

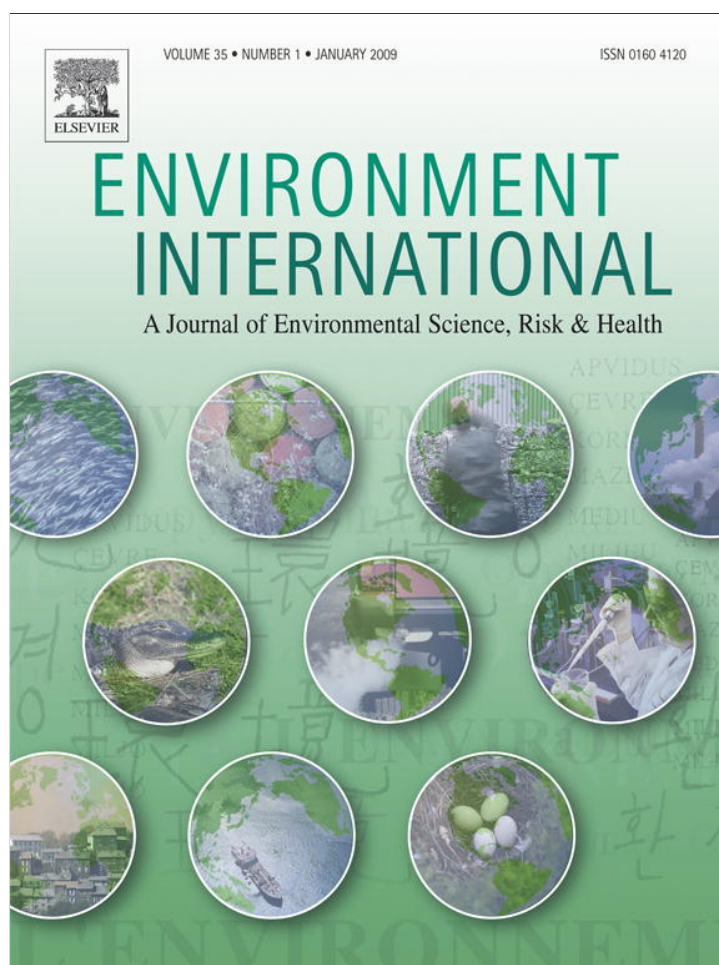
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## The relation between the estimated dietary intake of PCDD/Fs and levels in blood in a Flemish population (50–65 years)

Maaïke Bilau<sup>a,\*</sup>, Stefaan De Henauw<sup>a,b</sup>, Carmen Schroyen<sup>c</sup>, Liesbeth Bruckers<sup>d</sup>, Elly Den Hond<sup>e</sup>, Gudrun Koppen<sup>e</sup>, Christophe Matthys<sup>a,f</sup>, Els Van De Mieroop<sup>g</sup>, Hans Keune<sup>h</sup>, Willy Baeyens<sup>c</sup>, Vera Nelen<sup>g</sup>, Nik Van Larebeke<sup>i</sup>, Jan L. Willems<sup>a</sup>, Greet Schoeters<sup>e,j</sup>

<sup>a</sup> Department of Public Health, Ghent University, UZ 2 Blok A, De Pintelaan 185, B-9000 Ghent, Belgium

<sup>b</sup> Department of Health Sciences, Vesalius, Hogeschool Gent, Keramiekstraat 80, B-9000 Ghent, Belgium

<sup>c</sup> Department of Analytical and Environmental Chemistry, Vrije Universiteit Brussel (VUB), Pleinlaan 2, B-1050 Brussels, Belgium

<sup>d</sup> Center for Statistics, University Hasselt, Agoralaan 1, Building D, B-3590 Diepenbeek, Belgium

<sup>e</sup> Flemish Institute for Technological Research (VITO), Environmental Toxicology, Boeretang 200, B-2400 Mol, Belgium

<sup>f</sup> Institute of Food, Nutrition and Human Health, Massey University, Private Bag 102 904, North Shore Mail Centre, Auckland, New Zealand

<sup>g</sup> Provincial Institute of Hygiene (PIH) Kronenburgstraat 40, B-2000 Antwerp, Belgium

<sup>h</sup> Faculty of Political and Social Sciences, University of Antwerp, Sint Jacobstraat 2, B-2000 Antwerp, Belgium

<sup>i</sup> Study Centre of Carcinogenesis and Primary Prevention of Cancer, Ghent University, UZ 3K3, De Pintelaan 185, B-9000 Ghent, Belgium

<sup>j</sup> University of Antwerp, Universiteitsplein 1, B-2610, Antwerp, Belgium

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### ABSTRACT

Dioxin-like activity was measured in the serum of 1425 Flemish men and women via the CALUX assay. The adults, aged between 50 and 65 years, participated in a large biomonitoring program, executed by the Flemish Center of Expertise for Environment and Health between 2002 and 2006. Within the context of this biomonitoring program also dietary intake of dioxin-like contaminants was assessed through a food frequency questionnaire.

The relation between the estimated dietary intake and the dioxin-like activity in serum was evaluated using multivariate analyses: a logistic model was performed on the total population, while a linear regression analysis was done on the subsample with quantifiable dioxin activity levels in serum. Region, gender, age, BMI, smoking status, as well as dietary habits were entered in the model, with dioxin level as an outcome estimate.

Both the logistic and linear model confirmed the contribution of dietary intake to the dioxin activity measured in serum. Also BMI and region were found to be associated with dioxin activity levels.

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### 1. Introduction

Flanders, the Dutch-speaking, northern half of Belgium, is one of the most densely populated regions in Europe, with a dense traffic network and industrial activities close to habitation. In order to study the influence of environmental factors on certain health outcomes, a large biomonitoring study was executed by the Flemish Center of Expertise for Environment and Health of the Flemish Community, monitoring several biomarkers of exposure and effect. All public information on the project is available online ([www.milieu-en-gezondheid.be](http://www.milieu-en-gezondheid.be)).

One of the biomarkers under study was dioxin activity in serum, measured by the chemical-activated luciferase gene expression (CALUX) assay. The CALUX bioassay uses genetically modified mammalian hepatoma cells that contain a transfected Arylhydrocar-

bon Receptor (AhR) responsive luciferase reporter gene which responds to dioxin-like compounds with the induction of luciferase gene expression in a time, dose and chemical specific manner. The serum extract was pretreated to contain only the PCDD/F fraction and no longer the PCB fraction (Van Wouwe et al., 2004). Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) (further referred to as: dioxins) are a group of lipophilic contaminants, generated unintentionally as by-products from human activities, such as various industrial chemical reactions and combustion processes, including waste incineration. They recycle between air, water, soil, sediments and waste (Fries, 1995). Dioxins in air bind to small size particles and are deposited on fields and plants and are resistant to environmental and biological degradation. They are taken up by animals, concentrate in the lipid fraction of biota and are biomagnified in the food chain. Organisms at higher trophic levels in the food chain contain higher concentrations of lipophilic contaminants. As a consequence, human adipose tissue, serum and breast milk show relatively high levels of dioxins (Safe, 2000). Because of their persistence, resistance to

\* Corresponding author. Tel.: +32 9 332 24 23; fax: +32 9 332 49 94.

E-mail addresses: [maaike.bilau@ugent.be](mailto:maaike.bilau@ugent.be), [maaikebilau@yahoo.com](mailto:maaikebilau@yahoo.com) (M. Bilau).

degradation and fat solubility, levels of dioxins in the lipid component of body tissues and fluids are a good indicator of cumulative exposure (Arfi et al., 2001).

Increased levels of dioxin-like contaminants are associated with immune deficiency, dermal toxicity, reproductive effects and carcinogenicity as shown in animal and epidemiological studies on accidentally or occupationally exposed cohorts (WHO-ECEH-IPCS, 2000). Dioxin-like compounds have also endocrine disrupting properties. There is a lot of public concern on the health effects of the current environmental levels of dioxin-like compounds.

It is known that dietary intake is the most important source of human exposure: at least 90% of total exposure to dioxin-like contaminants can be attributed to dietary intake of food items of animal origin (Fries, 1995; Liem et al., 2000; Parzefall, 2002). Within the context of this biomonitoring program, a dietary intake assessment of animal fat has been evaluated, which allowed us to estimate the intake of dioxin-like contaminants through food (Bilau et al., 2008). Since the diet is known to be the most important source of dioxin-like substances in the human body and knowing that executing such a large biomonitoring program is an expensive and time-consuming task, this biomonitoring project is a unique opportunity to compare the estimated dioxin intake with measured levels of these contaminants in serum of adult men and women, in order to study the potential value of dietary assessment in predicting dioxin concentrations in the human body.

## 2. Materials and methods

### 2.1. Study population

Within the framework of the Flemish Center for Environment and Health, a biomonitoring program ran from 2002 till 2006 in eight geographical areas with different types of pollution pressure: two urban areas (city of Ghent and city of Antwerp), four areas with different types of industry (harbour, non-ferrous smelter, chemical industry, waste incinerator), a fruit growing area and a rural area. Three age groups were studied: adolescents (14–15 years), mothers and their newborn child, and adults (50–65 years). Only umbilical cord blood was analysed in the subpopulation of the mothers, and no dioxin-like activity was measured in the serum of the adolescents. Therefore, only data on the adult population will be presented.

Between September 2004 and June 2005, adults between 50 and 65 years old were sampled in municipalities, selected at random within the different study areas. The selected municipalities provided addresses of individuals who fell in the correct age range. Individuals were selected via stratified sampling in 3 age groups (50–54, 55–59 and 60–65). On the basis of a short questionnaire, it could be determined whether people fulfilled the inclusion criteria. Partners who met the inclusion criteria, could also participate in the study. People who did not meet the inclusion criteria, were randomly replaced by individuals from the same age group and the same sex.

The inclusion criteria for participants were the following: (1) they had to live for at least 5 years in the respective area, (2) they had to be between 50 and 65 years old at the time of the study, and (3) they had to be able to complete questionnaires in Dutch. All participants signed an informed consent.

**Table 1**  
Characteristics of the study population (group A)

	Total (N=1425)	Male (N=694)	Female (N=731)	<i>p</i> <sup>b</sup>
	Median (IQR) <sup>a</sup>	Median (IQR)	Median (IQR)	
Age (years)	57.7 (54.0–60.9)	58.5 (54.6–61.3)	57.0 (53.6–60.5)	<0.001
BMI (kg/m <sup>2</sup> )	26.4 (24.1–29.2)	26.9 (24.7–29.4)	25.7 (23.2–29.0)	<0.001
– BMI < 25	36%	28%	43%	
– 25 < BMI < 30	44%	51%	37%	
– 30 < BMI	20%	20%	20%	
Estimated intake (pg CALUX TEQ/d)	146 (106–199)	162 (120–226)	131 (96–179)	<0.001
Estimated intake (pg CALUX TEQ/kg bw/d)	1.96 (1.43–2.70)	2.01 (1.46–2.79)	1.93 (1.39–2.61)	0.042
PCDD/F level (pg CALUX TEQ/g serum)	0.12 (0.08–0.17)	0.12 (0.08–0.17)	0.12 (0.08–0.17)	0.592
PCDD/F level (pg CALUX TEQ/g serum fat)	23.0 (12.0–32.7)	22.4 (11.8–32.1)	23.4 (12.3–33.5)	0.399
Smoking habits				<0.001
– Current smokers	18%	21%	14%	
– Former smokers	37%	48%	25%	
– Never smokers	45%	29%	61%	

<sup>a</sup> IQR interquartile range.

<sup>b</sup> Differences between sexes (Mann–Whitney *U* test).

The participants provided a non-fasting blood and urine sample in which several biomarkers have been measured. They also completed an extensive questionnaire on dietary habits, residence history, education, occupation, lifestyle factors (smoking, hobbies, use of pesticides, ...) and risk perception. Body mass index (BMI) was calculated based on height and bodyweight, measured according to a standardized protocol (WHO, 1995).

In total 1582 adult men and women between 50 and 65 years old were included in the biomonitoring study. For our substudy, 157 subjects had to be excluded because no CALUX measurement has been performed. In total, 1425 subjects (48.7% males) were included in the study presented in this paper.

The biomonitoring study was approved by the Ethical Committee of the University of Antwerp, Belgium.

### 2.2. Dietary exposure assessment

A semi-quantitative Food Frequency Questionnaire (FFQ) was used to estimate the daily intake of lipophilic contaminants (in this case PCDD/Fs) via the consumption of (animal) fat containing food items. Food groups such as meat and meat products, fish and seafood, eggs and dairy products were extensively questioned, as well as added fats (e.g. baking and frying fat). This FFQ is described in detail elsewhere (Bilau et al., 2008).

Contamination data were provided by the Belgian Federal Agency for the Safety of the Food Chain ([www.favv.be](http://www.favv.be)). Food items were sampled between 2003 and 2006 and PCDD/Fs levels were determined using the chemical-activated luciferase gene expression (CALUX) assay. The methodology used is described elsewhere (Vanderperren et al., 2004). In order to perform the intake assessment, individual food items (1260 samples) were grouped into 21 food groups, based on fat content and fat origin. These groups were also chosen in view of the feasibility of combining consumption with contamination data. For each group, a mean PCDD/F concentration was calculated. Non-detects were assumed to be half of the limit of quantification (Bilau et al., 2008).

A simple distribution technique was used to estimate the dietary intake of PCDD/Fs, combining the observed distribution of individual food consumption data (food item level) with a point estimate for contaminant concentration (food group level) (Lambe, 2002).

### 2.3. Determination of PCDD/F in serum levels

Concentrations of dioxins and furans (PCDD/Fs) were determined by the CALUX *in vitro* bioassay in a non-fasting serum sample (5 mL). The methodology used is described in detail elsewhere (Schroijen et al., 2006; Van Wouwe et al., 2004). Summarily, since PCDD/Fs and PCBs are lipophilic, fat content of serum samples was extracted, using acetone, hexane and a celite column. After evaporation of the eluate, the amount of serum fat was gravimetrically determined. In a second step, other Arylhydrocarbon agonists were eliminated by using well specified mixtures of solvents (hexane, toluene, acetone and ethylacetate) and an acid silica column combined with an activated carbon column. In addition, PCDD/Fs were separated from PCBs: only the PCDD/F fractions were analysed in all individual samples via CALUX.

The PCDD/F concentrations were expressed per mL and per g serum fat. The CALUX analysis was performed with the pGudLuc 6.1 cell line supplied by Xenobiotic Detection Systems Inc (USA).

If dioxin concentrations were too low to be quantified, the samples were recoded to half of the limit of quantification (LOQ) in the data analysis (i.e. 0.03 pg CALUX TEQ/g serum).

This method facilitates the assessment of public health risks due to its high throughput rate and lower cost for the CALUX assay in Belgium compared to chemical analysis such as gas chromatography/mass spectrometry (GC/MS).

### 2.4. Statistical analysis

About 23% of samples were below the LOQ for CALUX measured in serum. They were considered as half the LOQ, i.e. 0.03 pg CALUX TEQ/g serum. Since such a large part

**Table 2**

Spearman Rank Correlations (*p*-values) between estimated dietary intake of PCDD/Fs (pg CALUX TEQ/d) for different food groups and CALUX levels in serum (pg CALUX TEQ/g serum) for group B

PCDD/F intake via	Total group (N=1092)	Change in dietary habits (N=395)	No change in dietary habits (N=677)
fish and seafood	0.072 (0.018)	0.038 (0.457)	0.115 (0.003)
dairy products	0.035 (0.252)	−0.027 (0.600)	0.071 (0.065)
meat and meat products	0.075 (0.014)	−0.012 (0.814)	0.124 (0.001)
eggs	0.056 (0.062)	0.039 (0.444)	0.072 (0.060)
added fats	0.007 (0.809)	−0.057 (0.260)	0.061 (0.115)
total diet	0.086 (0.004)	0.026 (0.612)	0.145 (<0.001)

of the CALUX levels were below the LOQ, different analyses were performed on two groups of the population: 1) group A, the whole study population (*N*=1425) and 2) group B, a subsample of the study population (*N*=1092) namely the subjects with quantifiable levels of PCDD/Fs in their serum. Normality was tested for both groups (Kolmogorov–Smirnov) and descriptive statistics were analysed for the whole study population (median; interquartile range).

#### 2.4.1. Univariate analysis

Non-parametric tests were used to study the relation between the estimated dietary intake of PCDD/Fs and measured dioxin activity levels (CALUX) in serum of group A. Mann–Withney *U* test was used to test for differences between both sexes, while Kruskal–Wallis (KW) test was used to test for differences between normal, overweight and obese individuals.

Spearman rank correlation was also performed on group B comparing correlations between estimated dietary intake and measured CALUX levels for a group of participants that have declared that their dietary habits have, versus have not, changed in the past 5 years.

#### 2.4.2. Multivariate analysis

Since PCDD/F serum levels in adult men and women are the result of a continuous accumulation of these substances throughout the entire life, several influencing factors have to be taken into account in a multivariate model.

In a first model, the 75th percentile (P75) of CALUX in serum was used to divide the whole study population (*N*=1425) in a highly exposed group (above P75) and a lower exposed group (below P75). A logistic regression was used to investigate the association of sex, age, region, dietary intake, BMI and smoking status with PCDD/F serum levels above P75 as the dependent variable, computing the odds ratios (ORs) as measures of association. This analysis was also performed for two subgroups: in order to compare the results of participants who have declared that their dietary habits have, versus have not, changed in the past 5 years.

In a second model, linear regression analysis was done on a subsample of the population (*N*=1092) with quantifiable dioxin levels in order to investigate the possibility that measured CALUX levels in serum could be predicted by dietary habits, sex, age, region, BMI and smoking status.

**Table 3**

Odds ratio (OR) and 95% confidence interval (CI) as a result of multivariate logistic regression analysis with dioxin levels (pg CALUX TEQ/g serum) above P75 as the response variable (group A; *N*=1425)

	OR	95% CI	<i>p</i> -value
Quartiles of dietary intake (pg PCDD/Fs TEQ/d)			0.020
Q1	0.619	0.431–0.890	0.010
Q2	0.606	0.424–0.865	0.006
Q3	0.778	0.554–1.095	0.150
Q4	1		
Region			<0.001
City of Ghent	0.396	0.256–0.612	<0.001
City of Antwerp	1.585	1.175–2.138	0.003
Harbour	0.918	0.654–1.289	0.622
Non-ferrous smelter (Olen)	0.961	0.693–1.334	0.814
Chemical industry (Albert Canal area)	0.744	0.523–1.059	0.101
Waste incinerator	1.352	0.996–1.835	0.053
Fruit growing area	1.372	0.985–1.912	0.061
Rural area	1.307	0.959–1.783	0.091
Sex (female as reference)	0.891	0.680–1.169	0.405
Age	0.960	0.969–1.030	0.999
BMI	1.035	1.004–1.066	0.025
Smoking status			0.542
Current smoker	0.851	0.594–1.221	0.381
Former smoker	0.868	0.650–1.160	0.339
Never smoker	1		
Constant	0.081		0.011

Data were analysed using the SPSS® software package (version 15.0). The level of statistical significance was set at *p*≤0.05.

### 3. Results

#### 3.1. Description of the population

Main characteristics of the study population (*N*=1425), measured PCDD/F serum concentrations and estimated PCDD/F intakes, are presented in Table 1. Regarding BMI, 511 participants (36%) have a normal weight, 626 participants (44%) are overweight (25<BMI<30) and 287 (20%) are obese.

#### 3.2. Univariate analysis

In group A (i.e. the whole study population) no differences were seen in CALUX levels between men and women (Mann–Whitney *U*). However, CALUX levels (pg TEQ/g serum) were significantly different between normal (BMI<25), overweight (25<BMI<30) and obese participants (30<BMI) (KW; *p*=0.036). The median CALUX levels were respectively 0.113, 0.122 and 0.122 pg CALUX TEQ/g serum. Also the estimated intake (pg TEQ/bw kg/d as well as pg TEQ/d) was different for normal, overweight and obese participants (KW; *p*<0.001). Their median estimated intakes were respectively 2.13, 1.96 and 1.70 pg CALUX TEQ/kg bw/d and 136, 151 and 152 pg CALUX TEQ/d.

To compare the estimated dietary intake of PCDD/Fs with CALUX levels in serum, Spearman Rank Correlation was determined (*r*=0.086, *p*=0.004) in group B, the group of participants with quantifiable levels of PCDD/Fs in their serum. Around 40% of that group stated that their dietary habits had been changed in the last 5 years. Spearman Rank Correlations were statistically different for people with or without changed dietary habits (see Table 2).

#### 3.3. Multivariate analysis

In a first stage, a logistic model was performed with CALUX levels in serum above the P75 as a response variable and dietary intake (quartile 1, 2 and 3 vs the highest quartile), region (average CALUX level as a reference), sex (female as a reference), smoking status (never smoker as a reference), age (continuous variable) and BMI (continuous variable) as predictive variables (group A, *N*=1425). Results for this model are presented in Table 3.

Odds ratios (OR) were statistically significant for dietary intake, region and BMI. The OR for having CALUX levels above the P75 was significantly lower for participants in the first and second quartile of estimated dietary intake. The city of Ghent had a 60% lower chance for having CALUX levels above the P75 than other regions, whereas the city of Antwerp had a significantly higher chance to have CALUX levels above the P75. There was also a significant increase of 3.5% per unit increase of BMI to have CALUX levels above the P75. No statistical significant relation was found with age, sex or smoking status.

Similar results were found when analysis was performed on the smaller subgroup of participants who stated that their dietary habits have not been changed in the past 5 years (OR (95% CI) 0.506 (0.317–0.806) for first quartile of dietary intake). However, ORs were not statistically significant for dietary intake for participants stating that they have changed their dietary habits.

**Table 4**

Multivariate linear regression: associations between dioxin CALUX levels in serum and selected explanatory variables among adults with quantifiable CALUX activity (group B)

	Unstandardized beta	Standardized beta	<i>p</i> -value
Dietary intake (pg PCDD/Fs TEQ/d)			
Intake via fish/seafood	0.00011	0.064	0.039
Intake via dairy	0.00004	0.013	0.678
Intake via meat/meat products	0.00044	0.068	0.039
Intake via egg	0.00030	0.019	0.532
Intake via added fats <sup>a</sup>	−0.00007	−0.027	0.431
Region <sup>b</sup>			
City of Antwerp	0.03013	0.134	0.002
Harbour	0.03501	0.144	0.001
Non-ferrous smelter (Olen)	0.02433	0.105	0.015
Chemical industry (Albert Canal area)	0.01114	0.049	0.261
Waste incinerators	0.03339	0.147	0.001
Fruit growing area	0.02739	0.112	0.008
Rural area	0.04466	0.194	<0.001
Sex <sup>b</sup>	0.00822	0.053	0.116
Age	0.00002	0.001	0.977
BMI	0.00139	0.073	0.018
Smoking status <sup>b</sup>	0.00124	0.008	0.804
Constant			0.100

<sup>a</sup> Added fats = spreads, baking and frying fats.

<sup>b</sup> City of Ghent (region), female (sex) and never smoking (smoking status) are references in the model.

In a second phase, linear regression was performed on the subjects with quantifiable levels of dioxin activity in their serum only (group B;  $N=1092$ ) with CALUX levels (pg TEQ/g serum) as the outcome variable. The  $R$  square of this model was 0.047. Coefficients and significance level of the introduced parameters are presented in Table 4.

#### 4. Discussion

It is widely accepted that the diet is responsible for more than 90% of the exposure to dioxin-like contaminants in the general population (Fries 1995; Liem et al., 2000; Pöpke, 1998; Parzefall 2002). Therefore, a dietary intake assessment is often performed as an exposure and risk estimate for the general population regarding this type of contaminants (Bilau et al., 2008; Bocio and Domingo, 2005; Charnley and Doull, 2005; Darnerud et al., 2006; Fattore et al., 2006; Kiviranta et al., 2004; Sasamoto et al., 2006; Taioli et al., 2005; Vrijens et al., 2002).

Measurement of the concentration of dioxin-like contaminants in adipose tissue, human breast milk or serum constitutes another exposure and surrogate risk estimate. The association however, between both exposure estimates, e.g. the serum concentrations, and the estimated dietary intake was mainly studied in specific subgroups of the population, such as recreational fishermen (Chen et al., 2003; Lee et al., 2006b; Turyk et al., 2005). Similar analyses in the general population are less often reported (Arfi et al., 2001; Hauser et al., 2005; Lee et al., 2007). Although not the primary target of the biomonitoring study, we found it of interest to study this association in our study population. The original aim of assessing the dietary exposure to dioxin-like contaminants, was to use the results of this intake estimation as an adjusting factor in the relations under study in the biomonitoring program.

##### 4.1. PCDD/F levels measured in serum

The reported median level of dioxin CALUX activity in serum was 23 pg CALUX TEQ/g serum lipid. In a previous Flanders Environmental and Health Study, PCDD/Fs were determined in serum fat of 200 women between 50 and 65 years using GC-HRMS (Koppen et al., 2002). The results reported were higher (48 pg TEQ/g serum fat) but a direct comparison remains difficult as the analytical methodology was different – a chemical analysis versus the CALUX test. According to Van Wouwe et al. (2004) and based on 341 plasma samples, a significant correlation was established between the bioassay and chemical method ( $R=0.64$ ). However, a proportional systematic error (CALUX was higher than GC-HRMS) was observed when the results obtained with the CALUX bioassay were regressed with the results from the GC-HRMS analyses. The limit of quantification (LOQ) used to calculate TEQ values from the GC-HRMS determinations, the use of the relative potency values instead of the toxic equivalent factor and the potential of CALUX bioassay to measure all compounds with affinity for the AhR may partly explain this proportional systematic error. Nevertheless, the present results suggest that the CALUX bioassay could be a promising valid screening method for human blood plasma analyses.

Overall, the levels reported in this paper, were equal to or lower than results found in other countries (Arisawa et al., 2005; Chen et al., 2007; Lee et al., 2006a). However, in countries where a younger population were studied, levels were somewhat lower (Cerna et al., 2007), which is easily explained by differences in age.

##### 4.2. Factors influencing PCDD/F levels

The present analysis confirmed that dietary intake contributes to the dioxin CALUX activity of serum. Logistic as well as linear regression models showed a statistically significant relation with dietary exposure. In the linear regression model, it was shown that the intake of meat and meat products on the one hand and fish and seafood on the other hand, have the largest influence within the diet.

Both food groups were expected to have an important contribution because of the large amounts consumed (meat/meat products) and the relatively high contaminant levels (fish/seafood) (Bilau et al., 2008; Vrijens et al., 2002).

The region in which people have been living for at least 5 years also is an explanatory factor. Participants living in the city of Ghent seem to have the lowest CALUX levels in serum, while the participants living in the city of Antwerp seem to have higher CALUX levels. An explanation for this difference is, however not obvious. Also the harbour and rural area have significantly higher CALUX levels. The region around waste incinerators as well as the fruit growing area showed a higher CALUX level, although not statistically significant. There might be some differences in the prevalence and/or amount of consumption of locally grown products (home grown vegetables, but also home produced eggs). The influence of the consumption of locally produced foods on serum levels of lipophilic contaminants, was also reported in other studies (Donato et al., 2006). However, the questionnaire did not provide enough detailed information to study this relationship adequately. It would be interesting to include in more detail the consumption of locally grown products in future questionnaires.

For individuals with a higher BMI, higher levels of dioxin activity in blood (pg CALUX TEQ/g serum) were found. However, this could not be explained by differences in dietary intake per kg bodyweight. The relation between BMI, dietary intake and CALUX levels in serum is a complex relation: people with a higher BMI are likely to have a higher dietary fat intake and thus a higher intake of fat-soluble contaminants. However, because of the higher amount of body fat expected, contaminants can be distributed over a larger volume of adipose tissue.

For bioaccumulative contaminants, such as PCDD/Fs, age is an important predictor of body burden. However, the age range in our study population was rather narrow (50–65 years). Moreover, body burden of all participants was assumed to have already reached the steady state level. Therefore, a relation between age and the serum concentration was not expected in this study population.

It was reported that smoking influences the metabolism of lipophilic contaminants. In our study, the chance to have a CALUX level above the P75 for current smokers was not significantly different, but chances for current and former smokers to have CALUX levels above the P75 tend to be smaller.

##### 4.3. Methodological considerations

###### 4.3.1. Calux measurement in serum

Although measuring PCDD/F levels in adipose tissue is known to be the best parameter for body burden, it was impossible to use this invasive measurement in a large biomonitoring study (Allam and Lucena, 2001). Analysing dioxin levels in serum by measuring CALUX activity is a reasonable proxy for estimating the PCDD/F body burden, certainly if no other media (such as adipose tissue) are present (Whitcomb et al., 2005).

The CALUX bioassay measures an activity level rather than an exact concentration of congeners, although with the clean-up procedure used in this study, a strong correlation between CALUX and gas chromatography-high resolution mass spectrometry (GC-HRMS) results was observed (Van Wouwe et al., 2004). Moreover, there might be some influence of short term dietary intake, since participants did not have to be fasting at the moment the blood sample was taken. Consequently it was possible that the levels in serum were not always in equilibrium with the levels in adipose tissue at the moment of sampling.

###### 4.3.2. Dietary intake assessment

On the other hand, the intake assessment is also prone to measurement error. It is, above all, an almost impossible task, to assess the life-long dietary habits of individuals at an age of 50 to

65 years old. As a proxy for life-long dietary intake, participants were asked to report dietary habits of the year before the study. Although one year is only a relatively short period when compared to the age reached at the moment of blood sampling, this is a long period when one considers the fact that the FFQ depends completely on the memory of participants.

Also, certain changes in dietary habits could have occurred throughout their life, resulting in incorrect estimation of life-long dietary intake. This was confirmed: almost 30% of the study population stated that their dietary habits have changed within the last 5 years. Of those 395 participants, 60% stated that their consumption of fish and seafood has increased over the last 5 years, while 81% stated that their consumption of meat and meat products has decreased over the last 5 years. The majority of the participants with changed dietary habits (54%) stated that their egg consumption decreased as well. However, in order to study this in detail, more information would be needed. Therefore, statistical analyses were done on the group who stated not to have changed their diet: the associations found were stronger, despite the smaller population under study. This uncertainty on dietary exposure may, in part, explain the relatively low  $R^2$  values in the regression models.

#### 4.3.3. Contaminant data

Also the contaminant levels in food items were affected by uncertainties, due to the sampling strategy, the methodology used and the evolution in time. For all participants the same contaminant levels were used, although it was possible that some participants consumed on average food items with higher contaminant levels than did others (e.g. participants consuming more or more often locally grown eggs).

## 5. Conclusion

Total dietary exposure (predominantly exposure via meat, meat products, fish and seafood), BMI and region were found to be associated with concentrations of PCDD/F, measured by CALUX in non-fasting serum samples of Flemish adults between 50 and 65 years old. However, estimated food intake in a general population with a rather homogenous dietary pattern seemed a less important factor in explaining the variation in dioxin activity in serum by CALUX compared to BMI and region, although the diet is the main contributor of PCDD/F exposure.

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