Factors influencing the PCDD/F levels in plasma of Belgian blood donors

Noemi Debacker[1], Nathalie Van Wouwe[1], Andre Sasse[1], Gauthier Eppe[2], Céline Xhrouet[2], Céline Xhrouet[2], Edwin De Pauw[2], Francis Sartor[1], Leo Goeyens[1], Herman Van Oyen[1]

[1]Scientific Institute Of Public Health
[2]CART, Liège

Introduction

In 1999 Belgium was the scene of a major food crisis due to the contamination of animal feed with 50 kilograms of polychlorinated biphenyls (PCBs) and 1 gram of polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs). To evaluate the impact of this incident on the PCDD/F body burden of Belgian citizens, plasma samples were collected after the crisis and compared to deep-frozen samples, stored by the Belgian Red Cross[1]. Possible influencing factors were defined from data on nutritional habits and lifestyle. The aim of the present abstract is to assess which factors do influence the PCDD/F levels in plasma of Belgian blood donors sampled in 2000-2001.

Material and Methods

Population sample

About 250 blood donors agreed to participate. They provided a second plasma sample during the last trimester of 2000; only a few samples originated from 2001.

At the moment of the plasma sampling a self-administered questionnaire was used to obtain information on factors possibly influencing the PCDD/F body burden: height, weight, smoking habits, vicinity of the residence to an incinerator, duration of present residence, use of herbicides and job activity. Nutritional habits were assessed by a qualitative food frequency questionnaire.

Blood Sampling

Plasma samples were collected in polyethylene bags and directly frozen at -80°C after blood donation. Their volume ranged from 90 to 650 ml depending on the donation. In February 2002, they were defrosted and divided into three aliquots: one of these was used for the analysis of 17 PCDD/F congeners (50-200ml). It was stored in polyethylene bottles and kept at -20°C until analyzed.

GC-HRMS analysis

The analysis method for the 17 PCDD/F congeners has been previously detailed[2]. Briefly, after addition of ^{13}C-labeled internal standards, 30-60 ml of sample were mixed with formic acid and water (1:1:1). This mixture was loaded on a preconditioned Isolute C18 cartridge and target analytes were eluted with hexane. The extract was cleaned on a Power-Prep system with an automated multi-column clean-up using disposable silica, alumina and carbon. Purified extract with recovery standard were then injected on a Hewlett Packard 6890 serie Gas Chromatography-AUTOSPEC ULTIMA High Resolution Mass Spectrometer.

Lipid determination

Because no data were available about the feeding state of the donors before plasma donations, values were reported on a lipid weight basis[3]. The lipid contents were determined by an enzymatic method.

Statistical analysis

The impact of various factors on the PCDD/F body burden was examined in two ways. First, we assessed their impact on the total amount of plasma PCDD/F levels and, secondly, their impact on the total toxicity or toxic equivalents (TEQ) caused by these compounds. The TEQ are based upon the 2,3,7,8-TCDD toxic equivalency factors (TEF) reported by the World Health Organization (WHO)[4]. All PCDD/F levels were log-transformed to achieve a normal distribution. The results provided are geometric means.

The analyses were carried out using Stata 8.2.

We used one-way analysis of variance (ANOVA) and multiple regression analysis to examine the effect of various factors on the PCDD/F body burden. With regard to the multiple regression analysis, it was first verified if the variables age, gender and body mass index (BMI) were to be maintained in the model. Afterwards the different nutritional and lifestyle factors were added one by one. The p-value was set at 0.05 for the variables to stay in the model.

The following variables were introduced into the model: the use of herbicides (users versus non-users), the vicinity of the residence to an incinerator (living within a radius of 10 km for more than 5 years or else), smoking habits (smokers versus non-smokers), the degree of urbanization and the frequency of consumption of full-cream milk, meat, poultry, eggs, cheese, fish, fruit and vegetables. The frequency of consumption was divided into three levels: 1, 2 and 3 stand

respectively for '< weekly', 'weekly' and 'daily' consumption. Furthermore, combinations were designed for regrouping the donors' consumption of dairy products and of meat products. These results were again divided into three levels: 1 'low consumers', 2 'intermediate consumers' and 3 'high consumers'.

Results and Discussion

221 donors provided both a plasma sample in 2000-2001 and a completed questionnaire.

The majority were males (76%). The mean age was 47 $\pm$ 10 years (range: 22-66 years) and the mean BMI was 26 $\pm$ 4 kg/m^2 (range: 18-42 kg/m^2). The population had rather high plasma lipid levels (mean: 6.8 $\pm$ 2.0 g/liter; range: 2.8-16.6 g/liter), which is probably due to the non-fasting state. The sum of the 17 PCDD/Fs in plasma amounted to 412 pg/g fat (range: 105-1856 pg/g fat). It was mainly constituted by OCDD (67%), followed by 1,2,3,6,7,8-HxCDD (8%) and 1,2,3,4,6,7,8-HpCDD (8%). The total TEQ amounted to 23.1 pg/g fat (range: 2.0-73.5 pg TEQ/g fat). It consisted mainly of 2,3,4,7,8-PeCDF (40%), 1,2,3,7,8-PeCDD (27%) and 1,2,3,6,7,8-HxCDD (14%).

As shown in Table 1 both age, BMI and gender are influencing the amount of PCDD/Fs found in plasma: the levels are higher in older donors, in obese subjects and in females.

As shown by the linear regression analysis both age, BMI and gender are independent variables (Table 2).

None of the examined food items affected the amount of PCDD/Fs present in plasma. The only variable of influence was smoking behavior: smokers tended to have lower PCDD/F levels than non-smokers (respective PCDD/F concentrations are 337 pg/g fat and 438 pg/g fat, $p<0.05$). This effect was independent from age, gender and BMI. When the number of cigarettes smoked is added into the model R-squared amounts to 23.7 (regression coefficient equals -0.014; $p<0.05$).

Table1: Sum of the 17 PCDD/Fs (amount and toxicity) by age, BMI and gender

	Amount (pg/g fat)	**p value**	**Toxicity (pg TEQ/g fat)**	**p value**
Age (years)		<0.0001		<0.0001
$\leq$ 26	178.1		7.7	
26-35	315.4		13.8	
36-45	386.0		22.8	
46-55	428.2		24.6	
56-66	544.3		32.8	
BMI (kg/m^2)		0.004		0.1
<25	359.5		21.1	
25-29	446.7		24.6	
$\geq$ 30	473.4		25.0	
Gender		0.03		1.0
Male	394.4		23.1	
Female	473.5		23.2	

Table 2: The results of the multiple regression analysis

Variable	**Amount (pg/g fat)**		**Toxicity (pg TEQ/g fat)**	
	β	**p value**	β	**p value**
Age	0.017	<0.001	0.027	<0.001
BMI	0.026	0.001	0.008	0.344
Gender[a]	0.223	0.004	0.002	0.982
R-squared	0.20		0.26	

a Referent category: male

With regard to the total PCDD/F TEQ in plasma only age exhibits a positive correlation (Table 1). The PCDD/F TEQ tends to be higher in obese subjects, but this is not significant. Multiple regression analysis confirms these results (Table 2). Hence, it was concluded to only keep age as an independent variable in the model.

Nutritional habits are slightly affecting the total PCDD/F TEQ in plasma. Multiple regression analysis reveals a negative correlation with the frequency of consumption of eggs and a positive correlation with the frequency of consumption of butter and of dairy products (constituted of full-cream milk, cheese and butter). There is also an effect of the degree of urbanization, but this effect disappears when dairy consumption is added into the model, indicating that these differences are due to different nutritional habits. We observed an interaction between the consumption of eggs and of dairy products: the donors rarely consuming eggs (N=36), present a positive correlation between the consumption of dairy products and the PCDD/F TEQ; this effect does not exist in the group of weekly egg consumers (N=121). The final model with the inclusion of age and the consumption of dairy products and eggs plus the interaction term leads to an R-squared of 0.36.

The age-dependent relation observed in our study is generally found[5] and reflects the bioaccumulation on the one

hand and the longer half-lives in elderly on the other hand[6].
No consequent findings exist on the effect of BMI on the PCDD/F body burden. However our results are supported by the observation of Flesch-Janys et al. (1996) that the PCDD/F's half-lives are increasing with increasing percent body fat[6].
The higher PCDD/F levels found in females were against the expectations since it is generally assumed that females tend to eliminate an important part of their PCDD/Fs through pregnancy and lactation. Higher female PCDD/F body burdens have been observed earlier however[5]. Differing nutritional habits between the genders are not a likely explanation in the present study since the effect of gender remains independent from the nutritional components.
The lower PCDD/F levels in smokers have been described before. This has been attributed to the shorter half-lives of PCDD/Fs in smokers due to enzymatic induction[6].
The variables studied are affecting the PCDD/F body burden in a different way as this is expressed as pg/g fat or as pg TEQ/g fat. This reflects the congener-specific contribution of the different nutritional and lifestyle factors: in our study the impact of cigarette smoking on OCDD is higher than on 2,3,4,7,8-PeCDF or on 1,2,3,7,8-PeCDD, while the contrary is true for dairy products. The latter finding is consequent with the congener distributions in Belgian foodstuffs: dairy products are important sources of the above-mentioned PeCDF and PeCDD[7]. Moreover our results confirm that dairy products are a major source of dioxin exposure in Belgium[7].
In conclusion the present results are largely in line with literature data, however, the effect of various factors on some specific PCDD/F congeners merits further investigation.

References

1. Debacker N., Sasse A., Van Wouwe N., Eppe G., Xhrouet C., Windal I., Carbonelle S., Van Overmeire I., De Pauw E., Francis S., Goeyens L., Van Oyen H. (2004) *Organohalogen Compounds*. 66: 2630-2636.
2. Focant JF, De Pauw E. (2002) *J Chromatogr B Analyt Technol Biomed Life Sci*. 776(2): 199-212.
3. Phillips D.L., Pirkle J.L., Burse V.W., Bernert J.T., Jr., Henderson L.O., Needham L.L. (1989) *Arch Environ Contam Toxicol*. 18(4): 495-500.
4. Van den Berg M., Birnbaum L., Bosveld A.T., Brunstrom B., Cook P., Feeley M., Giesy J.P., Hanberg A., Hasegawa R., Kennedy S.W., Kubiak T., Larsen J.C., van Leeuwen F.X., Liem A.K., Nolt C., Peterson R.E., Poellinger L., Safe S., Schrenk D., Tillitt D., Tysklind M., Younes M., Waern F., Zacharewski T. (1998) *Environ Health Perspect*. 106: 775-792.
5. Chen H.L., Liao P.C., Su H.J., Guo Y.L., Chen C.H., Lee C.C. (2005) *Sci Total Environ*. 337: 31-43.
6. Flesch-Janys D, Becher H, Gurn P, Jung D, Konietzko J, Manz A, Papke O. (1996) *J Toxicol Environ Health*. 47: 363-378.
7. Focant JF, Eppe G, Pirard C, Massart AC, Andre JE, De Pauw E. (2002) *Chemosphere*. 48: 167-179.