LEVELS OF PCDD/Fs AND DIOXIN-LIKE PCBs IN BREAST MILK FROM ITALIAN WOMEN LIVING IN THE PROVINCES OF CASERTA AND NAPLES

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Introduction

Several recent studies performed in Campania region, sited in the south of Italy, have documented a significant excesses of cancer mortality and congenital malformations in many municipalities of the Provinces of Naples and Caserta compared with rates seen in the whole region [1,2].

In the period 2001-2003 levels of dioxin in milk and dairy products from the same area were found significantly higher than those obtained from samples collected in the framework of the National Residues Surveillance Plan (NRSP)[3].

In this frame animal feed were collected from the area of study in order to identify the source of contamination. Results showed the correlation between the PCDD/Fs levels and patterns found in milk and animal feed samples[4].

In addition, the analysis of congener profiles suggested the possibility that PCDD/Fs contamination in milk could be linked to uncontrolled urban and industrial waste incineration

In contrast, no investigation has been performed in order to assess the PCDD/Fs exposure of population resident in the area of interest.

In 2006 the Ministry of Health financed an *ad hoc* study, coordinated by the Veterinary Institute Abruzzo and Molise (IZSAM) and the National Hospital "S. Anna e San Sebastiano" Caserta, in order to assess dioxin exposure in the Provinces of Naples and Caserta and to search for correlations with environmental and dietary factors by means of analytical determination of dioxin levels in breast milk. This biomonitoring programme is currently on going in the area of study.

In this paper we present preliminary data on PCDD/Fs and dioxin-like PCBs (dl-PCBs) levels in breast milk of women living in the Provinces of Naples and Caserta. In addition we investigate the role of residing area in the vicinity of dumping wastes as potential factor influencing the body burden of mothers.

Materials and methods

Sampling

The investigated area was identified according to a geographic study committed by the Italian Civil Protection where 196 municipalities in the Provinces of Naples and Caserta were divided into 5 groups of increasing intensity of exposure with regard to the degree of environmental pressure related to waste disposal [1].

Two areas were identified: the "Not Exposed Area" where the degree of environmental pressure was low (group 1 in the cited study) and the "Exposed Area" where the degree of environmental pressure was high and significant excesses of cancer mortality and congenital malformations were recorded (groups 4-5 in the cited study).

It was planned to collect 50 breast milk samples in each area for a total of 100 samples.

Volunteering mothers were selected according to the following criteria:

- primiparae mothers with a baby aged 2-8 weeks;
- mothers under 32 years;
- both mother and child apparently healthy and a normal pregnancy;
- exclusively breastfeeding one child (i.e. no twins);
- donors residing in the sampling area for the previous 5 years.

All participants read and signed a participant information sheet and consent form.
Data on individual characteristics, smoking and specific dietary habits (especially the consumption of meat, fish, dairy products and local food), professional activity and educational level, residential and working conditions, past history of diseases were collected through a questionnaire administered to the participants.
At least 50 ml of milk was collected either using a pump or by directly expressing the milk into the glass container that was provided to the volunteering mother by the study team. Samples were stored and shipped frozen to the IZSAM Chemical Laboratory.

Analysis
Milk samples from individual mothers (50-100 ml) were spiked with the specific PCDD/Fs and dl-PCBs standard solution, a mixture of $^{13}C_{12}$-labelled congeners (Wellington Laboratories, Ontario, Canada).
Milk was first added of ethyl alcohol and ammonia solution and then fat was extracted by means of a mixture of diethyl ether and petroleum ether 1:1 (v/v). After careful solvent evaporation, gravimetric lipid determination was performed. After a double liquid-liquid partitioning process (the first with sulphuric acid, the second with potassium hydroxide) the extract was purified by means of an automated clean-up process with Power-Prep® system (Fluid Management System, Waltham, Massachusetts)) using disposable columns (multilayer silica, alumina and carbon).
The fraction containing dl-PCBs was collected from the alumina column, while the fraction containing dioxins was collected from the carbon column. These fractions were concentrated, first under vacuum and then under nitrogen, and the remainders were dissolved in the corresponding recovery standards ($^{13}C_{12}$-labelled congeners).
For each batch of seven samples a laboratory blank and a control sample were analysed.
PCDD/Fs were separated by high resolution gas chromatography (HRGC) on a DB-5 MS capillary column (60 m x 0.25 mm, 0.10 μm film thickness, J&W Scientific, California) and determined by high resolution mass spectrometry (HRMS), at a resolution of 10000 operating with electron ionisation (EI) at 40 eV in the selected ion monitoring (SIM) mode. The HRGC/HRMS system consisted of a GC Trace Series 2000 coupled with a MAT 95 XP (Thermo Fischer, Bremen, Germany).
dl-PCBs were separated by HRGC on a VF-5 MS capillary column (60 m x 0.25 mm, 0.25 μm film thickness, Varian, California) and determined by HRMS, in the same operating conditions for PCDD/Fs above described. The HRGC/HRMS system consisted of a GC Trace Series 2000 coupled with a MAT 95 XL (Thermo Fischer, Bremen Germany).
Toxic equivalent (TEQ) values were calculated using the World Health Organization Toxic Equivalency Factors (WHO-TEFs)[5]. WHO-TEQs were calculated as upper bound concentrations assuming that all values of specific dioxins congeners below the limit of determination (LOD) are equal to the respective LOD.
Statistical analysis was performed using XLSTAT software version 7.5.2. Significance level was fixed at α=0.05. Mann-Whitney test was used to compare PCDD/Fs and dl-PCBs levels among the two groups. Numerical variables were described by arithmetic mean, median, 95th percentile, minimum and maximum values.

Results and discussion

Until now 44 samples have been analysed: 16 samples from the “Not Exposed Group” and 28 samples from the “Exposed Group”. Sampling collection is currently on going.
The PCDD/Fs and dl-PCBs levels in breast milk samples are reported as pg WHO-TEQ/g fat. The results are summarized in Table 1.
Dioxin contamination ranged between 5.5 and 14.9 pg WHO-TEQ/g fat (mean value 8.8 pg WHO-TEQ/g fat).
The dl-PCBs were in the range 4.0 – 21.2 pg WHO-TEQ/g fat (mean value 8.2 pg WHO-TEQ/g fat).
Figure 1 shows the relative contribution of PCDDs, PCDFs, non-ortho dl-PCBs and mono-ortho dl-PCBs to total toxicity. The contribution of PCDD/Fs in relation to dl-PCBs to total WHO-TEQ was 54:46 % for the “Exposed Group” and 47:53 % for the “Not Exposed Group”. These data are in agreement with those reported in a recent German study [6].
In relation to specific living area, contamination levels for each class of contaminants were compared between “Not Exposed Group” and “Exposed Group”. At a significance level α=0.05 the differences between groups were not statistically significant.

Recorded contamination levels are lower than those found in a number of Western European countries [7,8]
The congeners profiles are reported in Table 2. The most abundant congener is OCDD among dioxins followed by 2,3,4,7,8 PeCDF and 1,2,3,6,7,8 HxCDD. The prevalent congener is PCB-118 among dl-PCBs followed by PCB-156 and PCB-105.
The relative abundances of the single congeners for PCDD/Fs and non-ortho PCBs were quite similar to those generally observed in industrialised countries [9]. Since this study is still ongoing is not possible to reach definitive conclusions considering the restricted number of samples analysed.

Acknowledgement
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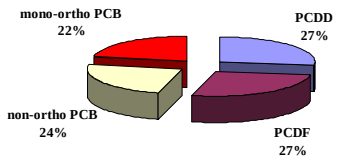
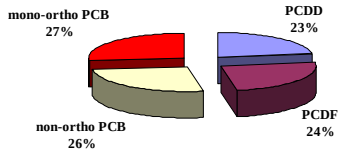
Figure 1. Contribution of PCDDs, PCDFs, non-ortho dl-PCBs and mono-ortho dl-PCBs to total WHO-TEQ



Exposed Group



Not Exposed Group

Table 1. PCDD/Fs and dl-PCBs levels for each group (Exposed/Not Exposed)

	Exposed		Not Exposed	
	WHO-TEQ (PCDD/Fs) (pg/g fat)	WHO-TEQ (dl-PCBs) (pg/g fat)	WHO-TEQ (PCDD/Fs) (pg/g fat)	WHO-TEQ (dl-PCBs) (pg/g fat)
Minimum	5.5	4.0	5.8	4.3
Mean	9.3	7.8	8.0	9.0
Median	9.1	7.1	7.9	8.2
95^{th} percentile	12.8	12.6	9.9	14.9
Maximum	14.9	21.2	10.2	16.5
N° samples	28		16	
Age (years)	26-32		26-32	

Table 2. Concentration (pg/g fat) of PCDDs, PCDFs and dl-PCBs for each group (Exposed/Not Exposed)

	Exposed	Not Exposed
	Median (Min-Max)	Median (Min-Max)
2378-TCDD	0.84 (0.35-1.6)	0.72 (0.53-0.90)
12378-PeCDD	2.8 (1.6-6.7)	2.3 (1.3-3.3)
123478-HxCDD	1.0 (0.37-3.3)	0.88 (0.30-1.8)
123678-HxCDD	5.9 (3.0-17)	5.3 (2.5-7.3)
123789-HxCDD	1.1 (0.047-3.4)	0.85 (0.43-1.7)
1234678-HpCDD	5.3 (2.4-18)	4.9 (2.4-12)
OCDD	37 (14-126)	28 (17-59)
2378-TCDF	0.41 (0.028-1.3)	0.41 (0.16-1.1)
12378-PeCDF	0.30 (0.031-1.1)	0.20 (0.078-0.53)
23478-PeCDF	7.8 (4.0-12)	7.1 (5.1-8.9)
123478-HxCDF	2.4 (1.4-4.3)	2.1 (1.3-3.4)
123678-HxCDF	2.1 (1.1-3.5)	2.0 (1.4-3.0)
234678-HxCDF	1.0 (0.029-2.2)	0.95 (0.43-1.4)
123789-HxCDF	0.013 (0.003-0.10)	0.0080 (0.001-0.14)
1234678-HpCDF	1.6 (0.24-15)	1.5 (0.62-4.4)
1234789-HpCDF	0.037 (0.003-0.17)	0.023 (0.001-0.13)
OCDF	0.28 (0.028-4.2)	0.21 (0.010-0.55)
PCB-77	3.9 (1.5-23)	4.5 (1.9-17)
PCB-81	1.8 (0.073-17)	1.9 (0.57-5.1)
PCB-126	37 (14-103)	39 (16-76)
PCB-169	21 (14-60)	25 (11-42)
PCB-105	1700 (1100-7200)	2000 (790-9300)
PCB-114	460 (300-1400)	530 (190-2100)
PCB-118	8500 (5300-30000)	9300 (3700-26000)
PCB-123	99 (52-420)	120 (38-370)
PCB-156	3500 (2200-9800)	3800 (1200-12000)
PCB-157	730 (420-2100)	830 (280-2900)
PCB-167	1000 (710-3500)	1200 (510-3000)
PCB-189	320 (140-750)	310 (110-790)