ORGANOHALOGEN CONTAMINANTS IN TWO DIFFERENT FAT COMPARTMENTS FROM OBESE INDIVIDUALS

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Introduction

Chemicals that are stored in lipid-rich compartments have the potential for long-term disruption of metabolic and endocrine processes. Given the evidence that persistent organic pollutants (POPs) might alter systemic metabolic, endocrine, and immune system functions, it implies that elevated chemical concentrations in abdominal fat may alter function of visceral organs through local chemical signalling. Obese individuals vary in their body fat distribution, their metabolic profile and the degree of associated cardiovascular and metabolic risk[1]. Yet, there are only few studies defining POPs concentrations in intra-abdominal fat from living humans. The body has several depots of white adipose tissue, of which the major two categories are subcutaneous and visceral fat. It is known that visceral fat correlates better with the development of insulin insensitivity and the metabolic syndrome, most probably related to a greater glucose uptake[2] and inflammation[3]. Most studies have shown relatively little difference in the fatty acid composition of the various fat stores, including fat in serum[4]. It is uncertain whether POPs distribute equally to all fat compartments, including visceral and subcutaneous fat.

The objective of this study was to determine the concentrations and accumulation features of organohalogen compounds, such as DDT and its metabolites, chlordanes (CHLs), hexachlorocyclohexane isomers (HCHs), hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDs) in visceral and subcutaneous abdominal fat samples from obese individuals in Belgium.

Materials and methods

***Sample collection*.** This study was approved by the Ethical Committee of the Antwerp University Hospital and all participants gave their informed consent. A total of 47 patients who were undergoing bariatric surgery at Antwerp University Hospital agreed to provide adipose tissue samples. In this group, paired visceral and subcutaneous abdominal fat samples were obtained between 2010 and 2011. Participant's age and extractable lipids are listed in the Table 1.

Chemical analysis. Analyses of organochlorines (OCs), PBDEs and HBCDs in visceral and subcutaneous abdominal fat samples were performed according to the methods described elsewhere[5], with slight modifications. Fat samples (~200 mg) were weighed, mixed with anhydrous Na_2SO_4 and spiked with internal standards (CB 143, BDE 77, BDE 128, ^{13}C-BDE 209, and ^{13}C-HBCDs). Further, the samples were extracted for 2 h by hot Soxhlet with 100 mL hexane/acetone (3:1, *v/v*). The lipid content was determined gravimetrically on an aliquot of the extract (105 °C, 1 h), while the rest of the extract was cleaned on ~8 g acidified silica (44%) and eluted with 20 mL hexane and 15 mL dichloromethane. The cleaned extract was evaporated to dryness, redissolved in 0.5 mL hexane and eluted from pre-packed silica cartridges (Varian). The first fraction (A) eluted with 6 mL hexane contained OCs and PBDEs, while the second fraction (B), containing HBCDs, eluted with 8 mL DCM. Both the fractions were evaporated to incipient dryness and re-dissolved in 100 µL *iso*-octane (Fraction A) and 100 µL methanol (Fraction B), respectively. Quantification of OCs and PBDEs were done using GC-MS, while HBCD isomers were quantified by LC-MS/MS. The analytical procedures were validated through the analysis of procedural blanks and certified material SRM 1945 (organics in whale blubber; NIST SRM catalog: http://www.nist.gov/srm/). Outliers and concentrations below the limit of quantification *(*LOQ) were assigned a value of ½*LOQ. The results are presented as mean, median with minimal and maximum values. The concentration of OCs, PBDEs and HBCDs were expressed in ng/g lipid weight (lw), basis unless otherwise specified.

Results and discussion

Contamination status. Organohalogen compounds were detected in variable quantities in all the Belgian fat samples in the order of PCBs > DDTs > HCHs > CHLs > HCB > HBCDs > PBDEs. This suggests a generalized exposure of the Belgian population to these contaminants. The levels and the patterns of POP distributions in the visceral fat (VF) and subcutaneous fat (SF) tissue depots were similar. The levels of individual POPs in a single individual is influenced by the degree and source of exposure, the time since that exposure occurred, genetic differences among individuals in the rates of metabolism, body mass index and, if female, number of pregnancies and whether or not the child was breast fed.

Residue levels and contamination status of OCs. Among OCs, PCBs (VF/SF; median: 270/255 ng/g lw) and DDTs (VF/SF; median: 145/150 ng/g lw) were the predominantly identified compounds in all the fat samples. Concentrations of other OC compounds (CHLs, HCHs and HCB) analyzed in this study were 1-3 orders of magnitude lower than those of PCBs and DDTs (Table 1). The overall mean concentrations of OCs (e.g., DDTs, CHLs, HCHs, and HCB) in visceral and subcutaneous Belgian fat samples were lower than those reported for other countries. Levels of PCBs (VF/SF; mean: 320/315 ng/g lw) measured in the present study were lower than those observed in previous Belgian studies on adipose tissue[5-7]. Concentrations of DDTs were comparable to other reported levels in Belgian data in human adipose tissue and milk[6,8]. Differences in concentrations for PCBs and DDTs between the populations are probably due to the differences in contaminant's load of the food items or to the possible present direct exposure.

Table 1. Concentrations (ng/g lw) of organohalogen compounds in human visceral and subcutaneous abdominal fat samples from Belgium.

	Visceral fat (*n*=47)			Scbcutaneous fat (*n*=47)		
	Mean	**Median**	**Range**	**Mean**	**Median**	**Range**
Age	40	40	19-59			
Lipid content (%)	88	89	72-96	87	88	73-98
Compounds						
CB-28	2.0	1.0	0.30-27	2.3	1.1	0.45-28
CB-52	1.3	1.2	0.48-4.0	1.8	1.5	0.62-8.0
CB-74	7.8	5.7	1.5-60	9.5	5.9	1.1-60
CB-99	7.3	5.3	1.7-30	7.2	5.8	0.45-30
CB-118	13	9.7	2.9-90	12	9.4	2.6-95
CB-146	9.6	7.5	1.4-40	9.4	7.4	1.2-40
CB-153	78	58	9.1-270	74	61	8.6-265
CB-138	42	33	5.9-142	40	33	5.0-140
CB-187	17	11	1.6-110	17	11	1.6-115
CB-183	7.5	5.8	0.90-30	7.3	5.8	0.93-30
CB-180	52	40	4.9-160	52	45	4.8-175
CB-170	24	18	2.4-80	24	19	2.3-70
Sum PCBs	**320**	**267**	**45-1230**	**313**	**253**	**39-1260**
p,p'-DDE	368	137	15-8400	377	140	16-9065
p,p'-DDD	3.2	0.94	0.48-55	1.8	0.9	0.39-20
p,p'-DDT	23	4.9	1.5-780	24	4.9	1.5-820
Sum DDTs	**393**	**143**	**17-9235**	**403**	**148**	**18-9900**
Oxy -CHL	6.5	5.5	1.1-15	7	6	1.0-20
Trans-nona	5.6	4.6	0.92-30	6	5	0.92-25
Sum CHLs	**12**	**10**	**2.0-45**	**12**	**11**	**1.9-40**
α-HCH	1.4	1.0	0.25-5.0	0.44	0.21	0.16-1.0
β-HCH	28	17	2.1-445	26	18	2.2-300
γ-HCH	0.81	0.67	0.47-2.0	0.85	0.94	0.22-1.5
Sum HCHs	**29**	**18**	**2.1-445**	**26**	**18**	**2.2-300**
HCB	**13**	**10**	**3.5-40**	**12**	**10**	**2.7-40**
BDE-28	0.081	0.044	0.015-0.45	0.08	0.06	0.010-0.40
BDE-47	1.0	0.70	0.12-6.0	1.0	0.47	0.090-5.5
BDE-100	0.37	0.21	0.059-2.0	0.42	0.22	0.066-2.0
BDE-99	0.33	0.21	0.053-1.7	0.35	0.22	0.073-1.5
BDE-154	0.53	0.5	0.026-2.0	0.55	0.51	0.019-2.0
BDE-153	0.98	0.81	0.26-2.5	1.24	0.89	0.22-10
BDE-183	0.29	0.12	0.025-6.0	0.20	0.15	0.039-0.74
Sum PBDEs (tri to hepta)	**3.6**	**2.8**	**1.0-13**	**3.8**	**2.6**	**0.81-14**
α-HBCD	5.6	3.1	0.91-45	5.5	3.4	0.89-40
β-HBCD	1.2	0.80	0.39-5.0	2.0	2.0	0.10-2.0
γ-HBCD	1.7	1.4	0.74-5.0	2.5	2.8	0.55-4.0
Sum HBCDs	**6.7**	**4.0**	**0.91-45**	**5.8**	**3.4**	**0.89-40**

PCB 153 (VF/SF: 27/26%) was the most dominant congener, followed by PCB 180 (VF/SF: 17/18%), PCB 138 (VF/SF: 15/14.5%) and PCB 170 (VF/SF: 8.1/8.4%) to the sum PCBs, respectively (Figure 1). As previously reported[5,7], CB 153 and CB 180 were the major PCB congeners in human adipose tissue, similar to profiles reported from current European reports/studies. Among DDT metabolites, *p,p'*-DDE was the predominant compound (VF/SF: 93/94%), suggesting exposure from historical sources from technical mixtures of DDT used in Belgium. Technical HCH, a popular formulation prior to the 1990s, contains only 10-12% of the active insecticide, γ-HCH, and is predominantly made up of non-insecticidal isomers α (60-70%), β (5-12%), δ (6-10%), and ε (3-4%)[9]; only β-HCH was detected in adipose tissue samples due to its greater persistence compared with other HCH isomers[10]. The relative enrichment of β-HCH might reflect enhanced affinity for particles, greater resistance to degradation, reduced volatility, or a combination of all three[10]. Among chlordane compounds, the most abundant were *trans*-nonachlor and oxychlordane (Table 1). Indeed, CHLs rapidly break down into metabolites such as oxychlordane, or into impurities such as trans-nonachlor and these breakdown products persist in the tissues of fish, birds and mammals[11].

Residue levels and contamination status of BFRs. Most of the VF/SF fat samples contained PBDE concentrations in the range of 2-8 ng/g lw. The total PBDE concentrations ranged between 0.81 and 14 ng/g lw (VF/SF; median: 2.8/2.6 ng/g lw). PBDE concentrations in the present study were similar to other reported concentrations in Belgium data in different human matrices[5,6,12]. Levels of PBDEs in Belgian individuals were similar to the Japanese studies on adipose tissue[13]. Generally, based on the available studies, PBDE levels in adipose tissue of the European population were considerably lower than those in samples from the United States[14]. This is probably due to a less usage of this group of BFRs in common goods and products. The differences between countries can be partially explained by the PBDE concentrations in the diet and especially in food items with higher contribution to the total PBDE intake[15]. However, other exposure pathways (e.g. indoor dust) have recently been identified for humans and their contribution to the total PBDE intake may be higher than expected for the Flemish population[16].

Among PBDEs, BDE 153 (VF/SF: 31/34%) was the dominant congener, followed by BDE 47 (VF/SF: 26/23%), BDE 154 (VF/SF: 16/16%), BDE 100 (VF/SF: 10/11%) and BDE 99 (VF/SF: 9/9%) (Figure 1). It has been reported that relatively higher proportion of BDE 153 was also observed in human adipose tissues from Belgium[5,7]. Interestingly, PBDE profiles in human fat samples found in this study were not identical to those observed in human milk samples in the available literature. While in human milk BDE 47 was clearly the most abundant congener[12], the more hydrophobic BDE 153, recognized as one the most persistent congeners, was the dominating congener in fat samples. Furthermore, the distribution of PBDE congeners between different tissues could be directly related to their half-lives, octanol-water partition coefficient, and the properties of the tissu[17].

Levels of HBCDs were 1-2 orders of magnitude lower than those of PCBs and DDTs. Sum of α-, β-, and γ-HBCD isomers were found in the human fat tissue samples at concentrations ranging from 0.89 to 41 (VF/SF; median: 4.0/3.4) ng/g lw (Table 1). To our knowledge, this is the first report on concentrations of HBCDs in human fat tissues from Belgium. The levels of HBCDs found in this study were comparable to Japan[18], but slightly higher than U.S.[14], and similar to other reported concentrations in Belgian data in different human matrices[12]. Human exposure to HBCDs occurs through multiple routes similar to PBDEs. For non-occupationally exposed persons, the major intake of HBCDs is probably from food and indoor air or dust[19]. However, the relevance of human HBCD exposure originating from house dust versus food-based HBCD exposure is still scarce[19].

The stable isomer, α-HBCD (VF/SF: 85/97%) was predominant followed by γ-HBCD (VF/SF: 11/3%) in both the fat samples (Figure 2). In contrast to the dominance of α-HBCD in this study as well as others[20], some studies have reported a higher percentage of γ-HBCD in human tissues, such as adipose tissue[14] and serum samples[21]. Although the reasons for the different isomer profiles in human tissues from different studies are not yet clear, it can be hypothesized they arise from a combination of differences in external exposures and inter-individual variations in metabolism.

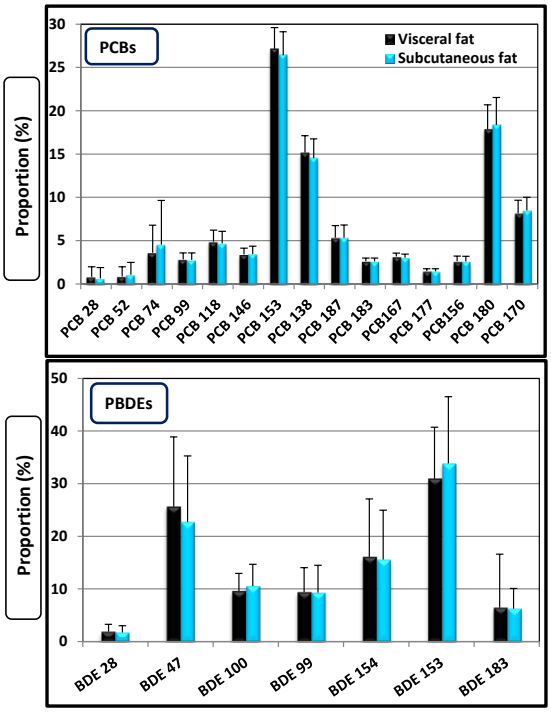

Figure 1. Congener profile of PCBs and PBDEs in human visceral and subcutaneous fat samples.

Correlation among POPs. In the present study, total PCB, PBDE and HBCD concentrations in human fat samples could not be correlated with age (r = 0.041; r = 0.128 and r = 0.019). The continuing exposure of all population groups to PBDEs and HBCDs from various sources (including the diet as well as inhalation either from indoor air or house dust as potential sources[19]) was probably responsible for the absence of a correlation between the age and levels in other similar studies in different human matrices conducted in the U.S., Japan and Belgium[14,13,6].

Contrary to BFRs and in agreement with many other studies, a significant correlation between age and the concentration of OCs (DDTs: r = 0.514, HCHs: r = 0.666, CHLs: r = 0.534 and HCB: r = 0.754) was observed in the present study (data now shown). For these contaminants, diet, particularly fish and other animal/dairy products, are the main exposure sources. Based on the differences in the exposure pathways and with regard to different pharmacokinetics, it was not surprising that there were no relationship among the concentrations of PBDEs, HBCDs and PCBs, in human adipose fat samples. Following the ban of PCBs in many countries, a slow successive decrease of PCBs may have occurred in the human food chain.

Levels of DDTs, HCHs, HCB and CHLs were significantly correlated each other. These correlations suggest that the contaminants share similar exposure routes, such as food ingestion and bioaccumulation in human bodies. No correlations were found among PBDE, HBCD and PCB concentrations in human fat samples. This may suggest differences in the sources of human exposures to OCs and BFRs or their differing pharmacokinetics in human body.

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