Levels of PCBs, Chlordane, DDE, HxCB and PBDE in human adipose tissue from Hungary compared to levels in Sweden

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Introduction

Levels of the traditional organochlorine compounds have been shown to decline since their peak levels in the 1970's. However newly introduced organic compounds including brominated flame retardants might still be increasing in human and other biological samples. A lot of data is available on the traditional POPs like PCBs, DDE and chlordanes. For brominated flame retardant information is however still limited and restricted to data from Western Europe and from Canada and the US. In the study presented here human adipose tissue samples from Hungary were analysed for both the traditional POPs and the brominated flame retardant PBDE. The levels were compared to a control group in a cancer study of which adipose samples were taken of healthy individuals at about the same time in Sweden (1998-2000). The samples from Hungary were taken in the year 2000 during autopsy of people who had died traumatically in sudden accidental deaths. The age span of the Hungarian subjects was from 18 to 47 years, 8 males and 20 females were included in the study making a total of 28 individuals. From a Swedish sample pool 53 individuals were selected for comparison.

Material and Methods

The human adipose tissue samples were homogenised in a mortar with sodium sulphate (1:5). About 1 g of the homogenised tissue was packed in Suprex standard extraction vessels (10 ml). An internal standard consisting of ^{13}C-labeled PBDE #77 and a mixture of several PCBs at each clorination level was added before supercritical fluid extraction (SFE) extraction. On the top of the sample about 4.5 g basic aluminium oxide (AlOx) was added as a fat retainer. The extractions were carried out on a Suprex Autoprep / Accutrap SFE using CO_2 as the supercritical fluid. The chamber temperature was 40°C and the pressure 280 bar during extraction at a flow rate of 2 ml/min for 25 minutes. All the analytes were trapped on a C18 solid sorbent (ODS, Octadecylsilica). The restrictor and trap temperatures were kept at 45°C and 40°C respectively[1,2]. After completion of the extraction the trap was rinsed with 3.5-ml hexane and 3.5 ml methylene chloride at a rate of 2 ml/min. After addition of the recovery standard containing ^{13}C-labeled PCB #128 and #178 in tetradecane the sample volume was reduced to 30 µl, producing an extract ready for GC/MS analysis. Separate lipid determination was performed by applying a part (~1g) of the homogenate on a small column and quantitative extraction with methylene chloride and hexane (1:1). The weight of the extracted lipids was determined gravimetrically.

Selected ions were recorded using a Agilent 6890 gas chromatograph coupled to an Agilent 5973 mass spectrometer (SIR HRGC/MS). Chromatographic separation was achieved by splitless injection of 2 µl on a non-polar DB-5 column using helium as the carrier gas. The GC oven was programmed as follows: 180°C initial hold for 2 min, increase at a rate of 15°C/min to 205°C, followed by an increase of 3.7°C/min to 300°C, final hold at 300°C for 15 minutes. The two most abundant ions of the molecular ion cluster were monitored for each compound in addition to one mass for the ^{13}C-labeled internal standard. Two quantification standards were used, one containing a mixture of TeBDE, PeBDE and HxBDE, and another containing several PCB congeners in addition to a mixture of chlordanes, HxCB and DDE and both the internal (IS) and recovery (RS) standards. The detection limit (DL) was calculated at a signal to noise ratio of 3 (S/N > 3). This DL depended on the amount of lipids extracted. Laboratory blanks were all well under 10% of the levels in the samples. All recoveries of the internal standards were within 50-120 %. One Hungarian sample was excluded from the data set, this sample did contained extremely low lipid levels and consisted most probably not of the requested adipose tissue.

Results and discussion

The results are summarised in Table 1, where the levels of both the Hungarian samples and the Swedish samples are given in ng/g normalised to the amount of lipids. The congener specific PCB analysis is given as the mean for 27 and 53 samples, respectively, together with the standard deviation (RSD) and the minimum and maximum amount detected in the samples. Two total PCB levels are given tot-PCB 1 is the summation of the 37 congeners analysed without including the detection limits (DLs), tot -PCB 2 is the summation of the same 37 isomers including the DL or less than values. The difference between tot-PCB 1 and tot-PCB 2 is an indication of the quality of the data and should be as small as possible.

Concentrating on tot-PCB 1 it can be seen that the levels in Hungary (200 ng/g) were significantly lower than in Sweden (660 ng/g). The RSDs were similar: 197 compared to 291, while the levels in Sweden showed a larger span (246-1646 ng/g) than the Hungarian samples (42-862 ng/g). The PCB pattern seems similar, although individual comparison of the results or multivariate statistical evaluation might give more insight if different commercial PCB formulations in both countries have resulted in different patterns in human samples[3,4]. Level were also lower than earlier levels reported from the Tarragona region in Spain[4].

For the DDE levels the situation is reversed and here the Hungarian samples showed the highest levels. The total DDE level in the Hungarian samples was 1735 ng/g, whereas the Swedish levels were 500 ng/g. One extreme value of more than 10 000 ng/g was measured in one Hungarian individual; surprisingly the levels of the other POPs in this individual were not extremely high. The levels of the different Chlordane isomers were higher in Sweden, especially the two dominating chlordanes (Oxychlordane and Trans-nonachlordane) with levels 4 and 9 times higher, respectively. Both the levels of HxCB and BDE #47 were similar in both groups of samples. This might be somewhat surprising as lower levels of PBDEs were expected for the Hungarian subjects. The mean values were very similar with minimum values of 0.15 ng/g and maximum value of 3.32 ng/g. In addition these PBDE levels are similar to levels reported from the Tarragona region in Spain[5].

Table 1. Levels in Human Adipose Tissue in Hungary and Sweden (ng/g lipids)

	Hungary (n = 27)				**Sweden (n = 53)**			
	Mean	RSD	Min	Max	Mean	RSD	Min	Max
Tri PCB # 28	2.76	6.83	0.34	36.60	2.29	2.33	0.06	11.70
Tetra PCB # 52	0.77	0.56	0.15	1.26	< 0.61	-	< 0.18	4.78
# 47	1.35	0.62	0.91	1.78	< 0.54	-	< 0.19	< 1.41
# 74	5.53	4.24	2.19	22.68	12.05	5.86	3.03	29.80
# 66	2.45	2.79	0.43	11.85	2.30	1.95	0.36	11.04
Penta PCB #101	0.40	0.40	0.12	1.98	1.30	1.41	0.21	9.71
# 99	3.28	2.37	0.34	10.58	11.79	6.78	2.10	31.57
#110	0.53	0.37	0.14	1.47	0.65	0.71	0.07	3.42
#118	5.51	4.49	1.37	20.94	25.21	13.56	4.86	73.67
#114	0.46	0.42	0.08	1.79	1.16	0.46	0.47	2.23
#105	1.34	0.92	0.36	3.72	4.33	2.84	0.75	17.40
Hexa PCB #153	42.76	37.14	8.54	155.51	171.80	73.81	56.53	431.33
#141	< 0.81	-	<0.33	<5.12	0.27	0.32	0.07	1.78
#138	30.46	23.11	8.42	89.57	131.13	62.53	42.42	346.33
#128/162	0.65	0.44	0.27	2.42	4.08	7.55	0.77	56.87
#156	4.57	4.12	0.71	18.04	17.52	9.63	6.76	53.21
#157	0.61	0.39	0.34	1.89	2.31	1.13	0.66	5.03
Hepta PCB #178	2.23	2.85	0.06	11.57	9.07	4.95	3.04	32.37
#182/187	9.71	11.96	0.32	53.57	34.02	18.90	12.83	102.98
#183	3.90	3.74	0.34	17.71	13.29	7.35	3.33	33.54
#174	2.01	2.34	0.42	11.02	7.87	5.87	0.62	38.88
# 177	1.54	1.74	0.33	7.69	5.40	3.37	1.95	19.80
#172/192	2.57	3.45	0.05	15.72	7.73	5.55	2.69	29.41
#180/193	40.83	55.00	6.78	234.47	104.69	50.33	40.12	274.62
#170/190	16.72	21.10	2.82	94.69	46.48	24.03	20.54	139.88
#189	1.30	2.95	0.09	14.58	1.55	0.90	0.50	4.61
Octa PCB # 202	0.96	1.23	0.14	4.94	3.00	1.27	1.07	6.17
# 200	0.15	0.12	0.07	0.52	0.43	0.27	0.09	1.41
# 199	0.75	0.99	0.05	2.69	0.19	0.13	0.07	0.65
# 201	3.65	6.21	0.40	28.20	7.62	3.90	2.89	21.15
# 196/203	3.63	5.42	0.29	24.79	8.30	3.64	3.43	17.63
# 195	1.29	1.99	0.20	9.21	3.08	1.48	1.24	9.11
# 194	6.26	9.55	0.96	45.02	10.53	5.53	3.92	32.70
Nona PCB #208	0.87	1.95	0.02	9.90	3.34	2.02	0.80	8.76
#207	0.28	0.46	0.01	2.31	1.11	0.65	0.32	3.18
#206	1.02	1.22	0.09	4.60	2.35	1.42	0.67	7.41
Deca PCB #209	1.35	1.87	0.12	10.05	3.16	1.89	1.10	10.13
tot PCB 1	**199.46**	**197.35**	**42.91**	**862.27**	**659.68**	**291.94**	**246.10**	**1646.22**
tot PCB 2	**226.59**	**197.20**	**53.26**	**875.00**	**661.87**	**293.35**	**247.18**	**1651.20**

Table 1. Continued

HxCBz	31.95	26.98	10.53	115.97	42.45	42.90	10.06	321.33
p,p-DDE	**1735.58**	**2143.25**	**77.96**	**10032.69**	**499.62**	**397.69**	**43.41**	**1567.76**
Heptachlorepoxide	0.75	0.91	0.09	4.42	1.34	1.75	0.22	8.40
Trans-Chlordane	0.22	0.39	0.05	1.73	0.46	0.23	0.08	1.16
Oxychlordane	4.56	8.02	0.89	40.12	18.53	12.04	2.96	60.26
MC6	0.80	0.55	0.27	2.81	6.80	3.31	1.82	16.94
Trans-Nonachlordane	4.38	4.41	0.81	22.96	35.05	20.07	7.25	89.73
Cis-Nonachlordane	0.45	0.55	0.05	2.66	2.08	1.38	0.21	5.52
Tetra BDE # 47	**0.75**	**0.83**	**0.14**	**3.32**	**0.87**	**0.64**	**0.15**	**3.02**

In summary the levels of both PCBs and chlordane were higher in the Swedish samples. The levels of DDE were higher in the samples from Hungary. Levels of both PBDEs and HxCB were very similar.

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