Busico F, et al., J Toxicol Cur Res 2019, 3: 009
DOI: 10.24966/TCR-3735/100009

HERALD

HSOA Journal of Toxicology: Current Research

Research Article

Monitoring of Persistent Organic Pollutants in Human Milk of Lazio Region

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Abstract

Direct or indirect prolonged exposure to organic contaminants diffused into the environment may lead to chronic toxicity through human milk. Infants and young children exposure during breast feeding can have a negative impact on adulthood. For this reason, some scientists express reservations on the benefits of lactation, despite the World Health Organization (WHO) and the American Academy of Pediatrics (AAP) continue to recommend breastfeeding. However, the human milk is the best way to monitor the level of exposure of these substances in the environment and in the food. This study studied the levels of Polychlorinated Dibenzodioxins, Polychlorinated Dibenzofurans (PCDD/F) and Polychlorinated Biphenyls (PCBs) in human milk in the Italian region of Lazio, which includes the provinces of Rome, Rieti, Viterbo, Latina, Frosinone, and correlated them with eating habits and residence of the donor mothers. We found no difference in Dioxins and PCB levels in human milk among the provinces of Lazio, with the exception of Rome, and no relationship between the levels of contamination detected in the milk and eating habits, confirming previously published literature. The levels of PCDD/F were compared with data obtained in other European countries between 1998 and 2015. We found a reduction of POPs in human milk compared to what has been detected in recent years in Italy and in other European countries and we conclude that the strict rules and the official controls in order to restrain the diffusion of those substances into the environment and into human food are gradually getting good results.

Keywords: Dioxins; Human milk; Lazio region; PCBs

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Citation: Busico F, Salvatori G, Pannone V, Berretta S, Mattei G, et al. (2019) Monitoring of Persistent Organic Pollutants in Human Milk of Lazio Region. J Toxicol Cur Res 3: 009.

Received: May 07, 2019; **Accepted:** May 21, 2019; **Published:** May 29, 2019

Introduction

Persistent Organic Pollutants (POPs) are a group of toxic substances that are persistent, bio-accumulative and can be diffused in the environment even over long distances. POPs are resistant to chemical and biological degradation, and may remain in the soil, waters, and living organisms for long periods of time due to their chemical-physical properties. Dioxins (polychlorinated dibenzodioxins and polychlorinated dibenzofurans), polychlorinated biphenyls, and chlorinated pesticides belong to a list originally comprised of 12 POPs that are regulated under the Stockholm Convention on POPs [1]. Within individual dioxin congeners, the toxicity is considerably diverse. Because of the Cl-substitution at the 2,3,7,8 positions, only 17 of the 210 congeners have significant toxicity. The most toxic congener is the 2,3,7,8 TCDD, which has chlorine at positions 2,3,7,8 and is taken as a reference to toxicity [2-4]. POPs are lipophilic compounds, stable against degradation and able to bio-accumulate, thus they are highly persistent and ubiquitous in the environment and in the food chain [4]. POPs cause chronic toxicity at low doses when absorbed daily with food (meat, fish, eggs, milk),main source of contamination. Humans are at the top of the food chain with risk of accumulation of pollutants within the lipid fraction of tissues. Because, women have higher percentage fat tissue than men [5], they are at higher risk. It is not easy to demonstrate the existence of a connection between the long-term exposure to a specific POP (or of a group of POPs) and its impact on human health. However, exposure during the fetal and nursing period is particularly critical. The main effects of the substances of the study, PCDD/F and Polychlorinated biphenyls are carcinogenicity, genotoxicity, mutagenicity and teratogenicity [6]. Furthermore, these contaminants may mimic the activity of endogenous substances, which interact with aryl hydrocarbons receptor, an intracellular soluble protein able to bind to numerous aromatic, halogenated and non-halogenated substances. As 2,3,7,8-TCDD has the maximum affinity to the receptor, thus the highest toxicity, the affinity decreases with increasing Cl-substituents. This is also indicated by the lower TE factors for higher chlorinated PCDD/F [7-10].

These substances may travel from the mother to the fetus during the gestational and the nursing period. Breast milk monitoring is a non-invasive mean to determine the levels of the chemicals of interest in humans [11]. Over the last decade, various international studies have been conducted to provide information on POPs burden in women of reproductive age, together with the perinatal exposure of the fetus and the nursing infant [12]. These studies highlighted a significant reduction in the levels of PCDD/F-PCBs in most European countries following the Stockholm Convention. Today in breast milk there is an average of 5pg/g fat PCDD/ F and PCB TEQ [13-24].

The Italian studies in the provinces of Venice, Rome, Milan, Piacenza and Naples demonstrated variable values between 4.37 and 12.56pg WHO-TEQ$_{2005}$/g fat [25,26]. The aim of this study was to define the levels of these pollutants in breast milk, and to correlate the types of pollutants with the mother's dietary habits within the Lazio region. This information could be used by the Ministry of Health to inform women on the benefits of a correct diet, thus reducing the exposure to these pollutants.

Materials and Methods

Samples

We studied eighty-one mothers who had given birth within 45 days in different birth centers in the Lazio region in 2014 or 2015 (Rieti, Latina, Viterbo, Frosinone e Roma), all of them living in Lazio, were studied: 16 samples from the Viterbo province, 24 from the Rome province, 13 from the Rieti province, 13 from the Latina province, and 15 from the Frosinone province. Mothers fit the following criteria: age between 25 and 40 years, resident in the examined provinces for at least 10 years, mother of healthy newborns born at due date (gestation 37-42 weeks) with a birth weight ranging within 2500g and 4000g after an uncomplicated pregnancy. All the enrolled mothers were provided with accurate instructions about the research's purpose and each of them signed written informed consent. Between 20g and 233g of milk were collected through a breast pump provided by the birth center. Immediately after sampling, the breast milk was frozen at -20°C until analysis.

Chemicalanalisys

In this study, a total of 35analytes were analyzed (10 PCDDs, 7 PCDFs,12 dioxin-like PCBs (8 mono-ortho PCBs and 4 non-ortho-PCBs), and6 non dioxin-like PCBs (IUPAC 28, 52, 101, 138, 153, 180, reported as a sum). All samples were processed in series of routinely analyzed samples(one method blank, one instrumental blank, one Quality Control (QC) and 6 unknown) in an ISO17025 accredited laboratory. Most isotope dilution calibration, internal, and recovery standards were purchased from Cambridge Isotope Laboratory (Cambridge Isotope Laboratories Inc., Andover, MS, USA. In order to analyze the PCDD, PCDF, dl-PCB and ndl-PCB, 80 g milk of individual mothers were spiked with a mixture of ^{13}C-labelled congeners (technic of isotopic dilution) homogenized for 2min, and submitted to lyophilization overnight [27]. The lyophilized milk was automatically extracted through a mixture of hexane-dichloromethane with aBÜCHI B-811 extractor (BÜCHI, Flawil, Switzerland). The extract obtained is evaporated and the fat is weighed, dissolved in hexane and passed on a column of silica acidified with sulfuric acid. The resulting extracted was mixed with standard automated cleanup 2,3,7,8-TCDD labeled at ^{37}Cl and PCB-79 labeled at ^{13}C (added to each extract to measure the efficiency of the cleanup process), followed by a process to purify-separate through the use of POWER-PREP® cleanup system (Fluid Management Systems, Waltham, MA, USA) with silica acid-based, alumina and activated carbon columns. From the separation two different extracts were obtained, one containing PCDD-PCDF and the other one containing dl-PCB- and ndl-PCB. The extracts are evaporated and added with three recovery standards, labeled at ^{13}C6 1,2,3,4 TCDD, labeled at ^{13}C12 1,2,3,4,6,8,9-HpCDF and labeled at ^{13}C 3,3, 5.5'-TetraCB 80. The final volume for each extract is 20 and 100 microlitres, for dioxins and PCBs respectively. For each analysis a blank sample and a spiked one were analyzed. A high-resolution mass spectrometer MAT95XP Thermo Finnigan® was used. PCDD and PCDF concentrations were analyzed using high-resolution gas chromatography through a split-less PTV injector with temperature programming and 280°C injection temperature, a capillary column Phenomenex® ZB-semi volatile phase 5% phenyl-arylene 95% dimethylpolysiloxane, and determined by high resolution mass spectrometry at a minimum resolution of 10000 in the selected MID (multiple Ion detection). For the determination of the PCB-dl and PCB-ndl, the same instrument was used, operating the chromatographic separation with a column SGE HT8 8% phenyl (equiv.) polycarborane-siloxane, and a split-splittles PTV injector in split-less mode at a temperature of 250°C. The analysis conditions in HRMS for dioxins and PCBs are those reported in the EPA 1613B and EPA 1668B [31,32]. The identification of a congener is considered positive if the exact masses of the monitored molecular ions and their ratio of isotopic abundance match. Relative retention times of each congener and its labeled congener were used for identification. The amount of each congener was determined using a five-point calibration curve as reported by the EPA 1613 method. The chromatograms of a human milk sample are shown in figures 1 and 2, they represent the MID (multiple ion detection) of PCDD, PCDF and PCBs. The ionic extracts (Figure 3 and 4) show a complete chromatographic and mass separation between the native and other interferers for 2,3,7,8-TCDD, 2,3,7,8TCDF and PCB 126, which represent the congeners with greater toxicity.

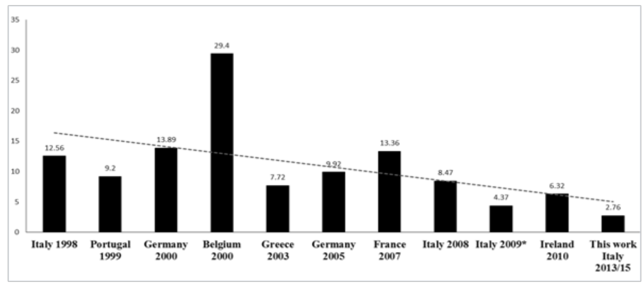

Figure 1: Concentrations of PCDD/F in human milk in several European countries found in the literature [12,16,17,19-21,24-26,29].

Note: *Concentration reported in pg/g fat WHO-TEQ$_{98}$.

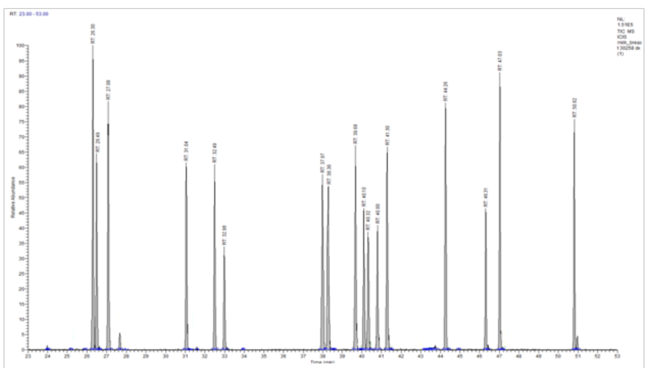

Figure 2: MID chromatogram in HRGC-HRMS, which represents the PCDD and PCDF content of a human milk sample. Tetra-substituted congeners (RT 26.30-27.08), penta-substituted congeners (RT 31.04-32.98), hexa-substituted congeners (RT 37.97-41.30), hepta-substituted congeners (RT 44.26-47.03), octa-substituted congeners (RT 50.82-50.91)

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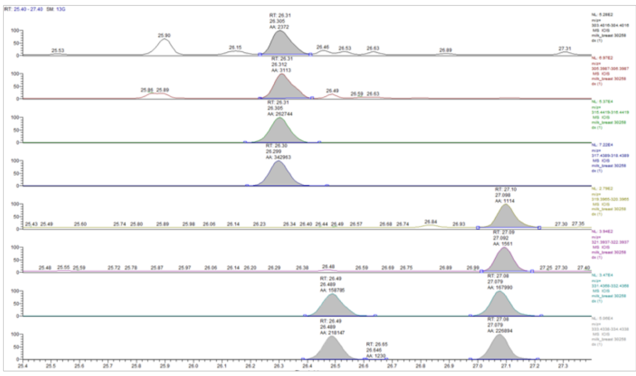

Figure 3: Ion extract of a breast milk sample shows the peaks of the native 2,3,7,8-TCDD (RT 27.10), the 2,3,7,8-TCDD labeled (RT 27.08), the 2,3,7 , 8-TCDF and 2,3,7,8-TCDF labeled (RT 26,30-26.31). At RT 26.49, the13C6 1,2,3,4 TCDD the recovery standard.

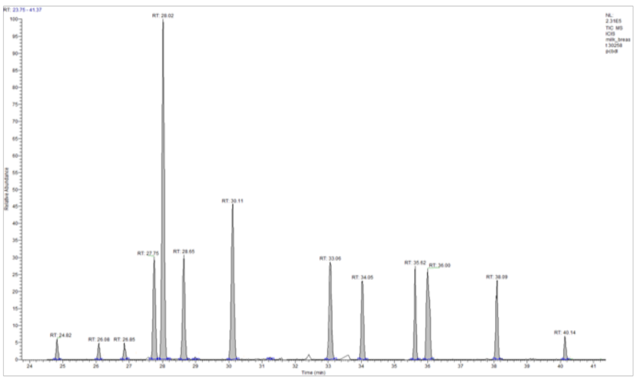

Figure 4: MID chromatogram in HRGC-HRMS, which represents the PCBs content of a human milk sample. Tetra-substituted congeners (RT 24.82-26.85), penta-substituted congeners (RT 27.75, 33.06), hexa-substituted congeners (RT 34.05-38.09), hepta-substituted congener (RT 40.14).

Statistical analysis

The amount of PCDD/F and PCB in breast milk of women enrolled was calculated according to the following parameters: age, Body Mass Index (BMI), number of children, smoking habits, province of residence, proximity to industrial areas, dietary habits (meat, fish, milk, cheese consumption). Table 1 shows all the characteristics collected through the questionnaires provided in the milk collection centers. Data is reported as average, median, Standard Deviation (SD), and range interquartile (IQR: 75°-25° centile). Discreet and qualitative data are reported as frequencies and percentages. The comparison within PCDD/F, PCB and other variables were obtained through the test U Mann Whitney. The multiple comparisons have been carried out through Kruskal-Wallis test, followed by a “post hoc” test. The associations and correlations have been carried out respectively through the Chi Quadro test and the Pearson correlation coefficient. For the statistics evaluations Stata v.12 software (StataSE, Texas, USA) was used. A value of $p<0.05$ is statistically considered quite important.

Expression of results and quality control

We used the toxic coefficients proposed by WHO both in 1998 and 2005, in which the concentration of PCDD/F and PCB-dl are expressed in pg per gram of lipid component of milk, to compare the obtained data with literature published before 2005. The value of toxicity has been expressed in upper bound, or better through, summing LOQ values for those analytes contained in the samples below this value. The quality of the data has been insured reporting, on control tables the value of WHO-TEQ98/05 per dioxin and PCB-dl obtained from the analysis made on spiked milk samples in each analysis section, and from the successful participation to PT organized by European laboratory of reference (EU-RL for Dioxins and PCBs in Feed and Food Freiburg). The method was validated in accordance with the Reg. (EC) 1883/2006 and the Decision 2002/657/CE. For the validation of the method, the following were considered: the linearity parameters (five concentration levels for each congener), specificity on twenty ten different bovine milk samples, limit of detection and limit of quantification on three concentration levels with six replicates, repeatability on three concentration levels with six replicates per level, reproducibility evaluated in two analytical sessions, accuracy (difference between the average value measured for each analyte in a material and its added value, expressed as a percentage of that value). The exactness seems to be compliant to validation parameters into limits of ±20% of all analytes. The extended margin of error of analytical method, calculated during the validation of metrological approach, is ±22% for dioxin and ±15% for PCB-dl. Values of concentration of WHO-TEF98/05 of PCDD, PCDF and PCB-dl found are expressed singularly for the five provinces of Lazio and globally for the full region, aimed to compare results with the ones of existing literature. In the literature for human milk there are no previous data corresponding to the region we have considered, the comparisons made by us concern other Italian and European countries (Table 2).

Parameter	N	Value	SD	Min	Max
Mother age	81	33	4.91	22	42
Body Mass Index (BMI)	81	22.5	3.14	16.7	29.6
Newborns age (days)	81	16.5	10.9	3	43
	N	**Children**	**N women**	**Percentage**	
Number of pregnancies		1	24	33.3	
		2	34	47.2	
		>2	14	19.4	
Resident in rural areas	81		29	39.2	
Smokers	81		2	2.74	

Table 1: Sampling parameters.

Results

Eighty-one different samples of human breast milk have been analyzed for dioxin, dioxin like PCBs and non-dioxin like PCB (six indicators). The obtained data has been represented in table 1, values of toxicity (WHO-TEQ1998-PCDD-PCDF, WHO-TEQ1998-dl-PCBWHO-TEQ2005-PCDD-PCDF, WHO-TEQ2005-dl-PCB) have been calculated by multiplying obtained results for each congeneric with both factors (TEF) proposed by World Health Organization 1997 [2], and reviewed in 2005 [4], in order to compare the obtained data with the ones published in literature.

The average concentrations of contaminants expressed in WHO-TEQ2005 were comparable for the province of Rieti, Viterbo, Latina and Frosinone, while the province of Rome showed higher values (Figure 5).

Pollutants	Concentration of the Pollutants					
	Average	Median	25th-75th Percentile		Min.	Max.
2,3,7,8-T4CDD	0.232	0.102	0.0176	0.393	0.064	1.22
1,2,3,7,8-P5CDD	0.809	0.769	0.148	1.28	0.01	3.4
1,2,3,4,7,8-H6CDD	0.367	0.305	0.0656	0.48	0.010	2.9
1,2,3,6,7,8-H6CDD	1.86	1.51	0.779	2.62	0.01	7.58
1,2,3,7,8,9-H6CDD	0.244	0.0481	0.0222	0.361	0.01	2.58
1,2,3,4,6,7,8-H7CDD	2.29	1.69	0.531	2.58	0.01	44.4
O8CDD	17.5	14.3	7.6	19.4	0.117	195
2,3,7,8-T4CDF	0.330	0.230	0.0353	0.495	0.01	1.79
1,2,3,7,8-P5CDF	0.155	0.064	0.0290	0.243	0.07	0.668
2,3,4,7,8-P5CDF	2.65	2.44	1.42	3.96	0.0221	8.48
1,2,3,4,7,8-H6CDF	0.766	0.694	0.294	1.13	0.01	4.37
1,2,3,6,7,8-H6CDF	0.763	0.776	0.230	1.08	0.01	3.16
1,2,3,7,8,9-H6CDF	0.196	0.0383	0.0231	0.204	0.01	1.25
2,3,4,6,7,8-H6CDF	0.242	0.0514	0.0275	0.383	0.012	2.03
1,2,3,4,6,7,8-H7CDF	0.792	0.585	0.215	0.911	0.01	7.06
1,2,3,4,7,8,9-H7CDF	0.043	0.0257	0.0146	0.0449	0.05	0.433
O8CDF	0.133	0.0332	0.0165	0.156	0.04	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
Pollutants	**Concentration of the Pollutants**					
	Average	**Median**	**25th-75th Percentile**		**Min.**	**Max**
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 (6 indicators sum) (ng/g fat)	72.5	61.6	37.7	96.7	20.1	359

Table 2: Concentrations of congeneric of PCDD-PCDF and PCB-dl and their sum found in samples of human milk collected within 2013 and 2015 from 81 donors resident in the five provinces of Lazio region expressed as pg/g of fat and as pg/g of fat TEQ-WHO.

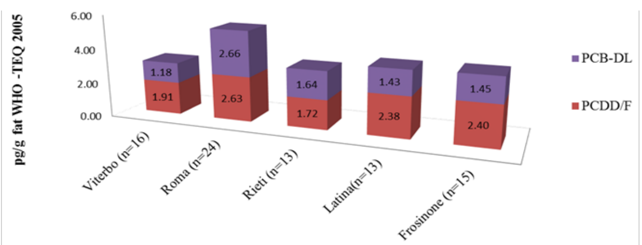

Figure 5: Average content of PCDD – PCDF – PCB-dl in the five provinces of Lazio expressed in pg/g fat TEQ –WHO$_{2005}$

To compare the profiles of the congeners obtained in our study with those found in literature, the most abundant component (O8CDD for the dioxins and PCB118 for dioxin like Polychlorinated biphenyls) is 100% and the other congeners reported according to their abundance (normalization to 100). This profile is consistent to all Lazio provinces (Figure 6 and 7), and 99% similar with the profile of human milk analyzed in Ireland in 2010 (Figure 8) [21]. The average concentrations in pg WHO-TEQ$_{1998}$/g fat for PCDD /F in the breast milk of Lazio were compared with data reported in the literature from 1998 to 2015 (Figure 1). There were not significant associations between provinces and the consumption of milk (p=0.166), cheese (p=0.123), fish (p=0.086) and meat (p=0.716), and between the consumption of food and the rural or urban living. Statistical analysis of the data showed no correlation between the levels of contamination of milk and feeding of donor mothers, as previously reported in the literature [26]. An important negative correlation was present between the number of pregnancies and the concentration of WHO-TEQ_{2005} PCDD/F (r=-0.966, p<0.05) and the sum of WHO-TEQ_{2005} PCB-dl+WHO_{2005}-TEQ PCDD/F (r=-0.867, p<0.05).

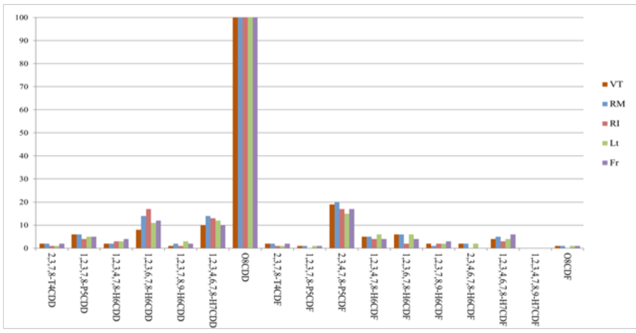

Figure 6: Comparison profile of PCDD/F congeners normalized for the five provinces of Lazio.

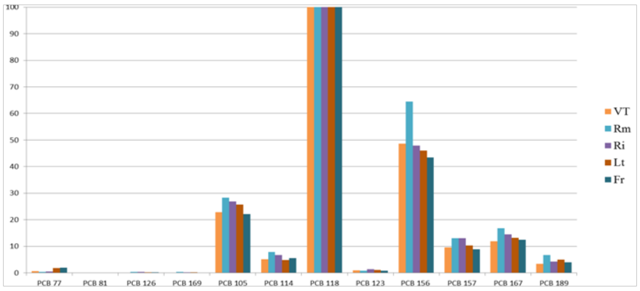

Figure 7: Comparison profile of PCB-dl congeners normalized for the five provinces of Lazio.

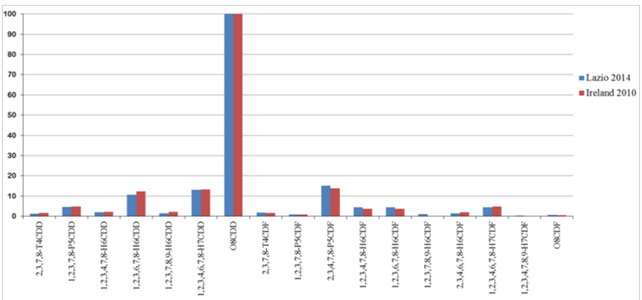

Figure 8: Match found between the profile of PCDD/F to Lazio and to a study carried out in Ireland in 2010. Comparing the profiles of PCDD/F content in human milk of Lazio and Ireland.

Discussion

The purpose of our study was to evaluate whether levels of PCDD-PCDF and PCB-dl in human milk of the Lazio provinces are different from the Italian and European average. The level of PCDD-PCDF and of dl-PCB found in Lazio's human milk samples seemed to be lower than what previously reported in the national and European average, confirming a reduction of those contaminants in human milk during the last decade, with an estimation of 57% and 20.5% reduction of WHO-TEQ PCDDs/Fs and PCB-dl values, respectively [28] and a reduction of 80% in the last 20 years [3]. However, those values are higher than maximum levels allowed by European regulation of baby food. Dioxin and the amount of PCB-dl in the area found in our research show a lower value than other Italian regions, showing a favorable trend over time [25,26,29]. The lack of an evident correlation of dioxin and PCB-dl concentration with food habits agrees with previously published results obtained from the city of Rome in 2008 [26], and could be correlated to the globalization of food market. We found no difference in Dioxins and PCB-dl levels in human milk among the provinces of Lazio, with the exception of Rome, which had higher level, probably due to the bigger extension of the territory together with higher population compared to the other provinces and a lower consumption of locally produced foods. Furthermore,in other provinces of Lazio more local food is consumed. The data obtained in this work are part of the temporal trend that leads to the reduction of pollutants in human milk in Italy and in other countries of the European community (Figure 1) [26,30]. Decrease reported here likely reflects the gradual reduction of dioxin levels in the environment and subsequent decline in food, resulting in reduction of human exposure. The decreasing trend is probably also due to the consumption of food of animal origin containing lesser amounts of dioxins and PCBs coming from farms in which the level of pollutants in feed is kept under control. The content of these pollutants in food for farm animals is controlled in Italy by the "National Plan for Animal Feeding", which keeps the content of these contaminants in check by removing food with pops content above the legal limits from the market.

Conclusion

The human milk continues to prove the best way to monitor exposure to these substances. Nevertheless, the recommendation to breastfeed children according to the WHO recommendations is still valid, independently of the maternal diet. Thus, from this type of study we can provide a valid element to raise the awareness of society for the environment safety to reduce the human milk contamination. Which it has been noticed is that in Lazio region the contaminants are more present in the city center, for the reasons linked to the geographic characteristics (more populated is more polluted). Moreover, this study has noted a reduction in the level of contaminants of these substances in the environment and in human foods over the last decade, probably due to the implementation of stricter legislation and controls in the European community.

Acknowledgment

This research was funded by The Ministry of Health and approved by the ethic committee of Hospital Bambino Gesù. The authors are grateful to the operators of breast milk collection centers and donor mothers.

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