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MONITORING OF ENVIRONMENTAL CONTAMINANTS IN BREAST MILK OF THE LAZIO REGION

F. Busico[1], A. Ubaldi[1], G. Mattei[1], S. Berretta[1], T. Mauti[1], G. Salvatori[2], V. Pannone[2], B. Neri[1]

[1]*Istituto Zooprofilattico Sperimentale delle regioni Lazio e Toscana "M.Aleandri", Rome*

[2]*Ospedale Bambino Gesù, Rome*

Introduction

Exposure to persistent organic contaminants of anthropogenic origin, direct and indirect, diffused into the environment, leading to chronic toxicity due to extremely low dose taken daily from the diet. The main effects of the substances of the study are carcinogenicity, genotoxicity, mutagenicity, teratogenicity. Other effects are subject of ongoing studies. Finally yet importantly and perhaps, the most insidious are the effects of contaminants such as dioxins and PCBs dioxin like that mimic the activity of endogenous substances of organisms (1). Doses taken by infants and young children through breast milk have a negative impact certainly different from that of an adult, for that purpose and contrast the benefits of lactation and breast milk. The study linked the concentration levels of pollutants in human milk in the provinces of Rome, Rieti, Viterbo, Latina and Frosinone, with the eating habits and the residence of the donor mothers. The results obtained are compared with other Italian and European studies.

Materials and methods

Breast milk samples (n = 81) issued from primiparae and multiparae mothers were collected between 2013 and 2015 in different birth centers of the Lazio region and assisted in the Bambino Gesù hospital in Rome. Nursing mothers have given birth to no more than 45 days before. The laboratory has collected 16 samples from the province of Viterbo, 24 from the province of Rome, 13 from the province of Rieti, 13 from the province of Latina and 15 from the province of Frosinone. The criteria used for the selection of donors included: maternal age between 25 and 40 years, residents at least 10 years in the provinces examined, mothers of healthy babies born at term (37-42 weeks EG) with birth weight between 2500 g and 4000 g. Statistical data were collected through a questionnaire concerning age, city and area of residence, whether the interviewees were smoker, exposure to contaminants during work-time, weight before pregnancy, weight gain during pregnancy, foods in the diet (portions of fish, meat and dairy products consumed during the week). The milk samples were collected by means of a breast pump provided by the birth center: volumes ranged between 20 and 233 g. Milk samples were placed in a glass container and kept in the refrigerator at + 4° C for a maximum of 48 hours, immediately after collection. The samples were individually subjected to the analysis of PCDD, PCDF and PCB-dl by HRGC-HRMS. Milk samples corresponding to volumes between 20 and 120 ml were thawed, added with mixtures (Wellington Laboratories inc.) of PCDD, PCDF and PCB-dl 13C-labelled (in isotope dilution technique), homogenized for 2 min. and entirely subjected to lyophilization in Petri dishes. Lyophilized extracted by solvent extraction (hexane-dichloromethane 80:20) with hot extraction mode (extractor BUCHI). The lipid extract was weighed and dissolved in hexane, added with standard cleanup 2,3,7,8-TCDD labelled to the 37Cl and PCB-79 to 13C-labeled, then subjected to purification-automatic and separation by POWER-Prep® purifier (Fluid Management Systems) with a mixed acid-basic silica, alumina and a carbon columns. The instrument used was a mass spectrometer high resolution MAT 95xp equipped with gas chromatograph Trace 2000 Series products from Thermo-Fisher ™, Bremen, Germany. Concentrations of PCDDs and PCDFs were determined by high-resolution gas chromatography using a PTV splitless injection system, a capillary column Phenomenex® ZB with semi-volatile phase consisting of 5% and 95% polysilarylene polymethylsiloxane, length 60m, internal diameter of 0.25mm and thickness of 0.25 µm film, and spectrometry high resolution acquisition MID (multiple ion detection) mass with minimum resolution of 10000. The liquid fraction containing PCBs-DL from the clean-up process was analyzed on a column with SGE HT8 phase (8% Phenylpolysiloxane-carborane, length 60m, diameter 0.25 mm, film thickness 0.25 µm).

Results and discussion

Equivalent toxic values (WHO-TEQ2005-PCDD-PCDF, WHO-TEQ2005 PCB-DL) were calculated multiplying the concentration obtained for each congener by the Toxic Equivalency Factors (TEF) proposed by the World Health Organization in 2005 (2). Equivalent toxicity value were expressed in

Upper bound mode considering the LOQ values for those analytes contained in the samples below this value. Expanded uncertainty of the analytical method was calculated during validation procedure by metrological approach and was calculated to be ± 22% for dioxins and ± 15% for PCB-DL for WHO-TEQs.

Fig. 1 shows WHO-TEQ05 means values (pg/g fat basis) of PCDDs/PCDFs and PCB-DL of the five provinces of Lazio. These results show that the levels of dioxins found in the five provinces of Lazio are lower than of 2008 for the region Campania (6.46 WHO-TEQ2005 pg/g on fat basis (3), and lower than recorded values for the city of Rome between 1998 and 2001 (WHO- TEQ98 PCDD/F of 9.40 pg/ g on fat basis (4)). The PCDD/Fs average value of 2.76 pg WHO-TEQ2005/g on fat basis in milk of women living in the Lazio region is significantly inferior to the data reported in the literature for other European countries (figure 2), while remaining high compared to the European standard limits for baby foods. The standard profile of dioxin congeners is in agreement with all the provinces of Lazio and found a correlation of 99% with the human milk profile analyzed in Ireland in 2010 (4) (figure 3). Information collected on the donor mothers have been linked with the data reported in Table 1 using the Mann-Whitney U test. Associations and correlations between the WHO-TEQs and the variables considered were evaluated by Chi Square test and the Pearson correlation coefficient, respectively, considering a statistically significant p value <0.05. Statistical evaluation of the data performed with the software Stata v.12 shows no correlation between the levels of contamination of milk and donor mothers' diet, as evidenced in the literature (4). A significant negative correlation was found between the number of pregnancies and WHO2005-TEQ PCDD/F concentrations ($r = -0{,}878$, $p <0.05$) and between the sum TEQ-WHO2005 PCB-dl and WHO2005-TEQ PCDD/F ($r = -0642$, $p <0.05$). The lack of clear correlation in the concentration of dioxins and PCB-dl with the eating habits agree with the results obtained for the city of Rome in 2008 (4) and may be attributed to the globalization of the food market and the variability of supplies even in the same dealer.

In conclusion, the present study shows the homogeneity of the concentration levels of PCDD/F and PCBs-DL in human milk for the provinces of Lazio, with the exception of the province of Rome (fig. 1), which shows higher values probably due to a larger size of the territory and a higher human activity and not least the sample size compared to the other provinces.

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Table 1. Levels of PCDF and dl-PCBs in human milk of women living in the Lazio region taken from 2013 to 2015.

Contaminants	Concentration of contaminants					
Dioxins and furans	Mean	Median	25th -75th percentile		Range	
2,3,7,8-T4CDD	0.232	0.102	0.0176	0.393	0.00637	1.22
1,2,3,7,8-P5CDD	0.809	0.769	0.148	1.28	0.001	3.4
1,2,3,4,7,8-H6CDD	0.367	0.305	0.0656	0.48	0.00101	2.9
1,2,3,6,7,8-H6CDD	1.864	1.51	0.779	2.62	0.001	7.58
1,2,3,7,8,9-H6CDD	0.244	0.0481	0.0222	0.361	0.001	2.58
1,2,3,4,6,7,8-H7CDD	2.292	1.69	0.531	2.58	0.001	44.4
O8CDD	17.479	14.3	7.60	19.4	0.117	195
2,3,7,8-T4CDF	0.330	0.23	0.0353	0.495	0.001	1.79
1,2,3,7,8-P5CDF	0.155	0.064	0.0290	0.243	0.007	0.668
2,3,4,7,8-P5CDF	2.655	2.44	1.42	3.965	0.0221	8.48
1,2,3,4,7,8-H6CDF	0.766	0.694	0.294	1.13	0.001	4.37
1,2,3,6,7,8-H6CDF	0.763	0.776	0.2305	1.08	0.001	3.16
1,2,3,7,8,9-H6CDF	0.196	0.0383	0.0231	0.204	0.001	1.25
2,3,4,6,7,8-H6CDF	0.242	0.0514	0.0275	0.383	0.0012	2.03
1,2,3,4,6,7,8-H7CDF	0.792	0.585	0.215	0.911	0.001	7.06
1,2,3,4,7,8,9-H7CDF	0.043	0.0257	0.0146	0.0449	0.0005	0.433
O8CDF	0.133	0.0332	0.0165	0.156	0.0004	1.27
WHO-TEQ$_{1998}$-PCDD-PCDF (pg/g WHO-TEQ fat)	2.76	2.50	0.851	3.79	0.03	10.4
WHO-TEQ$_{2005}$-PCDD-PCDF (pg/g WHO-TEQ fat)	2.26	2.00	1.15	3.24	0.023	8.71
Contaminants	Concentration of contaminants					
PCB	Mean	Median	25th-75th percentile		Range	
PCB 77	50,7	22,3	2.57	55.3	0.18	0.78
PCB 81	6,90	2,2	0.577	4.74	0.08	86.2
PCB 126	13,3	11,1	1.74	19.9	0.11	71.1
PCB 169	11,9	8,22	3.41	13.9	0.10	82
PCB 105	1090	896	583	1230	7.43	6930
PCB 114	293	229	146	416	0.16	1240
PCB 118	4420	3790	2150	5740	30.5	17500
PCB 123	42,5	40.2	23	57.9	0.18	126
PCB 156	2560	1980	1190	3830	18.1	7030
PCB 157	506	414	233	699	3.63	2390
PCB 167	685	557	371	948	5.93	23.20
PCB 189	258	184	113	382	1.85	1460
WHO-TEQ$_{1998}$- PCBdl (pg /g WHO-TEQ fat)	3.63	3.33	2.04	4.65	0.04	14.2
WHO-TEQ$_{2005}$-PCBdl (pg /g WHO-TEQ fat)	1.88	1.53	0.63	2.09	0.02	9.00
PCB-ndl (sum 6 marker) (ng/g fat)	72.5	61.6	37.7	96.7	20.1	359

Fig. 1. Mean concentrations in pg/g WHO-TEF$_{05}$ of fat in human milk of "dioxins" and pcb-dl for the five provinces of Lazio

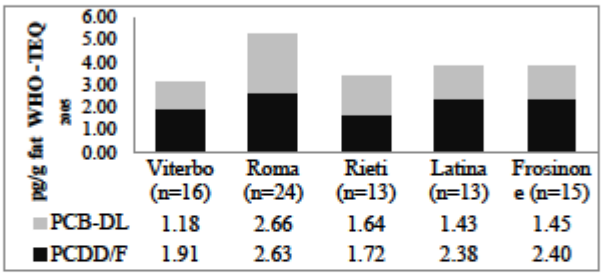

Fig.2. Report of work carried out in Europe since 1998 on the contents of PCDD/F in human milk

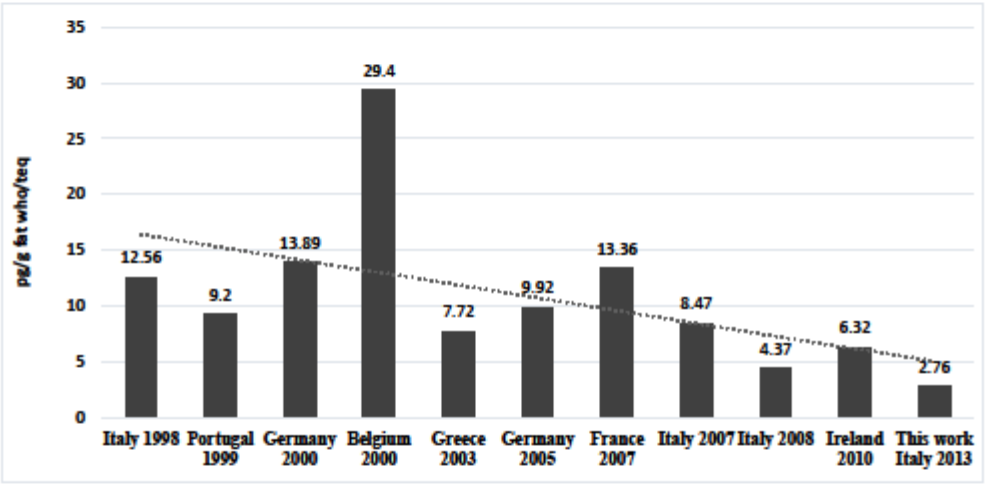

Fig.3. Match found between the profile (normalized) of PCDD/F in the breast milk of Lazio and to a study carried out in Ireland in 2010.