of the residues originated from PCBs. The most prominent congener was PCB 153, which contributed with 29% or more to the PCB content. With a few exceptions, the abundance of PCBs decreased in the order PCB 153 > PCB 180 > PCB 170 > PCB 138 > PCB 99. Remarkable exceptions were sample #3 muscle: PCB 180 contributed only <1% to sumPCBs. In all tissues, the contribution of PCB 138 to the PCB pattern in sample #4 was higher than in the other polar bears (~30% vs. 8-15%) and in liver PCB 138 was even more abundant than PCB 153. PCB 194

(GC/ECD abundance ~50% of PCB 170), PCB 209 and further congeners were also detected but not quantified in this study. Based on the lipid weight, the contamination with PCBs was generally highest in liver, it followed adipose tissue and muscle or kidney (**Table 1**). The contamination with PCBs decreased from sample #4 > sample #2 > sample #1 > sample #3 (**Table 1**).

Table 1: PCB levels (ng/g lipids) in tissues of polar bears stranded on Iceland

	Sample #1 14.5 y, female	**Sample #2 22.5 y, male**	**Sample #3 4.5 y, female**	**Sample #4 3.5 y, female**	**Mean value**
Adipose tissue	-	21,000	7,890	36,100	21,700
Liver	20,500	56,400	14,500	74,400	41,500
Kidney	10,300	6,150	4,590	25,800	11,700
Muscle	8,400	15,200	5,500	25,000	13,500

Polar bears are able to metabolize many of the PCBs.[4-6] Only one pentachloro congener (PCB 99) is slightly accumulated,[5] and PCB 99 was also detected in our samples. PCB 118 and PCB 105, which are usually as abundant as PCB 99 in mammals and birds are metabolized by the polar bear.[4-5] PCBs with unchlorinated *para*-positions such as PCBs 146, 187 and 201, which are persistent in mammals are also transformed by the polar bear.[5] The PCB congeners stored in the body of polar bears are substituted in both rings in 2,4-, 2,4,5-, 2,3,4- and 2,3,4,5-positions.[4] This applies for 2,2',4,4',5-pentaCB (PCB 99), 2,2',4,4',5,5'-hexaCB (PCB 153), 2,2',3,4,4',5'-hexaCB (PCB 138), 2,2',3,4,4',5,5'-heptaCB (PCB 180), and 2,2',3,3',4,4',5-heptaCB (PCB 170). Norstrom *et al.* noted that PCBs 99, 153, 138, 180, 170 and 194 represented ~93% of the PCB content.[4] These were also the most abundant congeners in our samples.

Contrary to our findings, PCB levels were reportedly highest in adipose tissue followed by liver, brain and blood.[7] Since all four polar bears were malnourished and had been starving prior to their arrival in Iceland[2], this could be the reason for the differences. Polischuk *et al.* noted that PCBs in adipose tissue increased during fasting for 47-68 days, in dependence of gender, age, as well as the constitution of the individual.[8] However, the calculated PCB load in the body remained unchanged. Accordingly, PCBs were neither excreeded nor metabolized during this period.[8]

PCB levels in adipose tissue of adult male polar bears from the East Greenland population (2006 and 2010) were on average at 12,650 ng/g lipids (95% confidence interval: 11,030-14,270 ng/g lipids). A total of 4,520 ng/g lipids (95% confidence interval: 3,900-5,140 ng/g lipids) originated from PCB 153 and 2,810 ng/g lipids (2,420-3,200 ng/g lipids) from PCB 180.[9] The PCB level of the only adult male individual in our study (sample #2) was much higher than the average of the samples analyzed by Dietz *et al.* (n=28).[9] This could be due to the high age of the individual and/or the effect of starvation prior to and during the swimming to Iceland. Adult females accumulated on average 7,630 ng/g lipids PCBs (5,740-9,510 ng/g lipids), i.e. slightly less than detected in juveniles. Juveniles accumulated on average 8,470 ng/g lipids PCBs (7,020-9,930 ng/g lipids) with 3,390 ng/g lipids (2,760-4,030 ng/g lipids) originating from PCB 153 and 1,780 ng/g lipids (1,450-2,100 ng/g lipids) from PCB 180.[9] PCB concentrations in sample #3 were on the same level whereas those of sample #4 were much higher (**Table 1**). Similar proportions (i.e. the same or higher values than in the literature) were also found for liver samples.

Brominated flame retardants. PBDEs were determined by GC/ECNI-MS-SIM using *m/z* 79 for quantification (formed by virtually all polybrominated compounds) and *m/z* 161 (restricted to polybrominated diphenyl ethers and their derivatives) for verification. All samples showed peaks at the retention times of BDE 47, BDE 153 and BDE 154. However, with the present GC setup BDE 154 was coeluting with PBB 153 and BDE 153 with PBB 138.[10] Based on the ratio of *m/z* 79/*m/z* 161 it was found that the first peak in our sample mainly originated from PBB 153 while BDE 154 only played a minor role. This was verified by means of the molecular ions (i.e. *m/z* 627 for PBB 153 and *m/z* 643 for BDE 154) for two samples (**Figure 1**).

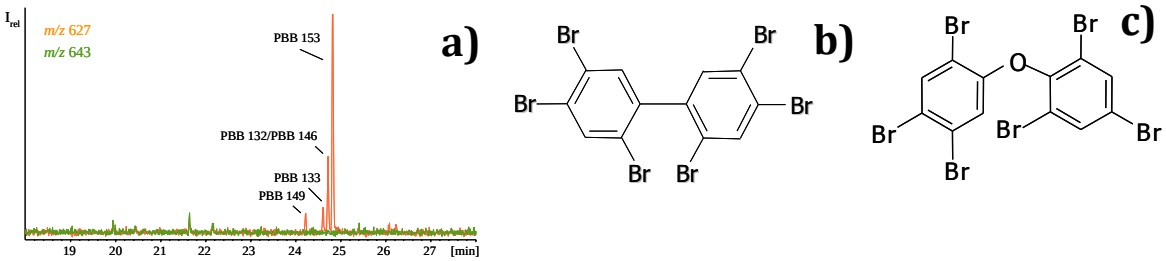

Figure 1: (a) GC/ECNI-MS-SIM chromatograms of *m/z* 627 (M^- of hexaBBs) and *m/z* 643 (M^- of hexaBDEs) in liver of polar bear sample #1 and structures of (b) PBB 153 and (c) BDE 154

Accordingly, the peak was assigned to PBB 153. Moreover, *m/z* 627 (M^- of hexaBBs) gave response to three further hexabromobiphenyl isomers. Based on the work of von der Recke and Vetter [11], these represented the coeluting pair PBB 132/PBB 146, as well as PBB 133 and PBB 149 (**Figure 1**). These PBB congeners are strong indicators for pollution with technical hexabromobiphenyl and not with decabromobiphenyl.[12] Accordingly, the major pollution source was North America (because in Europe, mainly decabromobiphenyl was used as BFR, which leads to the accumulation of PBB 154 and PBB 155 which were not detected in the polar bear samples). In addition, no response was detected for PBB 138 (which is in agreement with marine mammals.[11] Thus, the second peak detected in some chromatograms could be traced back to BDE 153, although *m/z* 161 could not be detected in either case due to the low abundance of the compound. As for PCBs, the highest PBDE level was found in sample #4, in which we also detected BDE 99 and BDE 100. However, the clear dominance of BDE 47 (250-1,030 ng/g lipids) was also a common feature in sample #4. In sample #1-#3, PBB 153 was higher concentrated than sumPBDEs. Likewise, highest levels on lipid basis were determined in liver, followed by adipose and muscle tissue. The kidney samples showed the lowest content of PBB 153 (**Table 2**). It followed in abundance BDE 47 (samples #1 and #2) which reached ~1/3 or the PBB 153 level and BDE 153 (~1/10 of PBB 153). Sample #3 was different in that the level of PBDEs in adipose tissue exceeded the one of PBB 153.
Muir *et al*. found that BDE 47 represented 65-82% of the PBDE contamination in polar bears from East Greenland and Spitsbergen.[13] A similar or higher contribution of this PBDE congener was also determined in our study. In addition, PBDE levels in polar bears from East Greenland and Spitsbergen were highest and dominated by BDE 47, 99 and 153.[14] Between 22-190 ng/g fresh weight PBDEs in adipose tissue (no correlation of the levels with gender and age) were determined in samples from East Greenland.[14]

Table 2: Concentration of different POPs (ng/g lipids) in polar bears stranded in Iceland (2008-2011)

	Sample #1	**Sample #2**	**Sample #3**	**Sample #4**
	fat/liver/kidney/muscle	fat/liver/kidney/muscle	fat/liver/kidney/muscle	fat/liver/kidney/muscle
PBDEs	- / 81 / 35 / 45	94/ 300/ 15 / 68	74/ 90 / 45/ 53	830 / 1470 / 520 / 410
PBB 153	- / 170 / 77 / 86	260 / 590 / 75 / 160	65 / 94 / 42 / 53	180 / 160 / 110 / 180
oxychlordane	- / 10,000 / 370 / 350	4,330 / 66,500 / 1,230 / 2,950	2,690 / 36,000 / 1,260 / 1,530	8,370 / 95,900 / 6,000 / 5,400
trans-chlordane	- / 1.4 / n.d. / n.d.	4.1 / 8.8 / n.d. / 2.9	2.4 / 12 / n.d. / 0.8	3.8 / 6.5 / 3.0 / 2.9
cis-chlordane	- / 21 / n.d. / n.d.	4.9 / 39 / n.d. / 3.9	3.3 / 12 / n.d. / 0.8	15 / 74 / 11 / 9.0
HCB	- / 400 / 220 / 160	330 / 400 / 220 / 160	270 / 170 / 130 / 100	1,840 / 1,500 / 1,220 / 350

Gebbink *et al.* reported that BDE 99 was tendentially higher concentrated in liver and BDE 153 in adipose tissue.[7] The low contribution of BDE 99 indicated that this congener may have been metabolized during the starving period of the polar bears prior to their arrival in Iceland.
In samples from East Greenland (2008-2011) PBDEs (43 ng/g lipids) were on the same level as PBB 153 (39 ng/g lipids) in samples from East-Greenland whereas in Spitzbergen, PBDEs (44 ng/g lipids) were two fold higher than PBB 153 (21 ng/g lipids).[15] McKinney *et al.* also suggested that PBB 153 was the most abundant single BFR congener.[15] This was also found in our individuals except for sample #4. This sample showed a much higher PBDE level than the literature samples.[16]

Chlordane and HCB. Oxychlordane was two orders of magnitude higher concentrated than *cis*- and *trans*-chlordane (**Table 2**). Typically, c*is*-chlordane dominated over *trans*-chlordane. According to literature, adipose tissue of male adults (2006-2010) contained on average 840 ng/g lipids oxychlordane and well as 13 and 1.8 ng/g ng/g lipids *cis*-chlordane and *trans*-chlordane.[9] The concentrations in juveniles were on the same level, and those of female adults were slightly higher.[9] Levels of *cis*-and *trans*-chlordane were in the literature range while oxychlordane was up to ten-fold higher concentrated. The samples also contained *trans*-nonachlor and nonachlor III [4,9], which were not quantified by us. In sample #1, HCB ranged from 40-180 ng/g lipids but in sample #4, HCB levles were tenfold higher. Level were highest in the youngest individuals (samples #3 and #4), i.e. younger females. HCB concentrations were lowest in muscle (**Table 2**).

Further polyhalogenated compounds. According to GC/ECNI-MS measurements, the samples contained further organochlorine compounds. For instance, 59 compounds were detected in liver sample #1. In the higher retention time range we determined a compound showing the molecular ion at *m/z* 610 which was present in several samples. This compound was recently described by Bendig *et al.* as chlordene plus.[17] Chlordene plus has been used as flame retardant but was also reported as an impurity in technical chlordane.[18] Since no standard was available to us we could not qunarify the compound. To give an estimate, the peak area in the liver of sample #1 corresponded with the one of PCB 170. Moreover, a related compound with M^- at *m/z* 576 (which corresponds with one Cl less than chlordene plus) was detected along with octachlorostyrene and two heptachloronaphthalene isomers.

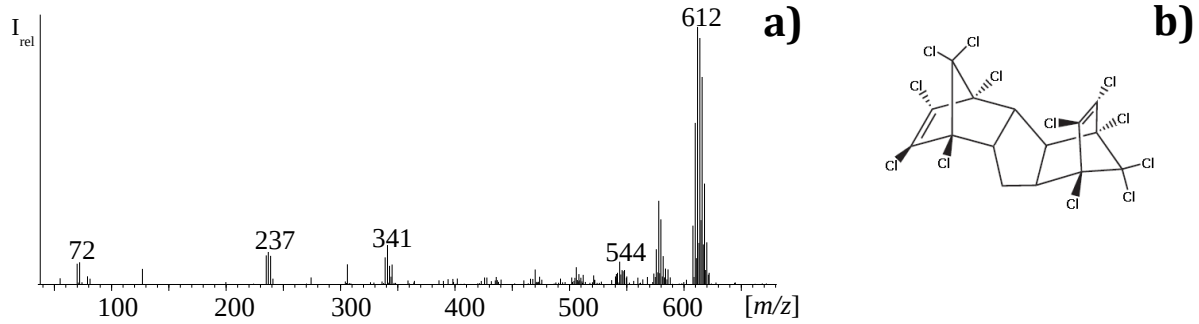

Figure 3: (a) GC/ECNI-MS mass spectrum and (b) structure of chlordene plus tentatively detected in the liver of a polar bear (sample #1) stranded in Iceland

Comparison of concentrations of different organohalogen compounds in the polar bear samples from Iceland.
In three out of four liver samples the concentration of oxychlordane exceeded those of the PCBs (**Tables 1** & **2**). Only in sample #1, oxychlordane (10,000 ng/g lipids) was lower concentrated than PCBs but still higher than PCB 153 (6,590 ng/g lipids). The dominance of oxychlordane over PCBs in liver further verified that the four polar bears originated from the East Greenland population.[19] By contrast, polar bears from Spitsbergen are characterized by higher burdens of PCBs compared to oxychlordane.[19] By contrast, HCB, *cis*- and *trans*-chlordane as well as polybrominated compounds were much lower concentrated than oxychlordane and PCBs.
According to literature, PBDE levels in polar bears are usually 2-3 orders of magnitude lower than PCBs.[15] This was also the case with our samples except for sample #4 which showed a comparably high burden of PBDEs (3-4.5% of PCBs). Within sub-populatios, relatively high PBDE concentrations were determined in Southern and Western Hudson Bay > East Greenland and Spitsbergen > Eastern Canadian arctic > Western arctic.[15] For samples #1-3, the PBDE concentrations further support the origins from East-Greenland while the high load with PCBs, oxychlordane and PBDEs in sample #4 may even suggest an origin closer to the North American continent.

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