COMPARISON OF CALUX-TEQ VALUES OF PCB'S AND DIOXINS IN HUMAN SERUM

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Introduction

The Calux bioassay can be used for biomonitoring purposes. Human serum samples have been analyzed and TEQ values were obtained for the dioxins and furans (PCDD/Fs) only[1,2] or for the combined PCCD/F and dioxin-like PCB fraction[3,4]. However it is also technically possible to analyze the dioxin-like PCBs separately. The greatest problems with the Calux PCB analysis are an insufficient extraction of the mono-ortho PCBs (about 70% recovery; data not published) and the low TEF values of the coplanar PCBs. The WHO-TEF values for PCB 126 and 118, the two most common coplanar PCBs in human serum, are respectively 0.1 and 0.00003[5]. A possible solution for this lower sensitivity of the Ah-Receptor for the coplanar PCBs is working with a linear calibration curve in the low concentration range[6].

Within the Human Biomonitoring Program 2002-2006, organized by The Centre for Environment and Health in Flanders (the Northern part of Belgium), the Calux assay was used for the analysis of PCDD/Fs and PCBs in adult's serum samples. The analyses were performed at the Free University of Brussels (VUB) (i.e. fat extraction and clean-up) and involved collaboration with the Scientific Institute of Public health (IPH) with respect to the Calux cell line. This paper presents a comparison of Calux-TEQ values of the dioxin-like PCB fraction and the PCCD/F fraction in human serum.

Materials and Methods

Study area and population

A part of the human biomonitoring program involved the recruitment of 1600 adults equally spread over nine areas in Flanders. The nine different study areas were selected on the basis of a characteristic, relevant and differing type of pollution pressure: the Antwerp agglomeration, the Ghent agglomeration, the industrial harbours of Antwerp (mainly petrochemical industry) and Ghent (mainly metallurgy), the industrial zone around the Albert canal (chemical industry), the industrial zone of Olen (nonferrous industry), the immediate surroundings of household waste incinerators, a rural area with intensive fruit cultivation, and a rural area devoid of highways and of important local industrial emissions and with a population density of less than 250 inhabitants per km². The total surface of the studied area is 3,036 km², corresponding to 22% of the total surface of Flanders (13,521 km²). Except for 'rural Flanders' and for 'waste incinerators', all study areas are contiguous geographical entities. From September 2004 till June 2005, individuals were selected taking into account the following inclusion criteria: being between 50 and 65 years old, living for at least five years in the same area, giving informed consent and being able to fill out Dutch questionnaires. Recruitment of this population group took place via home addressed letters and the sampling was carried out at specified dates in the municipalities. The mean percentage of participation was 27.4% and the recruitment resulted in a total of 1583 adults. PCDD/F results were determined for all the serum samples; dioxin-like PCB results were obtained for only 669 randomly selected serum samples by the Calux assay.

Calux analysis

The method for the analysis of the dioxin-like PCBs and the PCDD/Fs was previously described by Schroijen et al[2]. Briefly, the serum sample (5g) was mixed with acetone to destruct the proteins and extracted with hexane on a pre-conditioned celite column. The total extract was concentrated under a flow of pure air and the serum fat fraction was quantified. The fat extract was further purified on an acidified silica column and the dioxin-like compounds were isolated by passing the eluate over a carbon column (Xenobiotic Detection Systems Inc., USA). After washing the carbon column with hexane and hexane/acetone (9:1) the PCB fraction containing the dioxin-like PCBs and the dioxin fraction containing the polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) were separately eluted with a mixture of hexane/ethylacetate/toluene (8:1:1) and with toluene, respectively. Both fractions were concentrated to dryness in a vacuum centrifuge and resuspended in a know volume of hexane.

The Calux bioassay was performed using the mouse hepatoma H1L6.1 cell line (Xenobiotic Detection Systems Inc., USA). Details of the in vitro assay were previously described by Van Wouwe et al[1] and Schroijen et al[7]. Briefly, a calibration curve of 2,3,7,8-TCDD and sample extracts were transferred in DMSO and placed on a 96-well culture plate previously seeded with the cells. After 20-24 h of incubation, the luceferase activity was measured using a luminometer and the results, expressed in relative light units (RLU), were transformed into TEQ values. For the determination of the dioxin responses the Hill equation was used on the sigmoid calibration curve (LOQ = 10.9 pg TEQ / g fat)[2]; while for the determination of the dioxin-like PCBs a linear calibration curve in the lower concentration range was preferred (LOD = 3.6 pg TEQ / g fat)[6].

Statistical data treatment

Database management and statistical analysis were performed using SigmaStat 3.0. Data below the detection limit were set equal to half of the detection limit. Calux results in serum were transformed to their natural logarithm since the data were not normally distributed[8].The fact that the data are log-normal distributed is in agreement the distribution of environmental concentrations in general[9].

Spearman rank correlations were calculated between Calux PCBs and Calux PCDD/Fs in the whole study population and in each of the study areas. Raw data are reported as boxplots with median values, 25^{th}-75^{th} percentiles, outliers and extremes.

Differences in biomarkers of exposure between the nine areas were initially investigated using analysis of variance (ANOVA) on the ln-transformed data and were presented here on the original scale by the mean and standard deviation interval. A total exposure value for Flanders was determined by weighing the responses to inhabitant distribution. In this way, the importance of each study area was proportional to the number of total inhabitants in that area. The total exposure value can serve as a tool to describe the background exposure of the Flemish population and to identify areas in which the inhabitants have significantly different internal exposure. The mean value of each study area was compared with the total weighted mean and a statistical significance level of 5% was used.

Some variables can have an influence on the response. The significant relation ($p \leq 0.05$) of age, sex, smoking, BMI, education and income with the data was verified. Linear regression and ANOVA were applied to verify correlations and differences between respectively internal exposure levels of PCDD/Fs or dioxin-like PCBs and these variables.

Results and Discussion

Data treatment was performed with the dioxin-like PCB results and their corresponding PCDD/F results.

Spearman correlation

A significant correlation was observed between the ln Calux PCB results and the ln Calux PCDD/F results ($r=0.121$, $p=0.031$, $n=599$).

Comparison of the data within the individual areas showed only a significant correlation between the Calux PCDD/F results and the Calux PCB results in the rural area of Flanders ($r=0.271$, $p=0.017$, $n=77$). From these

correlation results can be concluded that on a toxicity base, the internal dose of PCDD/Fs in the human body of an individual is not or very weak correlated with the internal dose of dioxin-like PCBs.

Differences between the 9 areas

Significant differences between the nine areas were seen for the Calux PCDD/F results (p =0.004), but not for the Calux dioxin-like PCB results (p= 0.122) (Figure 1).

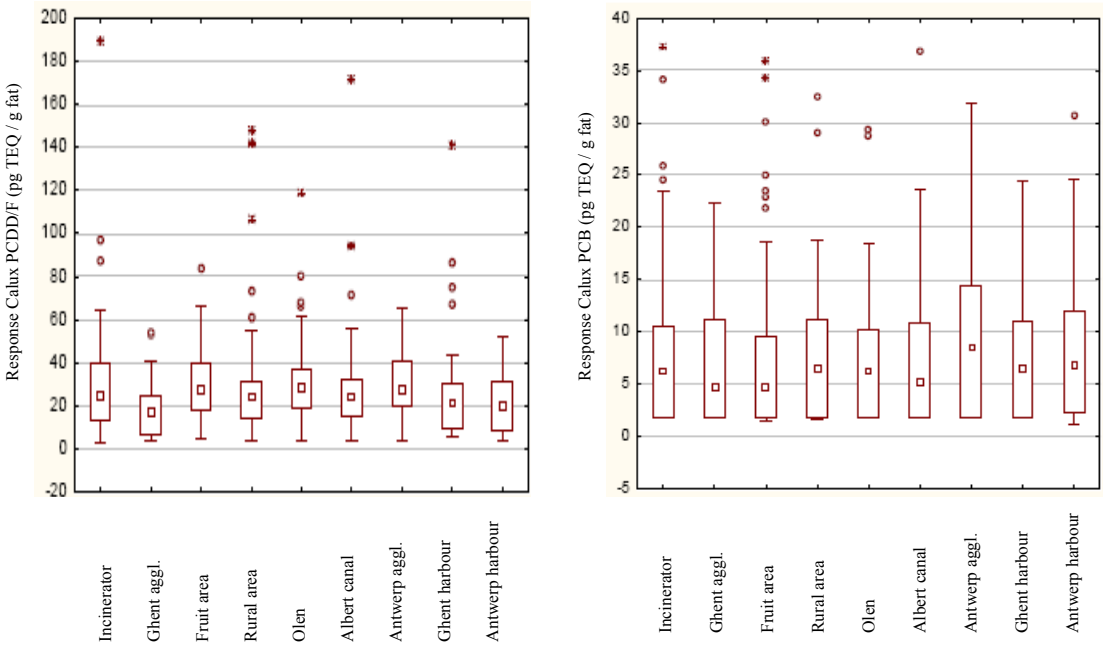

Figure 1: Boxplots of the Calux PCDD/F (left) and Calux PCB (right) results (pg TEQ / g fat) in the different areas

For the Calux PCDD/F fraction, the mean raw data results are significantly higher in the agglomeration of Antwerp, the fruit area and in Olen, and are significantly lower in the agglomeration of Ghent compared to the total weighted mean which can serve as a reference for the Flemish population. Although there were no significant differences between the different areas for the Calux dioxin-like PCBs, the highest mean TEQ value was found in the Antwerp agglomeration (nearly significant with p=0.053) and the lowest mean TEQ value was found in the agglomeration of Ghent, which is in agreement with the data observed for Calux PCDD/F results in these areas (Table 1).

Influence of variables

Both the ln Calux PCB results and the ln Calux PCDD/F results were not significantly influenced by the following variables: sex, age, smoking class (non-, ex- or current smoker), body mass index (BMI), education and income.

	Calux PCDD/F			Calux PCB		
Area	**Mean (SD interval)**	**p-value[A]**	**% < LOQ**	**Mean (SD interval)**	**p-value[A]**	**% < LOD**
Antwerp agglomeration	24.14 (11.88-49.06)	0.006	13.9	6.47 (2.36-17.73)	0.053	32.4
Antwerp harbour	17.01 (7.90-36.63)	0.450	27.1	5.27 (2.08-13.33)	0.909	38.2
Ghent agglomeration	13.85 (6.63-28.91)	0.002	34.2	4.67 (1.95-11.22)	0.312	40.2
Ghent harbour	20.17 (8.53-47.70)	0.572	27.6	4.87 (2.04-11.60)	0.703	38.7
Fruit area	23.45 (11.51-47.80)	0.011	15.4	4.70 (1.73-12.73)	0.319	46.9
Rural area	20.31 (9.01-45.79)	0.337	19.5	5.47 (2.33-12.86)	0.618	31.0
Olen	24.02 (11.37-50.75)	0.015	13.8	5.59 (2.15-14.51)	0.547	36.1
Albert canal zone	20.57 (10.00-42.31)	0.252	15.9	4.84 (1.92-12.23)	0.501	40.9
Incinerator	20.66 (8.47-50.40)	0.247	22.0	5.53 (2.29-13.37)	0.541	29.9
Total	**18.56 (8.62-39.99)[B]**		**20.4**	**5.19 (2.07-13.01)[B]**		**37.2**

Table 1: Mean (SD interval) of Calux PCDD/F and Calux PCB results (pg TEQ / g fat).
[A] Difference between mean values when compared with the total value of all areas weighted to population density. [B] Value weighted to population density. Areas with mean values significant different from the total mean are indicated in colour: light grey when significant lower and dark grey when significant higher.

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