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Relationship Between Age and Levels of Organochlorine Contaminants in Human Serum of a Belgian Population

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Polychlorinated biphenyls (PCBs) and organochlorine pesticides, such as hexachlorobenzene (HCB), 2,2-bis (4-chlorophenyl)-1,1,1-trichloroethane (DDT) and its metabolite 2,2-bis (4-chlorophenyl)-1,1-dichloroethylene (p,p'-DDE) are among the most prevalent environmental pollutants. Due to their lipophilicity and long elimination half-life, residues of these persistent organic pollutants (POPs) tend to bioaccumulate in the adipose tissues of biota and humans (De Voogt et al. 1990). It was shown that POPs are equally partitioned in the lipid compartment of different human tissues, such as liver, muscle, adipose tissue and blood (Mussalo-Rauhamaa 1991; Haddad et al. 2000). However, blood plasma or serum are the most convenient matrices for the monitoring of occupational (e.g. industrial cleaners) (Sinks et al. 1992), accidental (e.g. fires) (Papke et al. 1990; Dewailly et al. 1991) or background exposure (Glynn et al. 2000).

This paper presents background serum concentrations of organochlorine contaminants in a Belgian population. The serum samples were provided to the Toxicological Center (University of Antwerp) in relation to a fire at Brussels North Station (Belgium) in February 2001.

MATERIALS AND METHODS

Human serum samples (19 females and 113 males) were obtained from the Belgian Railway Company in February 2001 during the week following the incident. The age of subjects ranged from 20 to 53 years. Blood was collected in a vacuum system tube and centrifuged (15 min, 2000g) within 24 hours after collection. The serum was kept frozen at -20°C until analysis (March 2001).

Based on reported abundance in human serum, the following PCB congeners (IUPAC numbering) (Ballschmiter and Zell 1980) were targeted for analysis: 28, 52, 99, 101, 118, 138, 153, 170, 180 and 187. Additionally, we included HCB and p,p'-DDE, as the major organochlorine pesticides found in human serum. PCB 46 and PCB 143 were used as internal standards. Complete description of the analytical method and quality control has been reported previously (Covaci and Schepens 2001 a,b).

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Briefly, the available serum was spiked with internal standards (PCB 46 and 143). The serum was then mixed with a similar volume of formic acid and was equilibrated by ultrasonic treatment for 30 min. After sample loading on Empore™ disk cartridges (3M, USA), the disk cartridge was rinsed with 2 x 500 µl deionised water. The sorbent bed was dried thoroughly under a nitrogen stream and by centrifugation. The cartridge was then eluted with 2x 500 µl hexane and 500 µl of dichloromethane : hexane (1:1, *v/v*) mixture. The eluate from the C_{18} disk cartridge was applied to a cartridge filled with acid silica and the analytes were eluted with six ml hexane. The final eluate was concentrated under a gentle nitrogen stream to approximately 50 µl and transferred into an injection vial. Two µl of extract were injected into a GC-ECD system equipped with a 50 m x 0.25 mm x 0.10 µm, CP-Sil 5/C18 capillary column (Chrompack, The Netherlands). For confirmatory purposes, a HP 6890/5973 GC/MS equipped with a 50 m x 0.22 mm x 0.25 µm, HT-8 capillary column (SGE, Belgium). Three ions were monitored for each compound. Retention time, masses and relative abundance of the confirmation ions to the quantification ion were used as identification criteria. Internal quality control was ensured by regular analysis of an in-house control serum and of standard reference material (SRM 1589a). Recoveries of internal standards were monitored to ensure their maintenance at acceptable levels. The limit of quantification (LOQ) for the target analytes was set at 0.04 ng/ml.
For results below the LOQ, we computed values using the following calculations for each compound: [(1-p) x LOQ], where p = proportion of samples < LOQ. This provides a more realistic value than using LOQ, ½ LOQ, or zero.

Clinical parameters, such as triglycerides (TG), total cholesterol (TC), and high density lipoproteins (HDL) cholesterol were measured by standard enzymatic techniques (Vitros 950/950AT Operator's Guide 1998) by the Institute for Clinical Biology (Brussels, Belgium). White blood cell count, relative and absolute profiles of individual white blood cells (neutrophiles, eosinophiles, basophiles, lymphocytes and monocytes) and liver enzyme activity (e.g. aspartate amino transferase (AST), alanine amino transferase (ALT) and gamma glutamyl transferase (gamma-GT)) for each subject were also measured.

In order to compare the concentration of organochlorine contaminants in samples with different lipid content, concentration of total lipids per gram serum is necessary. Total lipid (TL) concentration calculated by summation of total cholesterol (TC), triglycerides (TG) and phospholipids (PL) gives a good estimation of total lipid content. However, PL determination is not routinely performed. Therefore, total serum lipids were estimated using the following formula (Philips et al. 1989): $TL = 2.27 \times TC + TG + 0.623$.

Pearson's correlation coefficients were calculated to display the correlation between different parameters (such as lipids, organochlorine concentrations and age). To compare the means of the relative PCB profile, a Student t-test was used.

RESULTS AND DISCUSSION

Most measurements of PCBs 28, 52 and 101 were below LOQ. HCB and PCBs 99, 118, 170 and 187 were sometimes found at concentrations below LOQ, while p,p'-DDE and PCB 138, 153 and 180 all had measurements above LOQ. Serum PCB concentrations were similar or lower than those found in other non-exposed populations (Table 1). The study of Pauwels et al. (2000), with a mean age comparable to the one in this study (32 yr) showed similar values, while another Belgian study (*Covaci; unpublished data*), with a higher mean age (58 yr), presented higher PCB concentrations. Compared with other countries (Sweden and USA), values observed in this study were lower. Moreover, a similar profile was observed (with PCB 138, 153 and 180 as the most important congeners).

Table 1. Comparison of PCB concentrations (ng/g lipid wt) in background exposed people from different countries

			PCB (ng/g lipid wt)							
Location	Mean Age	N	28	101	118	138	153	180	Sum PCBs	Reference
Belgium	40	132	n.d.	1	13	64	99	60	237	This study
Belgium	58	200	3.2	1.5	31	95	172	108	411	Covaci, unpub. data
Belgium	32	96	n.d.	n.d.	27	71	91	69	258	Pauwels et al. 2000
Sweden	63	7	3.8	3.6	37	134	269	207	654	Glynn et al. 2000
USA	46	100	<5	<5	94	280	340	200	920	DeVoto et al. 1997
Sweden	40	120	8	5	76	n.a.	580	400	n.a.	Asplund et al. 1994

n.d. = not detected; n.a. = not available

Organochlorine concentrations in human serum are age-dependent. Therefore, we divided all data into subgroups of 5-year intervals, obtaining 7 groups between 20 and 55 years. The mean for each subgroup, as well as the standard deviation, the median and the range of the values are presented in Table 2. This division into subgroups according to the age is necessary when profiling PCB background levels.

PCB 28 and 52 were only detected in 2 samples (or 1.5 %), which were in the higher age groups (45 – 49 years and 50 – 54 years). PCB 101 also had a very low frequency (10 samples out of 132 or 7.6 %). These low values can be explained by the rapid metabolism of these congeners in the human body (Alcock et al., 2000). Results of PCBs 28, 52 and 101 are not presented in Table 2. All other measured PCBs were detected in all age groups. Outliers were removed in the 20 – 24 yr age group, using Dixon's outlier test.

Pearson correlation coefficients have been calculated between all PCB concentrations and the age groups (Table 2). As expected from their bioaccumulation potential, high correlation between age and serum concentration of organochlorine contaminants were computed. Pearson's correlation coefficients ranged between 0.85 (for PCB 118) and 0.99 (for PCB 180) ($p<0.05$).

Table 2. PCB serum concentrations in relation to the age

Age Group (yr)	N		PCB 99 (ng/ml)	PCB 118 (ng/ml)	PCB 138 (ng/ml)	PCB 153 (ng/ml)	PCB 170 (ng/ml)	PCB 180 (ng/ml)	PCB 187 (ng/ml)
20-24	18	Mean (SD)	n.d.	0.05 (0.02)	0.20 (0.07)	0.25 (0.08)	0.06 (0.02)	0.17 (0.08)	0.05 (0.03)
		Median	n.d.	0.05	0.18	0.25	0.06	0.16	0.04
		Range	n.d. - 0.05	n.d.- 0.10	0.08 - 0.32	0.10 - 0.35	n.d. - 0.11	0.06 - 0.30	n.d.- 0.12
25-29	4	Mean (SD)	0.04 (0.02)	0.06 (0.03)	0.19 (0.09)	0.30 (0.14)	0.05 (0.01)	0.17 (0.06)	0.04 (0.01)
		Median	0.03	0.05	0.18	0.29	0.06	0.18	0.04
		Range	n.d. - 0.07	n.d. - 0.10	0.10 - 0.31	0.15 - 0.49	n.d. - 0.06	0.09 - 0.22	n.d. - 0.06
30-34	9	Mean (SD)	0.05 (0.03)	0.09 (0.09)	0.34 (0.15)	0.51 (0.21)	0.10 (0.05)	0.27 (0.12)	0.06 (0.03)
		Median	0.05	0.06	0.37	0.51	0.09	0.23	0.05
		Range	n.d. - 0.10	0.04 - 0.31	0.11 - 0.61	0.23 - 0.88	0.04 - 0.20	0.16 - 0.55	0.04 - 0.13
35-39	13	Mean (SD)	0.05 (0.01)	0.08 (0.03)	0.37 (0.14)	0.57 (0.20)	0.12 (0.06)	0.35 (0.15)	0.08 (0.02)
		Median	0.05	0.07	0.37	0.52	0.11	0.30	0.06
		Range	n.d. - 0.07	0.04 - 0.13	0.17 - 0.69	0.29 - 0.95	0.07 - 0.32	0.22 – 0.78	0.05 - 0.11
40-44	42	Mean (SD)	0.07 (0.03)	0.09 (0.05)	0.43 (0.18)	0.68 (0.23)	0.16 (0.07)	0.42 (0.16)	0.10 (0.04)
		Median	0.07	0.07	0.38	0.63	0.14	0.41	0.10
		Range	0.04 - 0.12	n.d. - 0.22	0.06 - 0.93	0.30 - 1.39	0.04 - 0.36	0.11 - 0.95	0.04 - 0.20
45-49	30	Mean (SD)	0.06 (0.03)	0.08 (0.04)	0.45 (0.15)	0.71 (0.21)	0.17 (0.06)	0.45 (0.14)	0.12 (0.04)
		Median	0.05	0.07	0.41	0.69	0.16	0.42	0.11
		Range	0.04 - 0.16	n.d. - 0.20	0.23 - 0.79	0.43 - 1.18	0.07 - 0.29	0.22 - 0.74	0.06 - 0.20
50-54	16	Mean (SD)	0.09 (0.05)	0.11 (0.07)	0.61 (0.22)	0.93 (0.30)	0.20 (0.07)	0.51 (0.15)	0.14 (0.06)
		Median	0.09	0.10	0.56	0.91	0.19	0.52	0.13
		Range	0.04 - 0.22	0.04 - 0.30	0.30 - 1.04	0.45 - 1.64	0.12 - 0.35	0.29 - 0.82	0.08 - 0.28
Correlation		Pearson coeff.	0.93*	0.85*	0.97*	0.98*	0.98*	0.99*	0.97*

n.d. = not detected ; * $p<0.05$

Table 2 continued. PCB serum concentrations in relation to the age

Age Group (yr)	N		Sum 7 Markers (ng/ml)	Sum all measured PCBs (ng/ml)	Sum 7 Markers (ng/g lipid wt)	Sum all measured PCBs (ng/g lipid wt)
24-25	18	Mean (SD)	0.65 (0.22)	0.78 (0.27)	123.0 (42.2)	145.9 (50.8)
		Median	0.64	0.76	130.8	161.7
		Range	0.27 - 0.93	0.33 - 1.16	43.3 - 188.1	53.2 - 229.3
25-29	4	Mean (SD)	0.75 (0.31)	0.85 (0.35)	145.8 (71.5)	164.1 (80.7)
		Median	0.72	0.81	133.9	151.2
		Range	0.41 - 1.17	0.46 - 1.31	72.8 - 242.7	82.3 - 271.8
30-34	9	Mean (SD)	1.25 (0.48)	1.43 (0.58)	188.1 (60.4)	213.2 (69.5)
		Median	1.22	1.43	153.5	176.0
		Range	0.60 - 1.92	0.66 - 2.25	123.2 - 266.7	141.8 - 311.4
35-39	13	Mean (SD)	1.42 (0.48)	1.63 (0.58)	229.9 (50.3)	262.9 (59.5)
		Median	1.43	1.57	225.6	255.5
		Range	0.78 - 2.58	0.90 - 3.10	142.5 - 300.4	158.3 - 349.1
40-44	42	Mean (SD)	1.67 (0.55)	1.94 (0.66)	257.8 (73.9)	299.0 (87.7)
		Median	1.58	1.81	251.4	295.9
		Range	0.70 - 2.36	0.75 - 3.96	120.4 - 415.8	129.3 - 472.9
45-49	30	Mean (SD)	1.77 (0.51)	2.05 (0.59)	278.1 (88.9)	322.7 (103.9)
		Median	1.70	1.99	252.0	294.9
		Range	1.01 - 2.97	1.14 - 3.39	141.5 - 512.4	158.7 - 613.9
50-54	16	Mean (SD)	2.21 (0.67)	2.61 (0.82)	305.4 (81.8)	359.5 (101.4)
		Median	2.21	2.50	292.0	362.9
		Range	1.09 - 3.60	1.35 - 4.17	161.2 - 471.3	199.8 - 586.6
Correlation		Pearson coeff.	0.99*	0.98*	0.99*	0.99*

n.d. = not detected; * $p<0.05$

For each age group, the difference between the mean and median of the same congener was below 18 % (Table 2). The maximal value was observed for the sum of all measured PCBs expressed as ng/g lipid wt in the age group of 30 – 34 years. This was due to the fact that this group was somewhat less homogenous and that the calculation was not corrected by the low number of samples (N = 9), which is rather low. The number of subjects varied throughout the different age groups, but this had no visible effect due to the absence of outlying values in the subgroups with low number of subjects.

Relative PCB profiles (calculated as the contribution of each congener to the sum of all PCB congeners) are shown in Figure 1. None of the measured PCB congeners was metabolized at a different rate with increase of age. If this was the case, percentages of the PCBs metabolized at a higher rate in the higher age groups would be relatively lower. Differences in the profiles were not statistically significant.

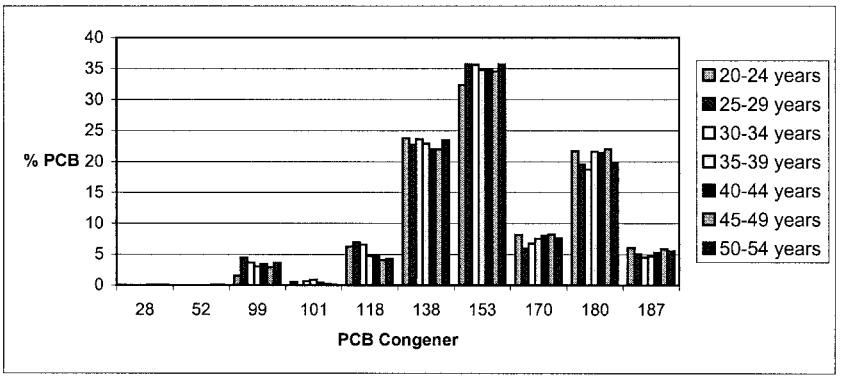

Figure 1: PCB profiles in human serum according to the age.

Table 3. Concentrations of organochlorine pesticides according to the age

Age group (yr)	N		p,p'-DDE (ng/ml)	HCB (ng/ml)	p,p'-DDE (ng/lipid wt)	HCB (ng/g lipid wt)
20-24	18	Mean (SD)	0.48 (0.25)	0.11 (0.05)	96.0 (56.4)	21.8 (9.1)
		Median	0.44	0.10	74.4	18.1
		Range	0.21 - 1.05	0.05 - 0.24	40.9 - 256.1	9.6 - 39.7
25-29	4	Mean (SD)	0.45 (0.25)	0.09 (0.01)	87.1 (55.0)	17.0 (2.3)
		Median	0.39	0.10	71.6	16.6
		Range	0.22 - 0.80	0.07 - 0.10	39.4 - 166.0	14.5 - 20.0
30-34	9	Mean (SD)	1.03 (0.88)	0.12 (0.03)	171.9 (135.2)	19.9 (4.4)
		Median	0.63	0.11	147.2	19.8
		Range	0.22 - 3.02	0.08 - 0.19	37.2 - 417.1	15.2 - 26.2
35-39	13	Mean (SD)	1.18 (0.72)	0.15 (0.05)	194.5 (121.5)	25.3 (10.7)
		Median	1.00	0.15	145.3	22.9
		Range	0.32 - 2.95	0.07 - 0.22	66.4 - 498.3	11.3 - 42.7
40-44	42	Mean (SD)	1.24 (0.61)	0.19 (0.11)	190.4 (88.8)	29.5 (18.2)
		Median	1.22	0.15	183.7	23.3
		Range	0.28 - 2.78	0.07 - 2.56	53.8 - 424.8	9.8 - 89.5
45-49	30	Mean (SD)	1.41 (0.97)	0.19 (0.13)	217.4 (146.4)	30.6 (20.4)
		Median	1.22	0.17	182.2	26.3
		Range	0.33 - 3.98	0.06 - 0.75	56.0 - 641.9	8.5 - 113.3
50-54	16	Mean (SD)	1.85 (1.12)	0.26 (0.15)	254.0 (140.5)	35.1 (19.0)
		Median	1.79	0.24	245.2	34.3
		Range	0.58 - 5.41	0.07 - 0.70	90.1 - 689.2	11.1 - 89.2
Correlation		Pearson coeff.	0.96*	0.94*	0.95*	0.91 (p = 0.064)

*p=0.05

Table 3 shows mean, standard deviation, median and range of measurements for HCB and p,p'-DDE for each age group. The values had a wider range than for PCBs. Serum levels of these pesticides were less predictable than PCB serum levels. This can be explained by differences in exposure (background vs. occupational) and half-life, which differed widely in the 3 groups of compounds discussed (PCBs, p,p'-DDE and HCB). High Pearson values ($p<0.05$) of 0.96 and 0.94 (0.95 and 0.91, respectively, when related to lipid wt) were calculated between age and p,p'-DDE and HCB, respectively. As for PCBs, this was expected because of their high bioaccumulative potential. Borderline significance was observed though, for HCB expressed per g lipid wt. The p value for this correlation was 0.064. Quantification of p,p'-DDE showed two subjects having very high serum levels of 5.48 ng/ml (or 1.07 µg/g lipid wt) and 22.64 ng/ml (or 4.07 µg/g lipid wt), respectively. Using Dixon's test, we excluded these values from the calculations.

Changes in clinical parameters, such as white blood cell count, blood profile (neutrophiles, eosinophiles, basophiles, lymphocytes, monocytes), or enzyme activity could not be demonstrated. Observed effects, which are not statistically significant, can come from ageing or from PCB exposure.

The obtained data provided us with a clear view on the background contamination with PCBs, p,p'-DDE and HCB of this selected Belgian population.

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