ANALYSIS OF POLYCHLORINATED BIPHENYLs (PCBs) IN BREAST TISSUE

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KEYWORDS

PCB, human breast tissue, analytical method, isomer specific.

INTRODUCTION

The analyses of PCBs in human tissues started with Sören Jensen's discovery of PCBs in eagles and human hair, in 1966. Most of the analyses are reported on total PCB basis. Nowadays more and more PCB analyses are being done isomer specifically due to the large variation in toxicity among the different congeners. The method reported in this paper is developed for isomer specific identification and quantification of PCBs in human tissue.

This is a pilot study in an ongoing project concerning PCBs, PCDDs, PCDFs and other pesticides, such as DDT, DDE, hexachlorobenzene, and their possible relations to the incidence of breast cancer.

OBJECTIVES

* Develop a method for monitoring isomer specific levels of PCBs in human breast tissue.
* Establish isomer specific background levels of PCBs in Swedish women.
* Study the age-related trend of PCB levels in human breast tissue.

MATERIAL AND METHODS

The breast tissues are collected from women, seeking hospital care for breast tumours. Some tumours are malignant (cases) and some benign (controls). The breast tissues are kept frozen until analysis. We report on the analyses of 16 out of totally 90 coded samples.

The breast tissues were mixed with anhydrous Na_2SO_4 and homogenised. To extract the fat, methylene chloride(MC) / cyclohexane(CH) (1/1) was used. The samples were spiked with 50 µl ^{13}C-PCBs #52, #101, #105, #118, #138, #153, #180 (1 ng/µl) and then evaporated to determine the lipid content (0.4g-1.5g).

The first clean up was made on an silica column containing anhydrous Na_2SO_4 and neutral-silica. The sample was eluted with MC/CH (1/1) onto a PX-21 carbon column. After the carbon column a PCB-fraction was collected. This fraction contained the "bulk" PCBs (all except the non-ortho PCBs). The PCDDs, PCDFs and the non-ortho PCBs were retained on the carbon column and dealt with and reported separately. Method blanks, one per every seven samples, were prepared in the same way.[1,2]

The PCB-fraction was split, 1/10 of each sample was used for further clean up. This splitted fraction was transferred to a silica column containing; non-activated KOH-silica, neutral-silica, H_2SO_4-silica (40%), H_2SO_4-silica (20%) and on top anhydrous Na_2SO_4. The sample was eluted with n-hexane.[3,4]

The final clean up was made on a basic alumina open column where the sample was first eluted with n-hexane and then with MC/n-hexane (1/99). These two fractions were pooled[3,4]. High resolution gas chromatography (HRGC), Fisons GC 8000 equipped with a 60 meter DB-5 column, coupled to a low resolution mass spectrometer (LRMS), MD-800, operating in electron impact (EI) mode at a resolution of 1000 Daltons, was used for identification and quantification.

RESULTS AND DISCUSSION

The standard recoveries for the PCBs in the breast tissues were between 70%-100%, most of them above 85%. The detection limits (DL) were 13 pg - 60 pg, for trichlorinated- to decachlorinated biphenyls. In the method blanks the reported congeners were under the detection limits. This method facilitated the detection and quantification of 34 PCBs, including the not earlier in human tissue reported octachlorinated- to decachlorinated biphenyls.

Table 1 shows the analysed congeners and their levels in breast tissue, expressed in ppb (ng/g) on lipid basis, for 16 Swedish women. The last two columns give the mean value and range for each congener. For the total of 34 analysed PCB isomers the mean value is 1.29 μg/g lipid and the range is 0.84 μg/g lipid - 2.10 μg/g lipid. In the case of co-elution between two or more PCB isomers, we have in Table 1 excluded the isomers not earlier reported in humans. This is based on previous experience and publications.[4,5]

Figure 1 shows the chromatograms from two of the samples, one woman at the age of 45 (a) and one at the age of 74 (b). The profiles of the two samples are similar but their PCB congener levels are different. The levels for the accumulating isomers #138 and #153 are for example, 149 ppb and 205 ppb for the younger woman and for the older one they are 444 ppb and 506 ppb.

The 16 analyses in Table 1 clearly show a tendency for increasing PCB levels with age. After all 90 analyses, we expect to find a significant correlation and calculate the correlation coefficient for levels and age. A recent publication[6] on data from a population in Georgia, U.S.A (28 deceased males and females) likewise shows an age-related trend between age and levels of PCBs as well as a publication from 1991 on data from a Finnish population.[7]

Table 1. The isomer specific levels of PCBs, ppb (ng/g) on lipid basis, analysed in breast tissues of 16 Swedish women.

PCB	71 yrs	68 yrs	51 yrs	62 yrs	73 yrs	50 yrs	54 yrs	56 yrs	73 yrs
# 28	6.7	3.5	75[1]	3.0	4.8	5.8	6.3	3.9	8.7
# 47	0.7	0.8	1.7	0.5	0.7	0.8	0.4	1.8	1.1
# 52	1.2	1.5	2.3	0.9	1.1	1.0	0.9	0.8	1.1
# 66	3.8	2.8	5.4	1.9	2.3	4.6	2.9	2.4	8.7
# 74	30	14	32	28	16	19	22	16	27
# 99	32	24	24	29	18	23	14	16	31
# 101	2.0	3.1	1.7	1.2	1.9	4.0	1.6	1.2	4.7
# 105	17	11	7.0	10	6.9	12	6.2	7.9	18
# 110	0.5	<0.04	0.5	0.3	<0.01	2.3	0.4	0.2	<0.02
# 114	4.2	2.2	2.9	4.5	2.0	2.9	2.9	2.7	4.6
# 118	87	50	39	53	43	60	36	42	93
# 138	308	257	320	319	216	236	171	219	366
# 153	354	296	400	399	260	291	231	264	443
# 156	39	28	37	46	25	30	32	32	50
# 157	5.5	4.7	5.3	6.5	3.4	4.1	4.1	4.1	7.4
# 128,167	14	11	11	13	9.2	11	8.9	10	20
# 170	112	89	115	136	79	91	100	113	147
# 171	9.9	8.2	11	11	7.6	8.2	7.6	9.3	12
# 172	11	10	13	15	7.4	8.8	9.6	11	16
# 177	17	18	18	19	13	12	8.0	15	22
# 180	204	167	227	259	150	165	193	205	286
# 183	30	30	34	30	25	26	17	25	37
# 187	59	57	75	75	39	46	38	54	75
# 193	9.0	7.3	9.9	11	5.4	7.3	8.4	8.5	14
# 189	3.8	3.2	4.1	4.9	2.6	3.0	3.8	4.0	5.5
# 194	23	20	27	34	16	21	29	32	35
# 195	6.2	5.5	7.0	7.5	4.8	5.6	5.2	7.5	7.9
# 201	18	17	22	24	12	14	17	21	27
# 203,196	25	23	30	32	18	22	23	29	33
# 202	6.2	4.9	7.5	7.4	3.9	4.7	5.6	6.7	9.0
# 206	6.1	5.1	7.1	8.0	5.7	4.6	5.8	8.3	7.8
# 207	1.9	<0.1	2.4	2.4	1.5	1.6	1.3	2.0	2.3
# 208	4.0	3.4	4.8	4.6	3.4	2.9	3.5	4.8	4.6
# 209	6.6	5.2	8.7	10	6.7	5.3	7.0	8.8	11
Sum of PCBs	1460	1180	1590	1610	1010	1160	1020	1190	1840

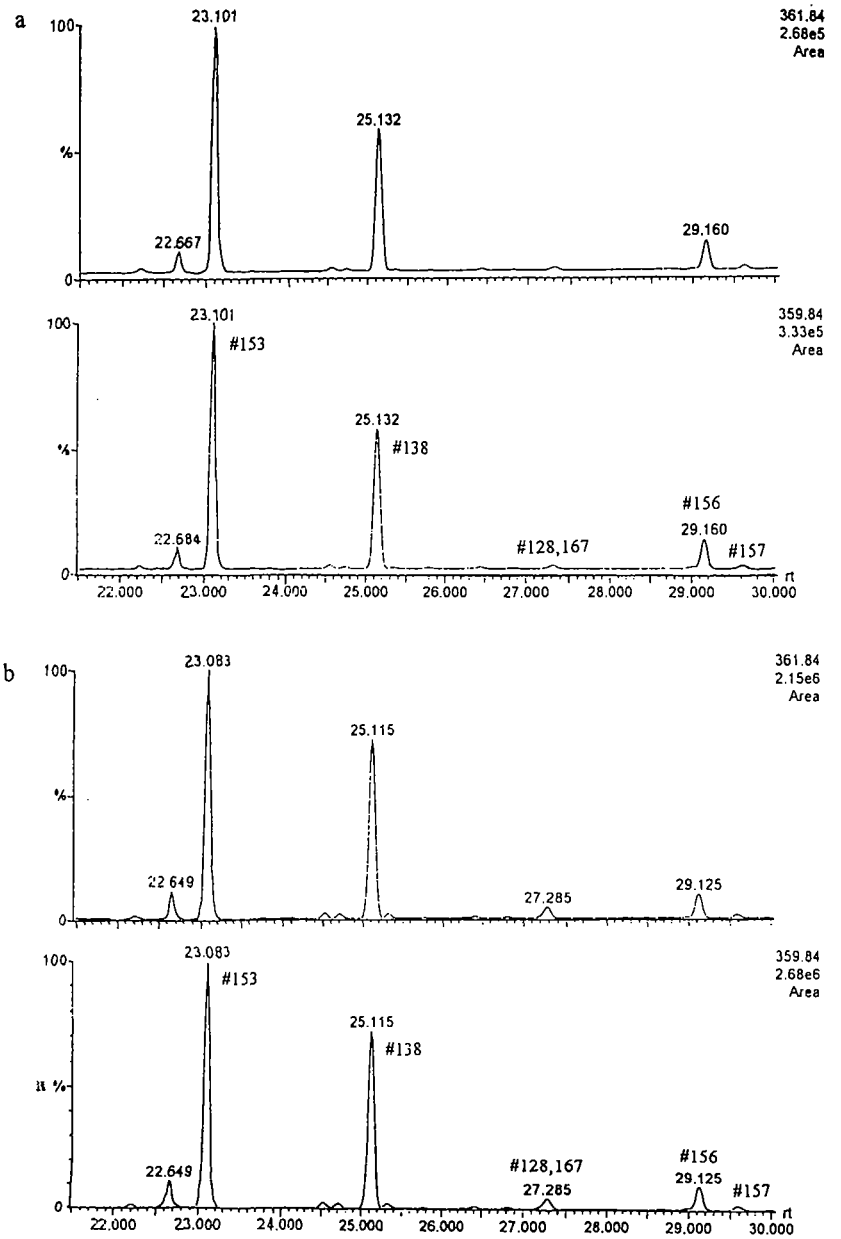
PCB	49 yrs	45 yrs	74 yrs	43 yrs	57 yrs	69 yrs	73 yrs	Mean	Range
# 28	5.5	<0.03	15	3.0	7.9	5.7	8.1	5.9	<0.03-15
# 47	0.8	<0.05	2.2	0.4	3.2	<0.01	0.7	1.0	<0.01-3.2
# 52	1.6	<0.05	1.1	1.1	0.6	<0.01	0.9	1.0	<0.01-2.3
# 66	2.4	<0.05	15	1.9	3.4	4.2	5.2	4.2	<0.05-15
# 74	8.9	4.3	34	17	11	21	18	20	4.3-34
# 99	12	9.1	50	17	21	14	28	23	9.1-14
# 101	<0.03[2]	<0.05	4.5	1.9	0.8	1.1	2.8	2.2	<0.05-4.7
# 105	<0.03[2]	1.4	34	6.5	9.6	11	13	11	1.4-34
# 110	<0.03	<0.05	1.1	<0.02	<0.01	<0.01	0.7	0.4	<0.01-2.3
# 114	1.2	<0.05	4.8	2.7	1.6	4.4	2.6	2.9	<0.05-4.8
# 118	29	7.5	132	44	44	55	58	55	7.5-132
# 138	181	149	444	240	234	228	239	258	149-444
# 153	219	205	506	302	256	287	268	311	205-506
# 156	20	25	48	31	24	45	24	34	20-50
# 157	2.8	3.2	7.3	4.1	3.3	5.6	3.5	4.7	2.8-7.4
# 128,167	7.6	3.4	24	10	12	14	10	12	3.4-24
# 170	68	86	143	99	88	145	73	105	68-147
# 171	7.2	7.2	13	9.0	8.8	10	7.1	9.2	7.1-13
# 172	7.1	8.3	16	9.7	9.2	16	8.2	11	7.1-16
# 177	12	7.5	26	14	15	13	16	15	7.5-26
# 180	134	181	276	187	156	286	143	201	134-286
# 183	25	16	48	26	34	19	27	28	16-48
# 187	41	38	97	47	50	65	51	57	38-97
# 193	7.0	9.5	15	7.8	6.7	14	8.0	9.3	5.4-15
# 189	2.6	3.6	5.0	3.5	2.3	6.3	2.4	3.8	2.3-6.3
# 194	16	22	31	21	19	47	17	26	16-47
# 195	4.7	4.4	8.6	5.6	6.2	7.1	4.7	6.2	4.4-8.6
# 201	12	16	26	14	15	31	14	19	12-31
# 203,196	20	19	36	21	23	33	19	25	18-36
# 202	4.2	5.3	8.7	4.5	4.3	9.7	4.5	6.1	3.9-9.7
# 206	5.3	2.8	7.7	4.1	4.7	10	4.6	6.1	2.8-10
# 207	<0.1	<0.2	2.9	1.2	1.6	1.1	1.9	1.5	<0.1-2.9
# 208	3.1	2.2	4.6	2.0	2.4	6.4	2.9	3.7	2.0-6.4
# 209	5.1	3.8	8.1	4.3	4.0	14	5.8	7.2	3.8-14
Sum of PCBs	866	841	2100	1160	1080	1430	1090	1290	841-2100

< = not detected.

[1]not included in the mean value, considered being an outlier.

[2]not included in the mean value, difficulties during the GC-MS run.

Figure 1. HRGC/LRMS chromatograms from a Fisons GC 8000 (DB-5), coupled to a MD 800 (EI, 1000 Da). The chromatograms show the ions of hexachlorobiphenyl, detected in two breast tissue samples. Trace a is from a 45-year old woman and b from a woman 74 years old. Their profiles are similar but the levels of their isomers are different. For example, PCBs #138 and #153 are for the younger one 149 ppb, 205 ppb and for the older one 444 ppb, 506 ppb. The levels of the rest of the isomers are reported in Table 1.



CONCLUSION

* The analytical method described in this short paper can with satisfaction be used to analyse PCBs (all but the non-ortho PCBs) isomer specifically in human tissue.

* Isomer specific background levels of PCBs have been established for 34 congeners, from trichlorinated- to decachlorinated biphenyls.

* The results from the 16 analyses show a tendency of increasing PCB levels with age.

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