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Hydroxylated PCB Metabolites and PCBs in Serum from Pregnant Faroese Women

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In the Faroe Islands in the North Atlantic, the traditional diet includes pilot whale meat and blubber and other marine food. Fatty fish and blubber of mammals may contain high concentrations of organohalogen substances (OHSs). Elevated levels of OHSs have been reported from the Faroe Islands, first documented in breast milk samples obtained in 1987. The aim of this study was to determine the concentrations of hydroxylated polychlorinated biphenyls (OH-PCBs) and polychlorinated biphenyls (PCBs) in serum samples from pregnant Faroese women known to differ in their dietary habits. High concentrations of OH-PCBs and PCBs were found in part of the human serum samples analyzed, and the relative OH-PCB and PCB congener distributions were similar to those observed elsewhere. There was a wide span between the lowest and highest OH-PCB and PCB concentrations in the serum samples analyzed, with ranges of 19–1,800 ng/g lipid weight (lw) and 150–22,000 ng/g lw, respectively. The ratio of Σ OH-PCB/ Σ PCB averaged about 10% and varied little. 4-Hydroxy-2,2',3,4',5,5',6-heptachlorobiphenyl was the most abundant OH-PCB metabolite in all samples analyzed, with four other OH-PCB congeners as dominating metabolites in the serum. More than 25 additional OH-PCBs were indicated. This study confirms the presence of high concentrations of organohalogen substances in populations or areas far removed from their sources. *Key words:* Faroe Islands, marine foods, polychlorinated biphenyls, polychlorobiphenyls, pregnancy. *Environ Health Perspect* 110:895–899 (2002). [Online 26 July 2002]

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Polychlorinated biphenyls (PCBs) remain an important class of environmental contaminants even though major legislative measures were taken by most industrialized countries in the early 1970s to stop their production and to restrict most their uses thereof (1). 2,2-Bis(4-chlorophenyl)-1,1,1-trichloroethane (4,4'-DDE) and persistent PCB congeners are in general and still the most abundant pollutants in wildlife and humans, although recent decreases have been reported (2,3). High concentrations of these substances have been determined even in populations living in remote areas (4,5), including people from the Faroe Islands, as first documented by analyses of four pools of human breast milk sampled from a birth cohort in 1987 (6). Similarly high PCB and 4,4'-DDE concentrations were obtained from a second Faroese birth cohort from 1994 (7). People who have a high consumption of fatty fish and live closer to the sources of contamination have high levels of PCBs and DDE (8).

Halogenated phenolic compounds (HPCs) are known to be strongly retained in human and wildlife blood (9), and these metabolites may be present in concentrations only slightly lower than those of the parent compounds (10–12). Among those compounds, a large number of polychlorobiphenyls (OH-PCBs) have earlier been reported in blood from humans, birds, seals, and polar bears (9–17). The concentration of

the OH-PCBs may exceed 10% of the total PCB concentration in human serum (5,9,12). As many as 38 OH-PCBs have been identified in human plasma (10), but only a few of these make up the major proportion of the OH-PCB concentrations in the human blood. OH-PCBs are formed via cytochrome P450-mediated oxidation of individual PCB congeners, and those being retained in the blood are metabolites of those that are most strongly accumulated in lipids—the most persistent PCB congeners. Several of the OH-PCBs compete with thyroxine (T_4) for its binding to transthyretin (TTR) (18). One of the major OH-PCBs, 2,3,3',4',5-pentachloro-4-biphenylol (4-OH-CB107), which has been identified in human blood (9), appears to possess adverse endocrine-related toxicity (19). OH-PCB metabolites are generally more hydrophilic than the parent compound and are therefore more easily eliminated from the body than their parent PCB congeners. Still, the physicochemical properties of some of the OH-PCBs suggest retention rather than excretion (9). However, it is not known whether the OH-PCBs occur along with the parent PCB congeners at similarly increased concentrations in subjects with high exposure to lipophilic contaminants from seafood as those with a lower exposure.

The Faroe Islands in the North Atlantic have a relatively uniform population of about 45,000 inhabitants. Besides fish (mainly cod),

pilot whale blubber and meat have traditionally been part of their diet (6). Seabirds and their eggs may also be included in the Faroese diet for part of the year. Certain types of marine food, especially fatty fish and blubber of mammals living in polluted waters, contain high concentrations of organohalogen substances (OHSs) (1). Persons who frequently consume contaminated seafood therefore exhibit increased concentrations of OHSs in serum and adipose tissue (8,12).

Our aim in this study was to determine the concentrations of OH-PCBs and PCBs in serum samples from pregnant Faroese women. Women were therefore selected to represent different exposure levels associated with different intakes of traditional food.

Materials and Methods

Samples. Serum samples were collected at the National Hospital in Tórshavn, Faroe Islands, in connection with the formation of a cohort of 182 mother–child pairs in 1994–1995. We collected maternal serum samples at the last antenatal consultation, approximately the 32nd week of pregnancy. Milk was obtained at 3–4 days postpartum and analyzed for major PCB congeners (7). Based on these results, we selected 36 subjects to represent the full range of PCB exposures. Stored serum was retrieved for analysis. We used a brief nutritional questionnaire to obtain information about the frequency of blubber and whale meat meals during pregnancy (number of meals per month). Although we made no attempt to assess lifetime intake levels, we categorized the subjects with respect to their recent consumption of

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pilot whale blubber as follows: none/low = 0–1 blubber meals/month ($n = 21$); moderate/high = 2–8 blubber meals/month ($n = 15$). This stratification provided reasonable numbers of subjects in each category.

Chemicals. The reference compounds, the abbreviations by which they are designated, and their sources of origin are given in Table 1. Hexane (Hx) and dichloromethane (DCM) were pesticide grade (Fisher Scientific, Leicestershire, UK). Potassium chloride, hydrochloric acid, sulfuric acid (Merck, Darmstadt, Germany), and potassium hydroxide (Eka Nobel, Bohus, Sweden) were all of pro analysis (p.a.) quality. 2-Propanol (p.a. grade; Prolabo, Cedex, France) and methyl *tert*-butyl ether (HPLC-grade; Rathburn, Walkerburn, Scotland) were glass distilled before use. Silica gel (< 0.063 mm) was purchased from Macherey-Nagel (Düren, Germany) and activated before use. Deionized water was produced in an Elga stat apparatus (Elga Ltd., Wycombe, England). Diazomethane was prepared from *N*-methyl-*N*-nitroso-*P*-toluenesulfonamide (Diazald) obtained from Sigma-Aldrich (Steinheim, Germany) (20).

Instruments. We used a vacuum centrifuge evaporator (Genevac SF50; Saler Development Ltd., Ipswich, UK) to evaporate solvents. A table centrifuge (Wifug Ltd., Bradford, England) operated at 3000 rpm was used to promote efficient phase separation.

Gas chromatography (GC) analyses were performed on a Varian 3400 gas chromatograph, equipped with a Varian 8200 autosampler (Varian Associates, Walnut Creek, CA, USA), an electron capture detector (ECD), and a split-splitless injector operated in the splitless mode. Hydrogen was used as carrier gas and nitrogen as make-up gas. For PCB analysis a DB-5 column (30 m × 0.25 mm internal diameter and 0.25 μm film thickness; J&W Scientific, Folsom, CA, USA) was used. The GC temperature program was initiated at 80°C (2 min) and then increased by 10°C/min to 300°C (10 min). The injector temperature was 250°C and the detector temperature 360°C. The analysis on the phenolic substances was performed on a CP-Sil 8-column (25 m × 0.15 mm internal diameter and 0.12 μm film thickness; Chrompack, EA Middelburg, The Netherlands). The column temperature was programmed as follows: 80°C (2 min), 50°C/min to 200°C, 1°C/min to 230°C, and 30°C/min to 330°C (3 min). The injector temperature was 280°C and the detector temperature 360°C. We collected and processed data using a PC-based ELDS Pro v2.0 system (Chromatographic Data System AB, Stockholm, Sweden).

Cleanup procedure. The extraction and cleanup procedure of the OHS from serum has been described in detail elsewhere (21). The surrogate standards, CB189 (4 ng) and

4-OH-CB193 (2 ng), were added before extraction. We determined the extracted lipid amount gravimetrically. The cleanup produced two fractions, one containing neutral and the other containing the phenolic substances (21). In addition to the cleanup described in the method, the neutral fraction was run through a pasteur pipette column (90 mm × 4 mm internal diameter) with activated silica gel (1 g), using hexane (5.5 mL) as the mobile phase. The neutral fraction was further cleaned up using a silica gel/sulfuric acid column (2:1, w:w, 0.5 g) with hexane (8 mL) as the mobile phase. The phenolic fraction was treated in the same way as described by Hovander et al. (21). Blank samples ($n = 9$) were run in parallel. Recoveries of the internal surrogate standards CB189 and 4-OH-CB193 were 83% (SD 5.1, $n = 36$) and 88% (SD 9.5, $n = 36$), which is in agreement with earlier studies (21).

Results

The concentrations of the major OH-PCBs, 4-OH-CB107, 3-OH-CB153, 4-OH-CB146, 3'-OH-CB138, and 4-OH-CB187, were added together, and median concentrations and 80th percentile ranges are given in Table 2. The ΣOH-PCB concentrations are reported both on a fresh-weight and a lipid-weight basis because these metabolites are not retained in the blood lipids like their parent compounds (Tables 2 and 3). The ΣOH-PCB ranges in the low and moderate/high blubber consumption groups were 19–750 ng/g lw and 25–1,800 ng/g lw, respectively. A typical GC/ECD chromatogram for OH-PCBs in serum from a pregnant Faroese woman is shown in Figure 1. The structures of the five OH-PCBs for which concentrations are reported (Table 2) are superimposed on the chromatogram. At least 20 other peaks

Table 1. Reference substances and internal surrogate standard (IS) used for quantification of PCBs and OH-PCBs in the human serum samples.

Substances	Abbreviation ^a	Source
Polychlorinated biphenyls (PCBs)		
2,3,3',4,4'-Pentachlorobiphenyl	CB105	<i>b</i>
2,3',4,4',5-Pentachlorobiphenyl	CB118	(34) ^c
2,2',3,4,4',5'-Hexachlorobiphenyl	CB138	<i>b</i>
2,2',3,4',5,5'-Hexachlorobiphenyl	CB146	<i>b</i>
2,2',4,4',5,5'-Hexachlorobiphenyl	CB153	(35) ^c
2,3,3',4,4',5-Hexachlorobiphenyl	CB156	(35) ^c
2,3,3',4,4',5'-Hexachlorobiphenyl	CB157	<i>b</i>
2,3',4,4',5,5'-Hexachlorobiphenyl	CB167	<i>b</i>
2,2',3,3',4,4',5-Heptachlorobiphenyl	CB170	<i>b</i>
2,2',3,3',4,5,5'-Heptachlorobiphenyl	CB172	(34) ^c
2,2',3,3',4',5,6-Heptachlorobiphenyl	CB177	(34) ^c
2,2',3,3',5,5',6-Heptachlorobiphenyl	CB178	<i>b</i>
2,2',3,4,4',5,5'-Heptachlorobiphenyl	CB180	<i>b</i>
2,2',3,4,4',5',6-Heptachlorobiphenyl	CB183	(35) ^c
2,2',3,4',5,5',6-Heptachlorobiphenyl	CB187	<i>b</i>
2,3,3',4,4',5,5'-Heptachlorobiphenyl	CB189 (IS)	(34) ^c
2,2',3,3',4,4',5,5'-Octachlorobiphenyl	CB194	(35) ^c
2,2',3,3',4,4',5,6-Octachlorobiphenyl	CB195	<i>b</i>
2,2',3,3',4,4',5,6'-Octachlorobiphenyl	CB196	<i>b</i>
Methoxy-PCBs		
4-Methoxy-2,3,3',4',5-pentachlorobiphenyl	4-MeO-CB107	(36) ^c
3'-Methoxy-2,2',3,4,4',5'-hexachlorobiphenyl	3'-MeO-CB138	(36) ^c
4-Methoxy-2,2',3,4',5,5'-hexachlorobiphenyl	4-MeO-CB146	(36) ^c
3-Methoxy-2,2',4,4',5,5'-hexachlorobiphenyl	3-MeO-CB153	(36) ^c
4-Methoxy-2,2',3,4',5,5',6-heptachlorobiphenyl	4-MeO-CB187	(36) ^c
4-Methoxy-2,3,3',4',5,5',6-heptachlorobiphenyl	4-MeO-CB193 (IS)	(36) ^c

^aAbbreviation according to PCB numbering system (37); MeO-PCBs were numbered according to Letcher et al. (9). ^bPromochem AB, Ulricehamn, Sweden. ^cSynthesized according to the reference given.

Table 2. Concentrations (ng/g lipid weight and ng/g fresh weight) of OH-PCB congeners in serum from pregnant women from the Faroe Islands according to recent pilot whale blubber consumption.

Compound	Concentration (ng/g lipid weight)				Concentration (ng/g fresh weight)			
	Low ^a		Moderate/high ^b		Low ^a		Moderate/high ^b	
	Median	80% ^c	Median	80% ^c	Median	80% ^c	Median	80% ^c
Lipid (%)	0.78	0.67–0.97	0.83	0.64–1.00				
4-OH-CB107	12	5.1–73	71	41–230	0.084	0.041–0.63	0.49	0.29–1.7
3-OH-CB153	13	4.0–75	64	16–190	0.10	0.031–0.58	0.49	0.11–1.3
4-OH-CB146	23	9.8–170	120	13–270	0.15	0.081–1.4	1.1	0.14–1.8
3'-OH-CB138	13	3.5–100	96	47–170	0.11	0.033–0.84	0.64	0.34–1.4
4-OH-CB187	37	18–210	190	23–470	0.27	0.14–1.6	1.6	0.23–3.0
ΣOH-PCB ^d	96	39–590	560	36–1300	0.75	0.34–4.6	5.0	0.37–8.1

The OH-PCB congeners are listed in elution order on a CP-Sil 8 column.

^a0–1 meals/month. ^b2–8 meals/month. ^c80% range (10th–90th percentiles). ^dSum of the OH-PCBs shown.

were detected, representing other OH-PCBs and dihydroxylated PCB congeners (10).

The serum concentrations, medians, and 80th percentile ranges of 18 PCB congeners, along with the Σ PCB (sum of detected congeners), are presented in Table 3. CB153 shows a concentration range of 35–2,200 ng/g lw and 58–5,700 ng/g lw in the low and moderate/high pilot whale blubber consumption groups, respectively, while Σ PCB ranges are 150–9,000 ng/g lw and 280–22,000 ng/g lw.

Discussion

This study amplifies previous evidence that increased PCB exposures occur in the Faroe Islands. In addition, new evidence is presented that OH-PCBs occur in conjunction with the PCB congeners normally observed as a result of dietary exposure to OHSs. As recently reported, likely sources of OHS exposures in the Faroe Islands are pilot whale blubber and muscle, wild birds, and bird eggs (22,23). This study did not attempt to quantify lifetime intakes of blubber or alternative exposure sources.

Instead, subjects were divided into two groups in relation to their recent consumption of pilot whale blubber, the only dietary-related parameter available describing potentially different exposures to persistent

organic pollutants and their metabolites. This division provided reasonable numbers of subjects in each category. When more information becomes available on OHS concentrations in Faroese subsistence foods and the actual intake

of these foods, it will be possible to improve the exposure assessment. Nonetheless, the present study shows a major difference in exposures of OH-PCBs and PCBs depending on the recent intake of pilot whale blubber.

Table 3. Concentrations (ng/g lipid weight) of 18 PCB congeners in serum from pregnant women from the Faroe Islands.

Compound	Low (0–1 meals/month)		Moderate/high (2–8 meals/month)	
	Median	80% ^a	Median	80% ^a
Lipid (%)	0.78	0.67–0.97	0.83	0.64–1.0
Polychlorinated biphenyls				
CB118	48	24–460	360	46–780
CB146	28	16–200	210	18–360
CB153	190	130–1,500	1,500	130–3,000
CB105	11	4.2–90	78	12–160
CB138	140	94–1,100	1,100	110–1,900
CB178	13	6.5–94	85	7.0–160
CB187	66	35–520	520	42–1,000
CB183	15	9.8–120	130	12–260
CB167	9.9	5.6–77	68	8.6–120
CB177	17	10–130	140	14–280
CB156	18	11–150	130	13–300
CB157	5.0	2.7–36	37	2.8–75
CB172	7.8	3.9–61	56	3.7–110
CB180	120	67–980	890	61–2,300
CB170	46	27–330	300	24–740
CB196	11	4.3–130	110	2.9–340
CB195	4.8	2.7–35	34	2.9–90
CB194	20	9.1–150	120	8.2–330
Σ PCB ^b	750	460–5,900	5,900	510–12,000

The PCB congeners are listed in elution order on a DB5 GC-column. ^a80% range (10th–90th percentiles). ^bSum of the PCBs shown.

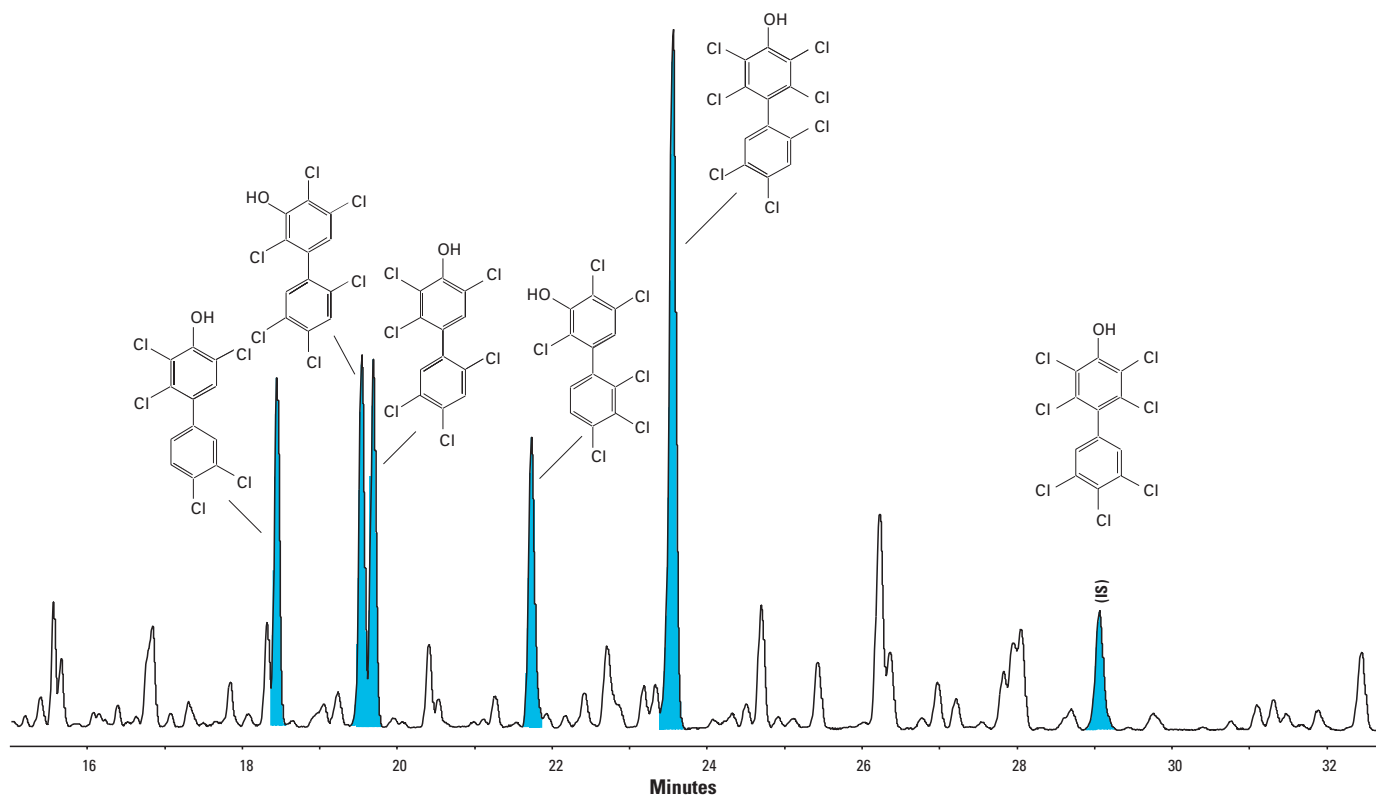


Figure 1. Gas chromatogram (GC/ECD) demonstrating the presence of hydroxylated polychlorinated biphenyls (OH-PCBs) in a serum sample from a Faroese woman with a recent moderate/high pilot whale blubber intake. The concentrations of the six most abundant OH-PCBs [4-OH-CB107, 3-OH-CB153, 4-OH-CB146, 3'-OH-CB138, 4-OH-CB187, and 4-OH-CB193 (IS)] were determined as their corresponding methyl ethers. The structures of the six most abundant OH-PCBs are superimposed in the figure.

The major OH-PCBs identified in the serum samples were 4-OH-CB107, 3-OH-CB153, 4-OH-CB146, 3'-OH-CB138 and 4-OH-CB187, the latter being the predominant hydroxylated PCB metabolite. The same metabolites have previously been reported as the major OH-PCBs present in human blood (5,9,12), with 4-OH-CB187 being the most abundant OH-PCB (12). However, other studies have reported that 4-OH-CB107 was the main metabolite (5,12). The OH-PCB peak pattern is shown in Figure 1. Estimation of the total OH-PCB concentrations in the samples indicate that the levels of the five major OH-PCBs account for 90% of the compounds. The 80th percentile of OH-PCBs in the moderate/high consumption group (36–1,300 ng/g lw) is almost in the same as the 80th percentile for CB153 in the low consumption group (130–1,500 ng/g lw; Tables 2 and 3).

Persistent PCB congeners, such as CB118, CB138, CB153, and CB187, have

been shown to form OH-PCB metabolites that are retained in blood (24). Overall, the Σ OH-PCB concentrations correspond to about 10% of the Σ PCB concentration in the blood serum samples, within a range of about 5–20%. This finding agrees with previous reports at lower OH-PCB/PCB exposures, where average ratios of 10% (5,12) have been observed (9). This ratio may possibly be higher for persons with relatively low levels of PCB, as indicated in Figure 2. The concentration dependence of the OH-PCB/PCB ratio indicates a faster elimination of OH-PCBs in humans at higher levels of PCB exposures, which may be explained by induction of phase II enzymes and conjugation reactions (9). However, the possibility also exists that dietary habits may influence the levels of OH-PCB in the body. Marine food such as pilot whale predominantly contains persistent PCB congeners and less of the lower chlorinated and the more rapidly metabolized PCB congeners. The formation

of the OH-PCBs depends on the metabolism and differences in metabolic capacity between individuals and may thus influence the pattern of OH-PCBs and their concentrations (9,25). Any conclusions in this regard are hampered by not knowing to what degree the OH-PCBs are only generated in humans or whether they are also retained from ingested food that contains these compounds, as is the case with eggs (22).

Some toxicologic data have become available on at least one of the OH-PCBs, 4-OH-CB107, which is one of the major OH-PCBs present in the blood (9,12,19). Thus, low-dose levels of 4-OH-CB107 induce *in vivo* disturbances in rats, influencing the estrous cycle and behavior of the offspring (19). Current exposure levels of OH-PCBs as determined in the present study appear to be close to the levels at which effects were observed in the rats. This conclusion is based on the assumption that all halogenated phenolic compounds are as toxic as 4-OH-CB107, an assumption that cannot be confirmed or rejected at this time.

Because of the possible interaction of OH-PCBs with transthyretine and thyroid metabolism (18), questions regarding the thyroid function have been addressed in the Faroese studies. In the full cohort of children born in 1994, thyroid function was assessed by analysis of serum from the mothers and from the umbilical cord. No association of thyroid hormone parameters with PCB exposure was found (7). In a previous cohort, no association was found between PCB concentrations in cord tissue and thyroxine and thyroid-stimulating hormone in neonatal serum (26). The Faroese rely heavily on a seafood diet, and their iodide intake would therefore likely be in the optimal range, but it is not known whether a high iodide intake may protect against adverse thyroid effects.

Possible adverse health effects have been considered in clinical examinations of Faroese children exposed to PCBs and related OHSs prenatally. Because of concomitant exposure to methylmercury, any neurotoxic effects of PCB have not been readily detectable (27). However, stratified analyses suggest that PCB-associated neurotoxicity may be more apparent when exposure to methylmercury is increased, thus suggesting a possible additive effect (26). In this regard, Σ PCB was used as a marker, which reflects a complex exposure that also includes OH-PCBs.

In the serum samples analyzed, CB153 was the most abundant PCB congener, followed by CB138 and CB180. The CB153 concentrations correlate well with the Σ PCB concentration ($r = 0.999$), with CB153 accounting for 25% of the Σ PCBs. Similar correlations have also previously been reported (28), although the relative CB153 concentration

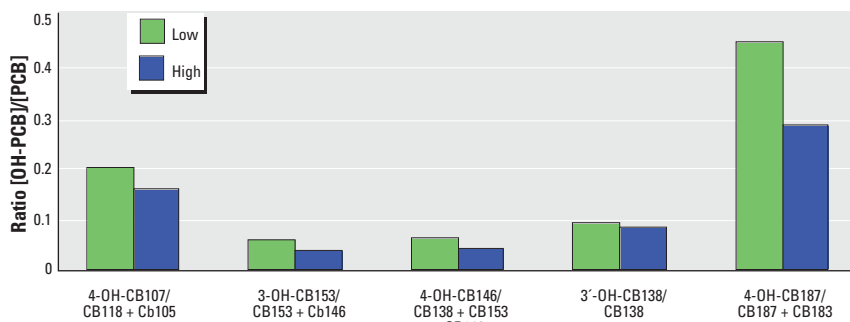


Figure 2. Ratio between OH-PCB metabolites and their corresponding parent PCB congener. Subjects were divided into groups according to recent pilot whale blubber consumption: low = 0–1 blubber meals/month and moderate/high = 2–8 blubber meals/month.

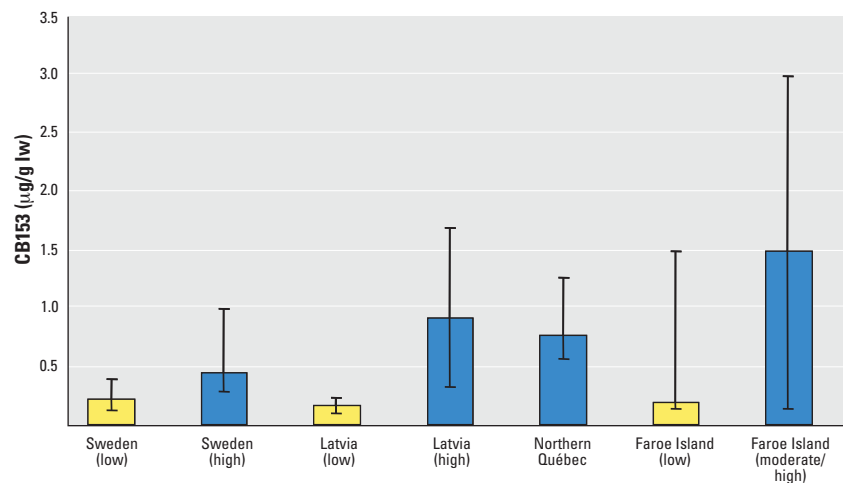


Figure 3. Median concentrations of CB153 ($\mu\text{g/g}$ lipid weight) in human plasma samples are given. Error bars indicate the 80% range (10th–90th percentiles). The Swedish and Latvian male subjects were divided into groups according to consumption of fatty fish: low = 0–1 fish meals/month and high \geq 12 fish meals/month (12). CB153 concentrations in plasma samples from northern Québec men are expressed as plasma equivalents and normalized for lipid content (5,29,30). The CB153 levels in women from the Faroe Islands (present study) were divided into low (0–1 blubber meals/month) and moderate/high (2–8 blubber meals/month).

has varied between 25% and 31% in previous studies (12,28). Undoubtedly, CB153 is a good indicator for Σ PCB concentrations in humans, but it is still important to determine the PCB congener pattern in samples analyzed because this may indicate origin of exposure or give other important details about the exposure.

The CB153 concentrations in the Faroese serum samples are compared to results obtained from Sweden, Latvia, and Arctic Québec in Figure 3. It is notable that the median level of CB153 in the Faroe Islands (moderate/high consumption group) is higher than in any of the other groups and also exceeds the concentrations from the Inuit population from northern Québec (5,29,30). Both the Inuit and the Faroese consume a seafood diet that includes marine mammals and seabirds (4,25). However, the Σ PCB concentrations in blubber from ringed seal (0.96–5.6 $\mu\text{g/g}$ lw) and beluga whale (0.31–1.5 $\mu\text{g/g}$ lw), which are part of the Inuit diet, are only moderately high (25,31) when compared to Σ PCB levels reported for pilot whale blubber (10–40 $\mu\text{g/g}$ lw) in the Faroese diet (32). Thus, even occasional intake of pilot whale blubber could cause a substantial exposure to PCBs. In this regard, it should be noted that the Faroese intake groups were based on recent dietary habits and that the Faroese may eat whale blubber for dinner (usually with whale meat or dried cod) but also eat thin slices of whale blubber as a snack.

Because of the potential adverse health effects caused by OHS contaminants, a recommendation was issued in 1998 by the Faroese health authorities: “The best way to protect fetuses against the potential harmful effects of PCBs, is if girls and women do not eat blubber until they have given birth to their children” (33). The present results, from samples collected before this statement was issued, reinforce this recommendation. However, further investigations are needed to qualify the present recommendation and to improve the risk assessment.

The present study shows some high concentrations of OH-PCBs and PCBs in human serum, possibly the highest so far reported in a population. The main source of OHS exposures in the Faroe Islands is pilot whale blubber, but consumption of seabirds and their eggs may also contribute to exposure, at least in some cases. It is notable that the Faroese population living in an Atlantic archipelago far away from OHS pollution sources show such high OHS burdens as reported in this study. The PCB exposure problem is thus not only a matter of the most contaminated areas, such as the Great Lakes and the Baltic Sea, but a worldwide problem linked to dietary habits.

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