PERSISTENT ORGANOCHLORINE COMPUNDS IN HUMAN SERUM OF 50-65 YEARS OLD WOMEN LIVING IN TWO REGIONS OF FLANDERS, BELGIUM

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Introduction

In 1999, a campaign of the Flemish Ministry of Health, Belgium, was set up to assess regional differences in pollutant (heavy metals, polyaromatic hydrocarbons, volatile organics and organochlorines) concentrations and related health effect biomarkers in humans. The study was called the 'Flanders Environmental and Health Study' (FLEHS). Concentrations of selected organochlorine pesticides, polychlorinated biphenyls (PCB) and polychlorinated dibenzo-*p*-dioxins (PCDD) and furans (PCDF) were measured by GC-MS in 47 pooled human serum samples originating from 200 women between 50 and 65 years living in two areas of Flanders, Belgium. Primary goals were: (i) evaluation of mean concentrations and regional differences of these pollutants, (ii) evaluation of pooling procedures in order to reduce the number of samples to be analysed, but to keep the resulting analytical information at acceptable levels, (iii) calculation of correlations between groups of organochlorine and (iv) comparison of TEQ values obtained by CALUX®-bioassay and gas chromatographic analysis. This is the first study with such goals done in Belgium and was considered a necessity due to high concentrations of organochlorines observed in the Belgian population (1,2).

Material and Methods

Study area and population

The rural area of Peer is situated 15-25 km from non-ferrous and chemical plants and lies away from motorways. The suburbs of Antwerp are located 11-13 km SE from the chemical and petrochemical industry established in the harbour of Antwerp, but close to a non-ferrous smelter, two municipal waste incinerators, a crematory, printing works, several small enterprises and a major motorway. The study group consisted of 200 healthy 50-65 old women from Antwerp (n=100) and Peer (n=100) randomly recruited between June and September 1999. The following four criteria had to be fulfilled: non- or ex-smoker, minimal residence time of 10 years in the study area, working in the town of residence or at home and exclusion of jobs with specific risks of exposure. Dietary information was obtained by a semi-quantitative food frequency questionnaire on meat, fish, eggs, milk and cheese.

Sample collection and pooling procedure

Approximately 40 mL of blood was collected from each individual and serum was separated and divided into one part for individual analysis of indicator PCBs congeners (3 mL) and CALUX-TEQ (2.5 mL) and the rest for pooling. Pooling was done by ranking the women in the order of decreasing daily intake of meat and fish, decreasing daily intake of eggs and milk, increasing total number of weeks of breast feeding and increasing body mass index. The available serum of 3 to 5 subsequently listed individuals was pooled to approximately 50 mL. Each of the resulting 47 pooled samples was divided in three aliquots for the analysis of PCDD/Fs (25 mL), PCBs/organochlorine pesticides (13 mL) and CALUX-TEQ (4 mL). All samples were stored in glass vials pre-cleaned with hexane and acetone, and kept at -20°C until analysis.

Materials and methods

In 200 individual serum samples, the indicator PCBs and CALUX-TEQ values were determined. In 47 pooled serum samples, the following PCB congeners were measured: mono-ortho PCBs (PCB 105, 118, 156, 157, 167), indicator PCBs (28, 52, 101, 138, 153, 180) and PCB 44, 66, 74, 99, 110, 128, 149, 170, 183, 187, 194, 199. The pooled samples were also analysed for non-ortho PCBs (77, 81, 126, 169) and the 17 PCDD/PCDF toxic congeners. The analysis of mono-ortho, non-ortho PCBs and PCDD/F congeners allowed the calculation TEQ values for each sample (3). Hexachlorobenzene (HCB), p,p'-DDT, p,p'-DDE, γ-HCH and pentachlorophenol (PCP) were also measured in the pooled samples as major organochlorine pesticides found in human serum. Serum concentrations of triglycerides, cholesterol and phospholipids were determined enzymatically. For all compounds, measurements under the detection limit were set at half of this detection limit. The chemical analysis methods for indicator PCBs (4), ortho-PCBs and pesticides (5), non-ortho PCBs, PCDD/Fs (1) have been previously described in detail. CALUX-activity was measured using a Chemical-Activated Luciferase gene eXpression (CALUX®) bioassay (BioDetection Systems BV, The Netherlands) variant of a previously described procedure (6).

Results and discussion

Concentrations and regional differences

The concentration of the 7 indicator PCBs was significantly higher in the urban region (392.0 ng/g fat versus 337.4 ng/g fat) (Table 1). While, concentrations of PCB 118 and 156 were significantly higher in the urban region, concentrations of PCB 170, 180, 187, 194 and 199 were not significantly different between the two regions. The total PCB concentration (sum of 27 congeners) was significantly higher in Antwerp (600.8 versus 498.6 ng/g fat, $p<0.005$). There was no statistical difference in the individual PCDD/F concentrations in the two regions. Non- and mono-ortho PCB TEQ values were significantly higher in the urban area (Table 1), while the PCDD/F TEQ and the total WHO-TEQ (sum of PCDDs, PCDFs, non-ortho and mono-ortho PCBs) were not statistically different for both regions. PCDDs and PCDFs contributed almost equally to the PCDD/F WHO-TEQ (average of 53% and 47%, respectively). PCDD/PCDFs contribution to the total WHO-TEQ value was on average 67%. The non- and mono-ortho PCBs contributed with 16 and 17 %, respectively to the total WHO-TEQ. The principal contributors to the total WHO-TEQ value were 2,3,4,7,8-P_5CDF (for PCDFs), 1,2,3,7,8-P_5CDD (for PCDDs), and PCB 126 (for non-ortho PCBs), PCB 156 and 118 (for mono-ortho PCBs). Concentrations of PCP, p,p'-DDE and γ-HCH were higher (but not significantly) in Peer, while the concentration of p,p'-DDT was significantly higher in that region (3.6 versus 2.0 ng/g fat, $p=0.003$). The hexachlorobenzene concentration was significantly higher in Antwerp (125.2 versus 95.6 ng/g fat, $p=0.001$).

Comparison with other countries

Mean concentrations and profiles of PCBs of all individual and pooled serum samples were comparable with recent individual results obtained from Sweden (7) and the Netherlands (8). A Canadian study (9) conducted in 1994 on 63 blood donors (33 females, 30 males) with mean age of 45 years showed approximately 3-fold lower levels of organochlorines than the present study. The burden of indicator PCBs was even 1.5-2 fold lower than presently found in the young Flemish women (10). This confirms that the PCB concentrations in Belgium and Europe remain a matter of concern.

The PCDD/F-TEQ values in the serum of the Flemish women (mean 49 pg WHO-TEQ/g fat) were higher than values found in other countries. The data should be interpreted with caution due to differences in: sampling years, age of individuals and TEF values used in the past. However, present levels in this Belgian age group can be compared with values obtained in other industrialised countries about 10 years ago. Moreover, a similar German population (43-71 years old) sampled in 1996 showed average values two times lower than PCDD/PCDF TEQ values measured in the Flemish population. As in the past, the PCDD/PCDF body burden values in Belgium remains higher than in our neighbouring countries (11).

Table 1. TEQ values for dioxin-like compounds and concentrations of PCBs in 47 pooled samples from women living in two regions of Flanders.

	Peer (N=22)	Antwerp (N=25)	Peer + Antwerp (N=47)
CALUX-TEQ (pg TEQ/g fat)	37.2 (13.1)	35.0 (16.5)	36.0 (14.9)
TEQ (pg TEQ/g fat)			
Mono-ortho PCB	11.6 (10.7-12.5)	14.2 (13.0-15.6)**	12.9 (12.1-13.8)
Non-ortho PCB	10.8 (9.3-12.4)	14.5 (12.4-16.9)**	12.6 (11.3-14.1)
PCB total	22.5 (20.3-24.9)	29.1 (26.1-32.4)**	25.8 (23.8-28.0)
PCDD	24.8 (22.5-27.2)	26.4 (24.0-29.1)	25.6 (24.0-27.4)
PCDF	23.2 (20.2-26.6)	23.1 (20.8-25.7)	23.2 (21.4-25.1)
PCDD+PCDF	47.9 (43.6-52.7)	49.2 (45.0-53.9)	48.6 (45.6-51.8)
Total	70.9 (65.3-76.9)	78.9 (72.7-85.6)	75.0 (70.8-79.5)
PCB (ng/g fat)			
PCB total	498.6 (460.6-539-7)	600.8 (544-663.6)**	550.6 (514.4-589.3)
Indicator PCB	337.4 (311.8-365.1)	392.0 (355.2-432.7)*	365.4 (342.2-390.2)

Mean (SD) or geometric mean (95% confidence interval) are given.

* and **: significant different than the other region with $p<0.05$ and $p<0.01$ respectively.

CALUX®

Another approach for assessing the total TEQ burden is the measurement of Ah-receptor activity in serum by CALUX®-bioassay. CALUX-TEQ values reflect the toxicity of all POPs having a synergistic, additive and/or antagonistic interaction with the Ah-receptor. Therefore, the CALUX-TEQ values differ from the chemically estimated TEQs, which are simply summated. In the present study the absolute value of the CALUX-TEQ was lower than the PCDD/F-TEQ, and about half of the total WHO-TEQ. No significant regional difference was observed in dioxin-like toxicity of the pooled serum samples (Table 1). The observed CALUX-TEQ was comparable to the CALUX-TEQ of the young Flemish women (12) and considerably lower than the CALUX-TEQ values (mean of 103.7 pg TEQ/g fat) measured in plasma of young Dutch women in 1990-1992 (13). It was clear that this assay might offer new possibilities in monitoring TEQ-values in human serum, but further interlaboratory validation is necessary.

Correlations between groups of organochlorines

The total WHO-TEQ value was in good correlation with the values of the individual contributors: mono-ortho PCBs (r=0.77), non-ortho PCBs (r=0.65) and PCDD/Fs (r=0.88) considering all serum pools in both regions. TEQ values from non-ortho PCBs were poorly correlated (r=0.23) with PCDD/F-TEQs. A better correlation was found with mono-ortho PCBs (r=0.65). TEQ values from mono-ortho PCBs also showed a higher correlation with PCDD/F (r=0.49).

Indicator PCB and CALUX-TEQ measurements could be two ways of estimating the total TEQ without need of large volumes of serum. Their correlation coefficients with the total TEQs were r= 0.70 and 0.57 respectively. Both increased to 0.77 and 0.73 when considering only the women in the urban region. The latter correlation layed in the range of what was found by comparing GC/MS determined total WHO-TEQs (including PCBs) and CALUX-TEQ in: human serum, r=0. 71 (14) and cow's milk, r=0.74 (15). Using the equation obtained in the regression analysis, the concentrations of total TEQ could be estimated from the concentrations of CALUX-TEQ or indicator PCBs. The difference between both concentrations was less than 20% in 77% or 85 % of the samples based on CALUX or indicator PCB measurements.

When correlation coefficients were higher than 0.70, linear regression was performed between the concentration of the potential marker substance and groups of polychlorinated aromatic hydrocarbons. The estimated concentration was calculated using the concentration of single

marker substances: PCB 153 (for indicator and total PCBs), PCB 118 (total PCB TEQ), PCB156 (mono-ortho PCBs and total TEQs), 12378-P_5CDD (PCDD/PCDF-TEQ). All of them were good markers with more than 50% of the pooled serum samples having a difference lower than 15% between observed and estimated concentrations of the respective compound groups concentrations. It would be therefore possible to 'predict' TEQ values (within a certain interval of confidence) for a background-exposed population by measuring only indicator compounds (9, 16).

Pooled samples versus individual samples

Serum concentrations of indicator PCBs and CALUX-TEQ values were measured both in each of the 200 women individual and in the 47 serum pools. As for the pooled samples, individual concentrations of the indicator PCBs were significantly higher in the urban area (423.6 versus 362.8 ng/g fat, p=0.002), when adjusted for age, animal fat and dairy consumption, and fasting status. For CALUX-TEQ there was no regional difference observed for the pooled samples. However, the individual CALUX-TEQs values were significantly higher in Peer (43.3 pg TEQ/g fat) compared to Antwerp (33.2 pg TEQ/g fat) (p=0.03). This was not due to adjustment with the confounder (numbers of weeks of lactation) in these individuals. Pooling of serum samples simply reduced sample size and power of finding differences in CALUX-TEQs. Therefore, pooling should be avoided if the purpose of the study is to compare pollutant concentrations between different areas. On the other hand, if the mean concentration of POPs in human serum of a population is of interest, the pooling procedure offers a good and cheaper alternative.

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