CONTRIBUTION OF HOST FACTORS AND ENVIRONMENTAL FACTORS TO CALUX- TEQ VALUES IN SERUM OF 50-65 YEARS OLD WOMEN

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Introduction

In 1999, the 'Flanders Environmental and Health Study' (FLEHS) was set up by the Flemish Ministry of Health, Belgium to measure a series of exposure and health effect biomarkers (1). Part of the study was the analysis of dioxin-like compounds in serum (CALUX-TEQ) in 200 adult women of 50-65 years old (2,3,4). Based on questionnaire data it was attempted to determine individual host factors and environmental factors as potential determinants of CALUX-TEQs in serum.

Material and Methods

Study population

The study group consisted of 200 healthy 50-65 old women living in eather suburbs of the port of Antwerp (n=100) and in the rural area of Peer (n=100). They were 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. The use of alcohol, intake of medicines, social class was asked in questionnaires. Dietary information was obtained by a semi-quantitative food frequency questionnaire on meat, fish, eggs, milk and cheese intake during the year preceding the study. From these results, the food frequencies per month were calculated. The amount of fat intake per day was computed from the recorded food frequencies using mean consumption portions and the Dutch food composition table (5). Body-mass index (BMI) was calculated by dividing body weight (kg), by body height in m^2. Body-fat content was calculated according to the formula of Deurenberg, viz. % body-fat = 1.20 x BMI + 0.23 x age - 10.8 x gender - 5.4, in which gender is 0 for females (6). Table 1 gives an overview of the study population characteristics.

Table 1. Description of studied population

	Rural area of Peer n=100	Suburbs of city Antwerp n=100	p*
Host factors			
Age (years)	59.0	58.0	NS

Weight (kg)	67	66	NS
Body mass index (kg/m^2)	26.4	26.2	NS
Serum fat (mg/dL)	692	705	NS
Total serum cholesterol	225	216	NS
Serum triglycerides	140	124	0.03
Body fat content (%)	40	39	NS
Environmental factors and lifestyle			
Number of years living in the region	41	35	NS
Number of years working	32	8	<0.001
Passive smoking (hours/day)	0.0	0.0	0.04
Dairy consumption (frequency/day)**	0.45	0.43	NS
Consumption of animal fat***(score, g/day)	0.53	0.53	NS
Consumption of local food (%)	72	28	<0.001
Total number of breastfeeding weeks	10	0	<0.001
Number of children	3.0	2.0	<0.001

NS – not significant for p>0.05
* Significant difference (p<0.05) between the 2 regions determined with the Mann-Whitney U-test or c^2-test for 2x2 tables.
** Average for milk, cheese and eggs.
*** Fat from fish, shrimps, mussels, meat, cheese, milk, eggs, based on the average daily consumption and average fat content of each nutrition group.

CALUX® bioassay

Approximately 5 mL of blood was collected for CALUX-TEQ analysis. Serum was spinned off and stored in glass vials pre-cleaned with hexane and acetone, and kept at -20 °C until analysis.

In the 200 individual serum samples, CALUX-activity was measured using a Chemical-Activated Luciferase gene eXpression (CALUXÒ) bioassay (BioDetection Systems BV, The Netherlands) variant of a previously described procedure (7) . Briefly, the method involved n-hexane extraction of the sample and removal of acid labile matrix components, fat and non-stable PCAHs by passage through a silica column containing concentrated H2SO4 (33 %, w/v). The extract was then quantitatively transferred to a conical vial for evaporation till almost dry, followed by adding 7.5 mL dimethylsulfoxide and dilution to a total volume of 750 mL with minimal essential medium. The CALUXâ assay uses rat hepatoma H4IIE cell line which is transfected with an AhR-controlled luciferase reporter gene construct. Cells were grown in 96-well plates in minimal essential medium with 10 % fetal calf serum at 37 °C and 5 % CO_2. When the cell layer reached 70 to 80 % confluency, the cells were dosed with 100 ml sample extract in triplicate together with TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) standards and then incubated at 37 °C for 24h. After removal of the medium, cells were washed with phosphate-buffered saline without Ca^{2+}/Mg^{2+} and 30 mL of a cell-lysis reagent was added. The well plates were shaken for 45 min and stored at -80°C for 1 h at least. The luciferase activity was determined after the cells in the well plates were thawed on ice and 100 µL luciferin assay mix was added at room temperature. The light production was measured by a luminometer. On each 96-well plate, standards of TCDD from 0.3 to 100 pmol/L were added to construct a TCDD-based calibration curve which is then used to quantify the TEQ content of the individual serum samples measured. Results were expressed in pg CALUX-TEQs per g serum fat. All 200 serum sample extracts were run in one experiment to avoid inter-experiment variation in cell

culture. The limit of detection was calculated as the light signal measured from the dimethylsulfoxide-control plus 3 times its standard deviation on each well plate, viz. 10±3 pg CALUX-TEQ /g serum fat.

Statistical Methods

Determants of the serum concentration of the dioxin-like compounds were identified by single regression. Subsequently multiple regression was used with the p-value set at 0.10 for the independent variables to stay in the model.

Results and discussion

Concentrations and regional differences

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. The observed median CALUX-TEQ of 42 pg TEQ/g fat (interquartile range: 19-63) was comparable to the CALUX-TEQ of young Flemish women (8) measured in 1998 and considerably higher than the CALUX-TEQ values in plasma of young Flemish adolescents measured in 1999 as part of the FLEHS study (median 32 pg TEQ/g fat) (9). For the women group of 50-65 years old, CALUX-TEQ values were significantly higher in the rural area Peer - namely 44 (31-65) pg CALUX-TEQ/g fat - compared to Antwerp, with a median concentration of 32 (12-60) pg TEQ/g fat. (p=0.03). If samples of the same women were pooled, the absolute values were comparable, but the regional differences dissapeared (3).

Host factors and environmental factors as potential determinants of CALUX-TEQs in serum

In the studied women group host factors (e.g. age, body-fat, blood fat) as well as environmental factors (e.g. life-style, dietary pattern, and geographical location) were analysed for contribution to the concentration of CALUX-TEQs in serum. Factors with p-value lower than 0.10 were: age and living in rural area Peer compared to the city of Antwerp (table 2). None of all other individual characteristics, dietary habits or other life style factors, were significant covariates. This means living area is an important covariate to be taken in to account. In another part of the FLEHS study, CALUX-TEQs were analysed in 200 17-18 years old adolescents. There, the serum levels showed to be negatively correlated with triceps skinfold (9). This negative correlation was explained as a dilution of dioxin-like compounds in the body fat during adolescence growth. Body fat was however no determinant fo the CALUX-TEQ in the 50-65 years old women, which might indicate loss of the dillution effect of the body fat.

Table 2. Covariates of the serum CALUX-TEQ level in single and multiple regression analysis (n=200)

Independent variables	Standardised Estimate (95% confidence interval)	Partial R^2	p
Single regression			
Environmental factor			
Residence in rural region of Peer[a]	0.22 (0.08-0.35)	0.22	0.002
Host factor			
Age	0.14 (0.004-0.28)	0.14	0.05

Multiple regression			
Residence in rural region of Peer[a]	0.21 (0.07-0.34)	0.21	0.003
Age	0.13 (-0.01-0.26)	0.13	0.07

[a]compared with living in suburbs of the port of Antwerp.

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