PCDD/Fs LEVELS IN HUMAN BLOOD OF DIFFERENT FLEMISH POPULATIONS: SOURCES AND EFFECTS

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Abstract

PCDD/Fs levels were measured by CALUX in cord blood plasma of 1196 newborns/mothers and blood plasma of 1583 adults (50-65 years old) in nine different areas of residence in Flanders (Belgium). The average level in the cord blood of the newborns/mothers (total of the dioxin-like compounds) was 23 pg CALUX-TEQ g^{-1} plasma lipid, while the lowest and highest values measured in the 9 areas of residence respectively amounted to 17 and 30 pg CALUX-TEQ g^{-1} plasma lipid. The sum of PCDD/Fs + dioxin-like PCBs (both measured separately) in the blood of the adults varied between 18.6 and 30.6 pg CALUX-TEQ g^{-1} plasma lipid in the 9 areas of residence with an average of 23.8 pg CALUX-TEQ g^{-1} plasma lipid.
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. BMI and residence area may also explain variability in dioxin activity in plasma measured by CALUX. Transcriptome analysis in human blood in relation to PCDD/Fs blood levels in adults showed significant correlations between the dioxins/furan levels and *CXCL1* and *DGAT2* gene expression.

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 carried out by the Flemish Centre of Expertise for Environment and Health of the Flemish Community, monitoring several biomarkers of exposure and effect. The biomonitoring program ran from 2002 till 2006 in eight geographical areas (Figure 1) with different types of pollution pressure: two urban areas (city of Ghent and city of Antwerp), four areas with different types of industry (harbours of Ghent and of Antwerp, non-ferrous smelter, chemical industry and waste incinerators), a fruit growing area and a rural area. At the start of the project the harbours of Antwerp and Ghent were considered together as one industrial zone, but in view of the results obtained, it seemed adequate to also consider the results for the harbours separately. One of the biomarkers under study was dioxin activity in plasma, measured by the chemical-activated luciferase gene expression (CALUX) assay. Participating sub-populations were respectively newborns/mothers (N=1196) and adults of 50 to 65 years old (N=1583).
All public information on the project is available online (http://www.milieu-en-gezondheid.be/English/index.html).

Dioxin levels in newborns and their mothers

For the first time, persistent compounds having dioxin-like activity (sum of PCDD/Fs and dioxin-like PCBs) were determined in Belgian cord blood plasma using the DR-CALUX bioassay[1]. We only found one

Japanese study reporting on ongoing measurements in cord blood using a similar assay[2]. The average concentration for all residence areas together amounts to 23 pg CALUX-TEQ g^{-1} plasma lipid while the lowest and highest values measured in the 9 areas of residence respectively amount to 17 and 30 pg CALUX-TEQ g^{-1} plasma lipid (Table 1). In most of our study regions, DR-CALUX values were moderate to well correlated with the other measured OCs (Organic Contaminants; r=0.21- 0.47). However, this correlation was low or absent in newborns from the non-ferro region of Ghent (data not shown). This could indicate a different pollutant profile of organohalogenated compounds compared to the other regions.

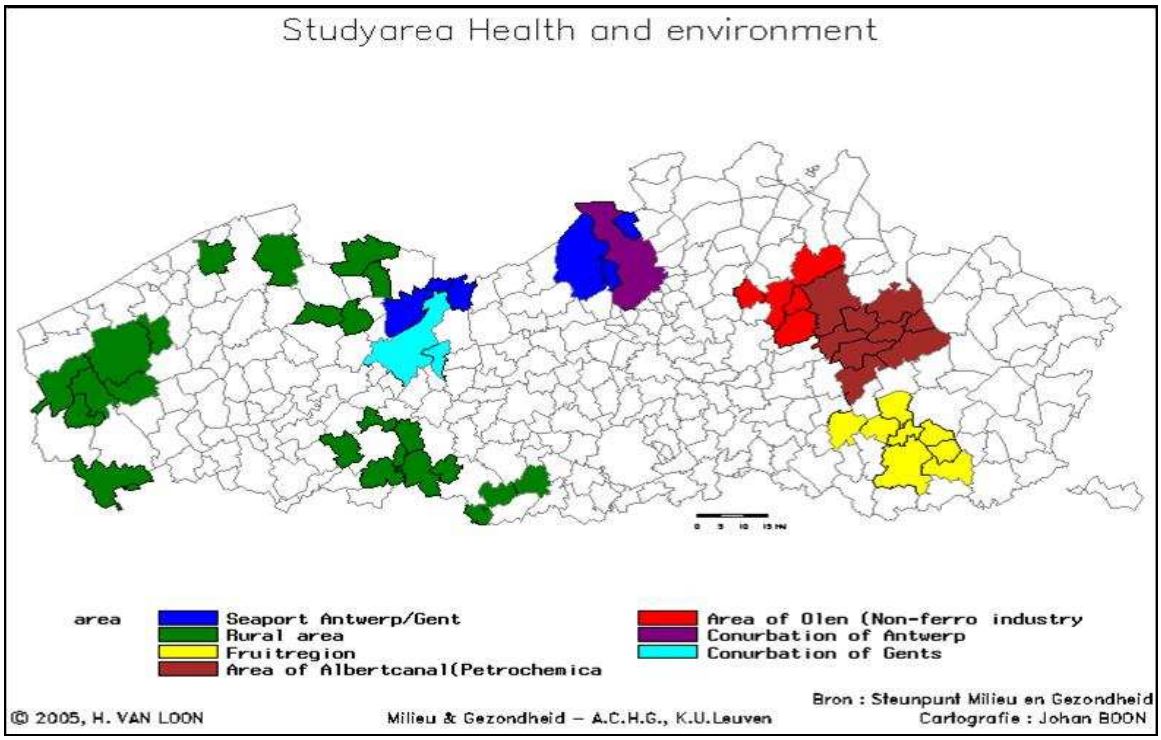

Figure 1: Map of the various residence areas in Flanders.

Limited data are available for evaluating trends of contaminants in Belgian cord blood. However, mother breast milk samples collected during the last WHO campaign of 2006, showed over a five-years-interval a decrease of 30-60 % to 10 pg WHO-TEQ g^{-1} lipids for PCDD/Fs and 80 ng g^{-1} lipids for the sum of six marker PCBs (PCB 28 + 52 + 101 + 138 + 153 +180)[3]. As already observed in other studies, one of the most important factors influencing the levels of OCs, was the age of the mother[4]. Our cohort included women from 18 to 44, with an average of 29 years old. OCs do not only accumulate with age, older mothers were also exposed to higher levels, since the levels of the measured OCs declined in time.

Area of residence explained most of the variation of the markers, especially for the OCs (between 10 and 22%). It was not expected that the residents of the rural areas would show the highest levels of OCs in cord blood (Figure 2, harbours considered together here). These rural areas were selected in our campaign because of what we considered 'low pollution pressure' i.e. low density of population (<250 inhabitants/km²), less than 5% of the area occupied by industry, no registered pollution sources, no major highways. The main route of intake of these OCs is supposed to be consumption of animal/fish products. However, intake of animal fat-containing food items showed little variation across the participating mothers (P25-P75 = 22 to 42 gram animal+fish fat per day) and did therefore not explain the higher cord blood levels of OCs in participants from the rural area. A weak but significant positive relationship between consumption of local diary/animal fat with levels of PCBs, pp'-DDE and CALUX TEQ in cord blood was observed. We have no systematic information on OC levels in regional food samples. But studies on free range chicken eggs suggest that concentrations in animal fat may be high.

	Number of newborns	Dioxin-like compounds new borns (pg CALUX TEQ/g lipid)	Number of adults	PCDD/Fs adults (pg CALUX TEQ/g lipid)	Number of adults	Dioxin-like PBCs adults (pg CALUX TEQ/g lipid)
Antwerp	**214**	**25**	**197**	**24.1**	**74**	**6.5**
Antwerp harbour	**87**	**27**	**92**	**17.0**	**55**	**5.3**
Fruit	**208**	**19**	**193**	**23.5!**	**87**	**4.7**
Olen	**134**	**22**	**203**	**24.0**	**31**	**5.6**
Ghent	**188**	**17**	**198**	**13.9**	**98**	**4.7**
Incineration	**25**	**25**	**198**	**20.7**	**87**	**5.5**
Rural	**205**	**30!**	**199**	**20.3**	**62**	**5.5**
Albert Canal	**62**	**20**	**196**	**20.6**	**88**	**4.8**
Ghent harbour	**73**	**17**	**107**	**20.2**	**87**	**4.9**
Reference mean	**1196**	**23**	**1397**	**18.6**	**669**	**5.2**

Table 1: mean dioxin-like TEQ values for the newborns and the adults in the different areas. Values significantly higher and lower then the reference mean are indicated in dark and light grey. The symbol (!) means that significantly more than 10% of the values is above the reference P-90. All values were corrected for age, sex, smoking and BMI.

Dioxin levels in adults (50 to 65 years old)

In these adults, the average concentration for all residence areas together amounts to 18.6 pg CALUX-TEQ g^{-1} plasma lipid while the lowest and highest values measured in the 9 areas of residence respectively amount to 13.9 and 24.1 pg CALUX-TEQ g^{-1} plasma lipid. For this population we determined separately the PCDD/Fs and the dioxin-like PCBs (Table 1). A significant correlation was observed between the ln CALUX PCB results and the ln CALUX PCDD/F results ($r=0.121$, $p=0.031$, $n=599$). 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 ($p= 0.122$). 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[5].

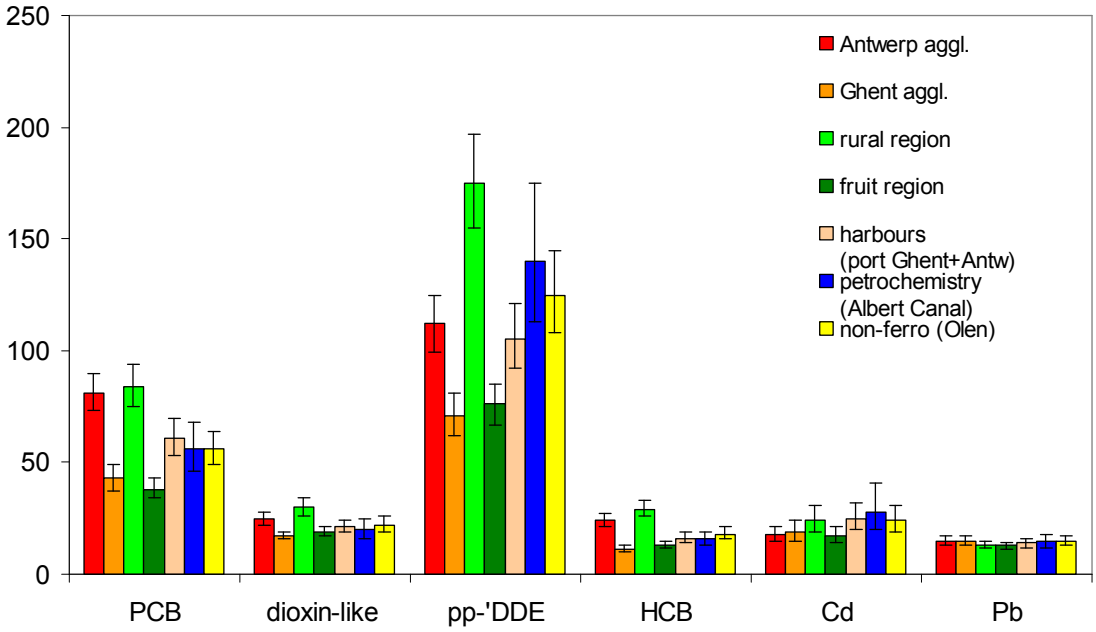

Figure 2. Regional differences in cord blood pollutants in Flanders. PCB138+153+180 (ng/g lipid), Dioxin-like: CALUX TEQ (pg/g lipid), ppDDE (ng/g lipid), HCB (ng/g lipid), Cd (E^{-2} µg/L), Pb (µg/L). All pollutant levels were adjusted for age of the mothers and for smoking habits before and during pregnancy.

Transcriptome analysis in human blood in relation to PCDD/Fs blood levels in adults

The expression levels of eight key genes, which were previously identified as promising biomarkers for environmental carcinogenesis - cytochrome P450 1B1 (*CYP1B1*), activating transcription factor 4 (*ATF4*), mitogenactivated protein kinase 14 (*MAPK14*), superoxide dismutase 2 (Mn) (*SOD2*), chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha) (*CXCL1*), diacylglycerol O-acyltransferase homolog 2 (mouse) (*DGAT2*), tigger transposable element derived 3 (*TIGD3*), and PTEN-induced putative kinase-1 (*PINK1*) - were measured in peripheral blood cells by means of quantitative PCR. Furthermore, associations with blood and urinary measures of biomarkers, including dioxins and furans were explored[6]. *CXCL1* is a chemokine whose gene expression was found to correlate significantly with blood measures of dioxins/furans. It has been associated with tumor growth and metastasis[7]. Furthermore, a positive significant correlation was found between dioxins/furans and *DGAT2* expression, a gene involved in triglyceride synthesis. The function of this gene as described in literature, however, does not indicate a relationship with environmental carcinogenesis.

Intake estimation of dioxin-like substances in adolescents, mothers and adults

The median estimated intake of PCDD/Fs and dioxin like-PCBs for the three Flemish subpopulations, adolescents, mothers and adults, is respectively 2.24, 2.09 and 1.74 pg CALUX-TEQ kg^{-1} bw d^{-1}. At the 95th percentile, the intake levels are respectively, 4.61, 4.26 and 3.53 pg CALUX-TEQ kg^{-1} bw d^{-1}. The estimated intakes of dioxin-like substances are thus decreasing with age. The consumption of total fat (g d^{-1}) is higher in the adolescent population than it is in the adult population, while the bodyweight of adolescents is lower than that of adults[8].

The relation between the estimated dietary intake of PCDD/Fs and levels in blood in adults

A detailed analysis confirmed that dietary intake contributes to the dioxin CALUX activity of plasma[9]. 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.
The area of residence is also an explanatory factor. Participants living in the city of Ghent seem to have the lowest CALUX levels in plasma, while the participants living in the city of Antwerp seem to have higher CALUX levels.
For individuals with a higher BMI, higher levels of dioxin activity in blood (pg CALUX TEQ g^{-1} plasma lipid) 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 plasma is a complex relation.

Conclusion

The main determinants for the levels of OCs present in cord blood were area of residence and age of the mother. Obviously area of residence is a broad 'umbrella, that covers differences in: habits, consumption, of locally grown foods, degree of urbanization, way of housing, traffic density, etc. In conclusion, even in a small territory as Flanders, the place of birth determines the load of pollutants at the start of life.
In adults (50 to 65 years old) a significant correlation was observed between the ln CALUX PCB results and the ln CALUX PCDD/F results ($r=0.121$, $p=0.031$, $n=599$). Also significant differences between the seven areas were seen for the CALUX PCDD/F results, but not for the CALUX dioxin-like PCB results.
Many correlations between gene expression and blood or urinary measures of biomarkers of exposure to environmental carcinogens were observed. Furthermore, we found evidence for the contribution of PCDD/F exposure to environmental carcinogenesis at the molecular level in terms of impact on the expression of genes related to metabolism and tumorigenesis.
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 plasma 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 plasma by CALUX compared to BMI and region, although the diet is the main contributor of PCDD/F exposure.

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