DIOXIN BODY BURDEN AMONG BLOOD DONORS BEFORE AND AFTER THE BELGIAN DIOXIN/PCB INCIDENT

Noemi Debacker[1], André Sasse[1], Nathalie Van Wouwe [1], Gauthier Eppe[2], Céline Xhrouet[2], Isabelle Windal[1], Sophie Carbonnelle[1], Ilse Van Overmeire[1], Edwin De Pauw[2], Francis Sartor[1], Leo Goeyens[1], Herman Van Oyen[1]

[1]Scientific Institute of Public Healht, Brussels
[2]Centre of Analysis of Residues in Traces, Luik

Introduction

In spring 1999, Belgium faced a severe food contamination when about 50 kg of PCBs containing 1 g of dioxins were introduced in the food chain(1). The possible public health impact of this food chain contamination was subject of a debate between different scientists. According to Vrijens et al.(2) and Bernard et al.(1) there was no significant public health impact, whereas van Larebeke et al.(3) presumed the opposite claiming a significant increase of cancer incidence. These risk assessments were based on measurements of dioxins/PCBs in contaminated foods and nutritional habits observed in a selected group of adolescents (14-18 years old) using simulation techniques(2) or on the estimation of the incremental cancer risk associated with the incremental levels of exposure to dioxins/PCBs during the incident(3).
In the meantime, an epidemiological survey has been carried out in order to measure the dioxin levels in plasma samples collected before and after the incident. The aim of this paper is to report the preliminary results of this epidemiological survey.

Materials and Methods

Population sample
Unused frozen plasma collected in November-December 1998 were available from about 250 blood donors who agreed, after written consent, to provide a second blood sample in 2000-2001.
In order to compare the investigated sample of blood donors with the population of the 1997 Health Interview Survey (HIS)(4) data have been obtained on height, weight, smoking habits, nutritional habits, education level and employment by fulfilling a questionnaire on the moment of the second plasma donation.
More specifically with regard to PCB/dioxin exposure data on vicinity of an incinerator, duration of present residence, use of herbicides for gardening, changes in nutritional habits after the incident, drug consumption, use of hypolipemic drugs and job activity have been collected.

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 for the analysis of 21 dioxin-like congeners (50-200ml), a second one for CALUX-TEQ analysis (20-60ml) and the last one for 7 PCBs markers analyses (20-60ml). These aliquots were stored in polyethylene bottles and kept at -20°C until analyzed.

GC-HRMS analysis
The method of the analyses of the 21 dioxin-like congeners (17 PCDD/Fs + 4 cPCBs) has been previously detailed[5]. 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 Helwett Packard 6890 serie Gas Chromatography- AUTOSPEC ULTIMA High Resolution Mass Spectrometer.

Lipid determination
Because no data were available about the feeding state of donors before plasma donations, values were reported on a lipid weight basis[6]. The lipid content of samples was determined by an enzymatic method.

Results and Discussion

A complete analysis on both plasma samples has been carried out for 232 donors, of which 172 were males (74%) and 60 were females (26%).
The mean age in 1998 for both males and females was 44.6 ± 10.7 years. The BMI was higher for males than for females in 1998 (respectively 27.1 versus 25.3 years, $t = 2.4$, $p<0.05$), as well as in 2000 (respectively 26.8 versus 24.7 years, $t = 3.4$, $p<0.001$).
Most blood donors originated from the southern part of Belgium (province Namur (31%) and Hainaut (21%)), followed by Brabant (31%), West-Vlaanderen (6%), Limburg (6%) and Antwerpen (5%).
In comparison with the sampled population having participated to the 1997 HIS[4], blood donors showed the following significant differences:
- male donors appeared to smoke less, to consume less frequently fish, fresh vegetables and a warm meal with vegetables and to consume more frequently skimmed and whole milk products and sugared snacks;
- female blood donors consumed more frequently skimmed milk products and sugared snacks.

At the moment of the survey a larger number of blood donors had a paid job compared to the HIS-population.
So the only differences between the HIS and the present sample of blood donors regarding consumed foodstuffs possibly influencing the dioxin body burden were fish, milk and sugared snacks.

In 1998 there was a significant difference in blood lipid levels between males and females (respectively 7.6 g/l versus 6.7 g/l, $t=3.1$, $p<0.05$), which was not seen in 2000 (respectively 6.9 g/l

versus 6.6 g/l, t=0.8, NS). Obviously, these high lipid levels are due to the non-fasting status of donors. Presently, it is not known whether these high levels of blood lipids may influence the blood dioxin levels. However, it has been shown that PCB levels measured in fasting and non-fasting blood samples were not significantly different after correction for total serum lipids[6]. Accordingly, it was decided to correct the results for the total plasma lipid levels.

The following values provided are geometric means since the levels of total blood dioxins are log-normally distributed.

The total dioxin body burden has significantly decreased between 1998 and 2000 (table 1). However, this difference was only attributable to the major contribution of OCDD to the total content of dioxins (about 70%). Indeed there was no significant difference between the total blood dioxins observed in 1998 and 2000 when OCDD levels were excluded. The significant decrease of the OCDD blood content between 1998 and 2000 is in accordance with the decline of environmental exposure to dioxins. Next to OCDD a slight but significant decrease was also observed for 2,3,7,8 TCDF (from 0.03 to 0.01 pg/g lip) and for 1,2,3,4,6,7,8 HpCDF (from 5.8 to 3.9 pg/g lip). Two congeners however exhibited a slight increase. 2,3,4,7,8 PeCDF, the most found congener in contaminated animal feed, poultry and culled cows[1], increased from 14.5 pg/g lip in 1998 to 17.9 pg/g lip in 2000. The concentration of 1,2,3,7,8 PeCDF, the third most found contaminating congener and only present in very low amounts in plasma samples, almost doubled ($3.6\ 10^{-3}$ pg/g lip in 1998 versus $6.3\ 10^{-3}$ pg/g lip in 2000). No difference in concentration was observed for 1,2,3,4,7,8 HxCDF, the second most found congener in contaminated poultry, pigs and cows[1]. For the other congeners no difference has been observed between 1998 and 2000.

Table 1: Geometric mean and range for the different dioxin congeners measured in 1998 and 2000 among 232 blood donors (pg / g lip)

Congener	1998		2000		Paired t-test
	Geometric mean	Range	Geometric mean	Range	
2,3,7,8 TCDD	0.8	0.0-7.0	0.6	0.0-5.5	1.1
1,2,3,7,8 PeCDD	5.0	0.0-23.9	4.4	0.0-20.2	0.8
1,2,3,4,7,8 HxCDD	3.9	0.0-27.0	3.5	0.0-33.4	0.8
1,2,3,6,7,8 HxCDD	31.9	0.0-113.1	28.6	0.0-99.3	1.3
1,2,3,7,8,9 HxCDD	2.2	0.0-21.7	2.1	0.0-17.5	0.2
1,2,3,4,6,7,8 HpCDD	32.7	0.9-180.4	30.0	0.0-181.7	1.2
OCDD	301.4	19.5-2611.4	277.1	40.8-1624.0	2.5*
2,3,7,8 TCDF	0.0	0.0-5.9	0.0	0.0-6.9	3.3*
1,2,3,7,8 PeCDF	0.0	0.0-3.3	0.0	0.0-5.4	-2.1*
2,3,4,7,8 PeCDF	14.5	0.0-79.4	17.9	1.4-65.9	-2.7*
1,2,3,4,7,8 HxCDF	5.8	0.0-24.2	5.1	0.0-24.5	1.1
1,2,3,6,7,8 HxCDF	7.0	0.0-32.4	6.1	0.0-28.9	1.4
1,2,3,7,8,9 HxCDF	0.0	0.0-5.1	0.0	0.0-10.6	-0.5
2,3,4,6,7,8 HxCDF	1.0	0.0-11.3	0.8	0.0-11.7	0.9
1,2,3,4,6,7,8 HpCDF	5.8	0.0-185.4	3.9	0.0-180.7	2.6*
1,2,3,4,7,8,9 HpCDF	0.0	0.0-5.7	0.0	0.0-11.3	-1.0
OCDF	0.0	0.00-23.7	0.0	0.0-30.4	0.0
Total - OCDD	133.4	28.0-454.4	129.6	28.1-473.9	0.9
Total	444.5	51.9-3002.1	416.9	72.0-1855.8	2.1*

* $p<0.05$

In both years higher levels of OCDD, leading to a higher total dioxin level, have been observed in females compared to males (tables 2 and 3). On the other hand in 1998 the 1,2,3,4,6,7,8 HpCDF level was twice as high in males than in females. The reason for this difference is not known. No other differences between males and females have been seen.

Table 2: Geometric mean and range of the different dioxin congeners in 1998 for males and females separately (pg/g lip)

Congener	Males (N=172)		Females (N=60)		Two sample t-test
	Geometric mean	Range	Geometric mean	Range	
2,3,7,8 TCDD	0.8	0.0-7.0	0.8	0.0-6.6	0.1
1,2,3,7,8 PeCDD	5.3	0.0-23.9	4.1	0.0-20.6	0.9
1,2,3,4,7,8 HxCDD	4.0	0.0-27.0	3.7	0.0-13.4	0.3
1,2,3,6,7,8 HxCDD	33.6	6.1-113.1	27.3	0.0-91.4	1.6
1,2,3,7,8,9 HxCDD	2.1	0.0-14.5	2.7	0.0-21.7	-0.7
1,2,3,4,6,7,8 HpCDD	31.0	0.9-180.4	38.0	4.8-127.9	-1.7
OCDD	282.7	19.5-2611.4	362.0	71.7-1615.1	-2.5*
2,3,7,8 TCDF	0.0	0.0-5.9	0.0	0.0-3.4	0.7
1,2,3,7,8 PeCDF	0.0	0.0-2.0	0.0	0.0-3.3	-1.8
2,3,4,7,8 PeCDF	15.3	0.0-79.4	12.5	0.0-46.6	1.0
1,2,3,4,7,8 HxCDF	6.3	0.0-24.2	4.7	0.0-19.6	1.8
1,2,3,6,7,8 HxCDF	7.6	0.0-32.4	5.5	0.0-19.8	1.9
1,2,3,7,8,9 HxCDF	0.0	0.0-1.9	0.0	0.0-5.1	0.7
2,3,4,6,7,8 HxCDF	1.1	0.0-11.3	0.7	0.0-6.1	1.0
1,2,3,4,6,7,8 HpCDF	7.0	0.0-185.4	3.4	0.0-35.6	3.4**
1,2,3,4,7,8,9 HpCDF	0.0	0.0-2.2	0.0	0.0-5.7	0.5
OCDF	0.0	0.00-23.7	0.0	0.0-17.9	0.4

* p<0.05; ** p<0.001

Table 3: Geometric mean and range of the different dioxin congeners in 2000 for males and females separately (pg/g lip)

Congener	Males (N=172)		Females (N=60)		Two sample t-test
	Geometric mean	Range	Geometric mean	Range	
2,3,7,8 TCDD	0.5	0.0-4.4	1.0	0.0-5.5	-1.6
1,2,3,7,8 PeCDD	4.5	0.0-17.8	3.9	0.0-20.2	0.5
1,2,3,4,7,8 HxCDD	3.8	0.0-33.4	2.9	0.0-16.0	1.1
1,2,3,6,7,8 HxCDD	27.4	0.0-88.1	32.1	4.2-99.3	-0.9
1,2,3,7,8,9 HxCDD	1.9	0.0-14.2	2.6	0.0-17.5	-0.8
1,2,3,4,6,7,8 HpCDD	27.9	0.0-138.4	36.7	1.8-181.7	-1.5
OCDD	256.7	40.8-1624.0	345.0	68.4-1440.1	-3.1*
2,3,7,8 TCDF	0.0	0.0-4.0	0.0	0.0-6.9	0.5
1,2,3,7,8 PeCDF	0.0	0.0-3.4	0.0	0.0-5.4	-1.4
2,3,4,7,8 PeCDF	17.9	1.4-65.9	18.2	2.5-61.5	-0.2
1,2,3,4,7,8 HxCDF	5.2	0.0-19.1	4.8	0.0-24.5	0.3
1,2,3,6,7,8 HxCDF	6.2	0.0-23.7	5.7	0.0-28.9	0.4
1,2,3,7,8,9 HxCDF	0.0	0.0-10.6	0.0	0.0-1.0	-0.1
2,3,4,6,7,8 HxCDF	0.9	0.0-10.4	0.6	0.0-11.7	0.9
1,2,3,4,6,7,8 HpCDF	4.5	0.0-180.7	2.5	0.0-22.3	1.9
1,2,3,4,7,8,9 HpCDF	0.0	0.0-11.3	0.0	0.0-3.5	0.7
OCDF	0.0	0.00-30.4	0.0	0.0-12.1	0.6

* p<0.05

OCDD, a typical congener present due to background environmental exposure, was the most important part (69%) of the total dioxin level in human blood (table 4). It was followed by 1,2,3,4,6,7,8 HpCDD (8%) and 1,2,3,6,7,8 HxCDD (8%). The other congeners contributed very little to the total dioxin content with 1,2,3,7,8 PeCDF, 1,2,3,4,7,8,9 HpCDF and 1,2,3,7,8,9 HxCDF being the least present (respectively 0.03, 0.02 and 0.02% in 1998 and 0.07, 0.04 and 0.03% in 2000).
The contribution of OCDD and 2,3,7,8 TCDF has slightly but significantly decreased from 1998 to 2000. On the other hand the contribution of 1,2,3,7,8 PeCDF and 2,3,4,7,8 PeCDF, both present during the PCB contamination, and of OCDF has significantly increased: from 0.03 to 0.07%, from 4.2 to 5.0% and from 0.4 to 0.5% respectively.
These small changes can be attributed to the PCB-contamination. Still it is worthwhile to mention 10 year old data from a German non-exposed population{Schecter, Furst, et al. 1994 17 /id}{Phillips, Pirkle, et al. 1989 10 /id}[7] revealing a higher percentage contribution of 1,2,3,7,8 PeCDF and a comparable contribution of 2,3,4,7,8 PeCDF and OCDF for a higher mean total dioxin level: 895 pg/g lip (German data) versus 444 pg/g lip (Belgian data).

Table 4: Percent contribution of individual congeners to total PCDDs and PCDFs in human blood plasma (pg/g lip)

Congener	1998		2000		Paired t-test
	% Contribution	CI	% Contribution	CI	
2,3,7,8 TCDD	0.44	0.39-0.49	0.41	0.37-0.44	1.2
1,2,3,7,8 PeCDD	1.63	1.51-1.74	1.70	1.57-1.82	-1.2
1,2,3,4,7,8 HxCDD	1.19	1.10-1.28	1.28	1.14-1.42	-1.1
1,2,3,6,7,8 HxCDD	8.10	7.63-8.56	8.14	7.67-8.62	-0.3
1,2,3,7,8,9 HxCDD	1.02	0.96-1.08	1.04	0.98-1.11	-0.6
1,2,3,4,6,7,8 HpCDD	8.14	7.67-8.61	8.34	7.92-8.76	-1.0
OCDD	68.59	67.34-69.83	67.32	66.00-68.63	2.7*
2,3,7,8 TCDF	0.16	0.12-0.19	0.10	0.08-0.13	2.2*
1,2,3,7,8 PeCDF	0.03	0.02-0.05	0.07	0.05-0.09	-2.5*
2,3,4,7,8 PeCDF	4.22	3.88-4.55	4.99	4.64-5.34	-5.8**
1,2,3,4,7,8 HxCDF	1.60	1.50-1.71	1.66	1.55-1.77	-1.1
1,2,3,6,7,8 HxCDF	1.93	1.80-2.05	1.92	1.80-2.05	0.0
1,2,3,7,8,9 HxCDF	0.02	0.00-0.04	0.03	0.00-0.05	-0.7
2,3,4,6,7,8 HxCDF	0.57	0.52-0.62	0.58	0.52-0.63	-0.2
1,2,3,4,6,7,8 HpCDF	1.99	1.71-2.28	1.84	1.56-2.12	1.5
1,2,3,4,7,8,9 HpCDF	0.02	0.00-0.04	0.04	0.01-0.08	-1.3
OCDF	0.36	0.26-0.46	0.53	0.39-0.67	-2.2*

* $p<0.05$; ** $p<0.001$

In conclusion, the population sample of blood donors, investigated in the present study appeared to be a representative group of the Belgian population excepting a few characteristics (consumption of milk, fish, sugared snacks, fresh vegetables and a warm meal, and smoking behavior).
After removing the impact of OCDD, of which the concentration decreased significantly between 1998 and 2000, the mean total dioxin content did not change in this group. A significant, although

small, increase has been found for 2 congeners involved in the incident. These small changes could be attributed to the contamination of the entire food chain. However, these increases did not affect the total body burden owing their small contributions to the total dioxin content.
These data suggest that the public health impact of the dioxin/PCB incident is negligible, as already agreed by most experts, since the incident was too limited in time and scale. However, assuming that contaminated food may not have been equally distributed across the country, it is possible that the studied population was not affected by the incident. Also, it cannot be excluded that some subgroups such as farmers consuming their own products had a higher probability of exposure.

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