DETERMINATION OF POP'S IN HUMAN SERUM SAMPLES OF ADOLESCENTS FROM THE FLEMISH ENVIRONMENT AND HEALTH STUDY (FLEHS II)

Croes K[1,8*], Den Hond E[2], Bruckers L[3], Colles A[2], Koppen G[2], Loots I[4], Nelen V[5], Schoeters G[2], Nawrot T[6,7], Van Larebeke N[8], Covaci A[9], Van Langenhove K[1], Vandermarken T[1], Elskens M[1], Baeyens W[1]

[1]Vrije Universiteit Brussel, Department of Analytical and Environmental Chemistry (ANCH), Pleinlaan 2, 1050 Brussels, Belgium; [2]VITO, Environmental Health and Risk, Boeretang 200, 2400 Mol, Belgium; [3]Interuniversity Institute for Biostatistics and Statistical Bioinformatics, Hasselt University, Belgium; [4]University of Antwerp, Political and Social Sciences, Antwerp, Belgium; [5]Provincial Institute for Hygiene, Antwerp, Belgium; [6]Occupational & Environmental Medicine, Unit of Lung Toxicology, K.U.Leuven, Belgium; [7]Centre for Environmental Sciences, Hasselt University, Diepenbeek Belgium.; [8]Ghent University Hospital, Study Centre for Carcinogenesis and Primary Prevention of Cancer, Ghent, Belgium; [9] Toxicological Center, University of Antwerp, Belgium

Introduction:

As part of the 'Decree on Preventive Health Care as a legal recognition of environmental health', voted by the Flemish government in 2003, the Flemish human biomonitoring program was continued in 2007 with a second cycle (FLEHS II). The main purpose of this new campaign (2007-2011) was generating reference values for several biomarkers and determining the pollution pressure in selected hotspot areas (i.e. geographical areas or population groups with a concern for environmental pollution pressure)[1]. In total, more than 40 biomarkers of exposure and 10 effect markers (i.e. hormones, asthma and allergies and sexual development) were measured in around 650 reference samples, recruited from 14-15 year-old adolescents (n=200), adults between 20-40 years of age (n=200) and mother-child pairs (n=250). Among the biomarkers of exposure were some well known pollutants, like metals and POPs, but also some new emerging pollutants like perfluorinated compounds and new generation flame retardants (TBBPA and HBCD). The results of the reference population were used as control values for the two adolescent biomonitoring campaigns (n=200) in the hot spot areas Genk-Zuid (an industrial area around a stainless steel plant in the South-East of Flanders) and Menen (an industrial area around a shredder, near the French border in the South-West of Flanders). The group of adolescents was chosen for the hotspot biomonitoring, since younger people reflect recent exposure to pollutants (compared to adults who show a cumulative exposure for certain pollutants like PCDD/Fs and PCBs). Therefore, adolescents are the most interesting group for investigating the role of the current industry in the hotspot region on the health of people living in that area. In this paper, the results of the POPs (PCDD/Fs, dl-PCBs, marker PCBs, p,p'-DDE and HCB), measured in the serum of adolescents, are discussed.

Materials and methods:

For the adolescent study of the reference biominotoring campaign, 1269 14-15 year-old adolescents were invited to participate. 51.8 % of the adolescents replied to the letter and 69.5 % of those that replied gave consent. Within the group that gave consent (n=456), 210 participants were selected, after stratification for province, sex and educational level. From May 2008 to May 2009, the selected adolescents were recruited in ten schools in Flanders. The one-year sampling period was chosen to have seasonal variation. All adolescents filled in a questionnaire and collected a urine sample at home (first morning urine) at the day of the examination. The examination performed at the schools comprised blood and hair sampling, weight and height measurements and computer tests (NES or Neurobehaviour Examination Survey). During the field examination, a short questionnaire on recent exposure and a questionnaire with possibly sensitive questions on smoking, drugs, etc. were answered by the adolescent (without supervision of the parents). In the hotspots Genk-Zuid and Menen, a similar approach was used to recruit around 200 adolescents. Since the number of 14-15 year-old adolescents living in the selected areas was rather low, recruitment was not only done in the schools, but also via home visits with technical support of the local community. In Genk-Zuid, 197 adolescents were examined between January to November 2010, while in Menen 199 adolescents were recruited between May 2010 and February 2011. The study design was approved by the medical-ethical committee of the University of Antwerp.

More details about population characteristics can be found in the final reports, published on the website of the Flemish Centre of Expertise on Environment and Health (http://www.milieu-en-gezondheid.be/rapporten.html).

The PCDD/Fs and dl-PCBs were analyzed with the CALUX bioassay at the VUB, according to the method described by Croes et al. (2011)[2]. The marker PCBs, DDE and HCB were analyzed with GC-MS at the University of Antwerp, according to the protocols described by Covaci and Schepens (2001)[3] and by Covaci and Voorspoels (2005)[4].
Geometric means (after Ln transformation) were calculated using SAS 9.2. To determine the factors that influence the POP levels in the serum samples, first, univariate regression relationships were calculated. To compare the reference values with the hotspot measurements, multiple regression analysis with correction for pre-defined confounders (sex, age, BMI, smoking behaviour and amount of blood fat when expressed per amount of serum) and significant covariates was done. For samples below the limit of quantification (LOQ), half of the LOQ was used.

Results and discussion:
In Table 1, an overview of the geometric mean POP levels in human serum samples of adolescents (14-15 years old) is given. All POPs could be measured in more than 90% of the serum samples. In both hotspot regions (Genk-Zuid and Menen) all POPs, except HCB, were significantly lower compared to the reference mean.
Possible explanations for these lower POP values could be the use of local food (e.g. eggs), the consumption of fat-rich food in general (e.g. meat, milk, eggs, etc.) and the time trend (measurements in Genk and Menen were done two years later compared to the general, reference population). Information from the questionnaires showed that adolescents residing in Menen en Genk consumed less frequently local eggs ($p<0.0001$), vegetables ($p=0.0002$) and fruit ($p<0.0001$), and also eat less eggs ($p=0.02$), compared to the adolescent from the reference group. Especially in Menen, a very low number of participant consumed local eggs (only 19.3%, compared to 45.0% in the reference group). Furthermore, adolescents from Menen were also less frequently being breastfed as a newborn, compared to Flanders ($p=0.009$).

Pollutants	Reference values Flanders	Hotspot Genk-Zuid	% difference with Flanders	p-value	Hotspot Menen	% difference with Flanders	p-value
Sum PCBs	218	138	-37%	<0.001	166	-24%	<0.001
p,p'-DDE	309	211	-32%	<0.001	213	-31%	<0.001
HCB	36.7	34.5	-6%	0.25	34.8	-5%	0.32
PCDD/Fs	108	48.1	-55%	<0.001	70.0	-35%	<0.001
dl-PCBs	32.1	10.9	-66%	<0.001	29.1	-9%	0.03

Table 1: Geometric mean POP levels in human serum: overview of the reference values for Flanders and comparison with two hotspot regions (raw data). Sum PCBs, p,p'-DDE and HCB are expressed in ng L^{-1}, PCDD/Fs and dl-PCBs are expressed in pg CALUX-BEQ per g lipid. Sum PCBs = sum of PCB 138, PCB 153 and PCB 180

After correction for pre-defined confounders and significant covariates, the concentrations measured in both hotspot areas were still significantly lower than the reference mean (Table 2). However, when an extra correction was applied for consumption of local eggs, the concentration levels for sum of PCBs and for p,p'-DDE were respectively, only 19% and 21% lower in Menen compared to the reference group. This means that the lower concentrations measured in Menen can partly be explained by the lower consumption of local eggs in this region. For the dl-PCBs and the PCDD/Fs, this additional statistical correction could however not explain the differences between the two areas (data not shown).

	Hotspot Genk-Zuid			Hotspot Menen		
Pollutants	**% difference with Flanders**	**p-value**	**Corrected for**	**% difference with Flanders**	**p-value**	**Corrected for**
Sum PCBs	-28%	<0.001	Confounders, education level, breastfeeding, local eggs, season	-28%	<0.001	Confounders, education level, self-caught fish
P,p'-DDE	-22%	0.002	Confounders, breastfeeding, local eggs	-30%	<0.001	Confounders, self-caught fish
HCB	-7%	0.17	Confounders, education level, breastfeeding, self-caught fish, season	-4%	0.40	Confounders, education level, self-caught fish
PCDD/Fs	-55%	<0.001	Confounders, season	-39%	<0.001	Confounders, domestic heating
dl-PCBs	-64%	<0.001	Confounders, season	-17%	<0.001	Confounders, domestic heating

Table 2: comparison between POP levels in Flanders and in the hotspots, after correction for pre-defined confounders and significant covariates. Sum PCBs = sum of PCB 138, PCB 153 and PCB 180

The concentration levels measured in Flanders in this study were lower compared to the concentrations levels found five years earlier (FLEHS I, 2001-2006, Flanders). The corrected geometric means for the sum of PCBs, p,p'-DDE and HCB were respectively 23%, 26% and 60% lower in this study than in FLEHS I. The dl-PCBs and PCDD/Fs were only measured for the first time in adolescents in Flanders and could thus not be compared with other Flemish data.

When comparing our data to the US NHANES study[5] (12-19 year-old adolescents, 2003-2004) and the German GerES program[6] (12-14 year-old adolescents, 2003-2006), comparable results were found for the sum of PCBs, DDE and HCB. For the serum PCBs, the levels in Flanders (218 ng L^{-1} or 50 ng g^{-1} lipids) were comparable to the concentrations found in Germany (266 ng L^{-1}), but higher than in the US (12.9 ng g^{-1} lipids). The p,p'-DDE concentrations were higher in Flanders (309 ng L^{-1} or 70 ng g^{-1} lipids) than in Germany (190 ng L^{-1}) and lower than in the US (105 ng g^{-1} lipids). For HCB, higher concentrations were found in Germany (91 ng $^{L-1}$) and in the US (13.3 ng g^{-1} lipids) compared to the concentrations measured in Flanders (36.7 ng L^{-1} or 8.2 ng g^{-1} lipids). For the dl-PCBs and PCDD/Fs a broad range of results have been reported in literature. These differences are probably not only due to geographic and population differences, since also the used sample analysis protocol is of importance. Often different techniques are used (CALUX rat cells, CALUX mouse cells, GC-HRMS and analysis with or without separation of PCDD/Fs and dioxin-like PCBs), which makes clear interpretation and comparison of the results difficult. An extensive overview of PCDD/F and dl-PCBs levels in human serum was published by Croes et al. (2011)[2].

Overall it can be concluded that the POP concentration levels are decreasing over time. This is probably due to a more strict legislation on the disposal and emission of these POPs. However, although the use and production of PCBs, DDT and HCB was banned more than 25 years ago, these compounds can still be detected in the serum of most of the adolescents participating in our study. Follow up of the concentrations levels of these ancient pollutants in the human body is thus still advisable.

Acknowledgements:
This study was commissioned, financed and steered by the Ministry of the Flemish Community (Department of Economics, Science and Innovation; Flemish Agency for Care and Health; and Department of Environment, Nature and Energy).

References:
1. Schoeters G, Den Hond E, Colles A, Loots I, Morrens B, Keune H, Bruckers L, Nawrot T, Sioen I, De Coster S, Van Larebeke N, Nelen V, Van de Mieroop E, Vrijens J, Croes K, Goeyens K, Baeyens W. (2012); *Int J Hyg Environ Health* 215:102-108.
2. Croes K, Van Langenhove K, Den Hond E, Bruckers L, Colles A, Koppen G, Loots I, Nelen V, Schoeters G, Nawrot T, Van Larebeke N, Denison M, Vandermarken T, Elskens M, Baeyens W. (2011); *Talanta* 85: 2484-2491.
3. Covaci A, Schepens P. (2001); *Chemosphere* 43: 439-447.
4. Covaci A., Voorspoels S. (2005); *J Chromatogr B* 827: 216-223.
5. CDC, Centre for Disease Control and Prevention. Department of Health and Human Services (2009); Atlanta, US, http://www.cdc.gov/exposurereport.
6. Becker K, Müssig-Zufika M, Conrad A, Lüdecke A, Schulz C, Seiwert M, Kolossa-Gehring M. (2008); *WaBoLu-Hefte 01/08*, Federal Environment Agency, Dessau-Roßlau, ISSN: 1862-4340, http://www.uba.de/gesundheit-e/survey/us03/uprog.htm.
.