Chemosphere 94 (2014) 76–84

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Dioxins levels in breast milk of women living in Caserta and Naples : Assessment of environmental risk factors

Armando Giovannini [a,*], Gaetano Rivezzi [b], Pietro Carideo [b], Roberta Ceci [a], Gianfranco Diletti [a], Carla Ippoliti [a], Giacomo Migliorati [a], Prisco Piscitelli [c], Alessandro Ripani [a], Romolo Salini [a], Giampiero Scortichini [a]

[a] Istituto Zooprofilattico Sperimentale "G. Caporale", via Campo Boario, 64100 Teramo, Italy
[b] Azienda Ospedaliera Sant'Anna e San Sebastiano, Via F. Palasciano, 81100 Caserta, Italy
[c] Department of Internal Medicine, University of Florence, Italy

HIGHLIGHTS

- We measured dioxins in breast milk from 100 primiparae from Naples and Caserta.
- Dioxin levels were related to a set of environmental and individual risk factors.
- Dioxin in breast milk is correlated to environmental contamination and age of donor.
- Illegal burning of solid waste in the vicinage is also a significant risk factor.

ARTICLE INFO

Article history:
Received 5 March 2013
Received in revised form 21 August 2013
Accepted 2 September 2013
Available online 9 October 2013

Keywords:
Dioxin
Breast milk
Ordinary Kriging
Campania region
Italy

ABSTRACT

Naples and Caserta provinces are extensively affected by the illegal dumping of hazardous and urban wastes, which were periodically set to fire. Several studies were made on the possible health impact of this illegal waste management. The aim of the study was to detect dioxins levels in breast milk of volunteer primiparae and to assess the possible source of dioxins in the affected areas. The authors determined dioxins levels in breast milk from 100 primiparae from the study area and collected anamnestic information on donors. We determined dioxins levels in breast milk from 100 primiparae from the study area and collected anamnestic information on donors. As a measure of environmental risk of dioxins (EDR) we used the interpolated values of dioxins concentration in buffalo milk samples collected in the study area. Correlations between the EDR, age of the mother, smoking habit, cheese consumption, occupation in activity at risk, presence of plants for the disposal of toxic waste or illegal burning of solid waste near the residence of the donor and dioxin level in breast milk were investigated. The dioxin level in breast milk is significantly correlated to the EDR, the age of the sampled women and the presence of illegal burning of solid waste.

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs), commonly referred to as dioxins, is a group of organochlorine compounds that belong to a list originally comprised of 12 persistent organic pollutants (POPs) that are regulated under the Stockholm Convention on POPs.

The toxicity of individual dioxin congeners differs considerably. Among 210 congeners, only 17 have significant toxicity because of the substitution of hydrogen with chlorine atoms at least in the 2,3,7,8-positions (Van den Berg et al., 1998, 2006).

These compounds have a similar toxicological profile to that of the most toxic congener (2,3,7,8-TCDD).

The toxic responses include dermal toxicity, immunotoxicity, carcinogenicity, and adverse effects on reproduction, embryo development, and endocrine functions (IARC, 1997, 2012; Schecter et al., 2006; Lundqvist et al., 2006; Yamada et al., 2006; Cordier et al., 2010; Imura et al., 2010; Puga, 2011).

Carcinogenic effects of dioxins have been described and documented at high dose exposures, such as after the Seveso accident (Consonni et al., 2008; Pesatori et al., 2009; Warner et al., 2011)

* Corresponding author. Tel.: +39 861 332240; fax: +39 861 332251.
E-mail address: a.giovannini@izs.it (A. Giovannini).

0045-6535/$ - see front matter © 2013 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.chemosphere.2013.09.017

or the exposure to the Agent Orange during Vietnam War, although with mixed and inconsistent results among the different publications (Ketchum et al., 1999; Pavuk et al., 2005; Michalek and Pavuk, 2008). At low doses, however, dioxins are known to be responsible of adverse health effects, even if of a completely different nature than the onset of tumours. In fact, *in utero* and lactational exposure of children to relatively low dioxin doses can permanently reduce sperm quality (Mocarelli et al., 2011).

Human milk is recognized as the preferred matrix because has several important advantages. Biomonitoring of human milk data can provide information on the exposure of the mother as well as the infants. More recently, it has been recognized that human milk is an ideal matrix to generally monitor levels of persistent organic pollutants in the environment (WHO, 2007). Globally, a number of papers have been published on the measurement of PCDD/Fs in human milk (see Table 1). The study designs vary considerably from study to study:

- In some study several milk samples are pooled together before the analysis, in other studies samples are analysed individually.
- Parity of the milk donor is also important because the higher the parity the more PCDD/Fs have been depleted, therefore many studies tend to prefer primiparous donors, or at least donors are separated according to their parity.
- The age of the donor is also relevant for POPs since they are subject to accumulation along the life.
- The origin of the donor -or at least her residence in a specific place for a specified duration of time- is important for the donor to be representative of a certain geographical area.
- Various sources of environmental and occupational exposure may affect the body burden of PCDD/Fs and many studies are devoted to compare exposure factors each other or to compare the presence and absence of such factors.

All the listed factors must be taken into consideration in designing a study on the burden of PCDD/Fs and, depending on the objectives of each study, different solutions and different study design may be implemented. In Table 1 is a summary of different designs implemented in different countries during the last 15 years. An important guideline in designing a study based on the testing of breast milk to evaluate the human exposure to PCDD/Fs, is given by a protocol prepared by WHO in 2007 (WHO, 2007). In Table 1, the listed studies are sorted according to their compliance to the WHO guideline. This does not mean that the studies are sorted according to their overall quality, because the specific objectives of the studies may suggest derogating, even substantially, from the WHO guideline.

A vast area of the provinces of Caserta and Naples in the Campania region of Italy has been extensively affected in the last 30 years by the illegal dumping of hazardous and urban wastes, which were eventually set to fire.

In this area, an excess mortality for various types of tumours has been observed (Martuzzi et al., 2009), and it is suspected that such phenomenon is linked to the environmental pollution by various chemicals, including dioxin. The level of exposure of human beings to dioxins in the study area was unknown.

In the framework of the National Residues Surveillance Plan activities, dioxin levels exceeding the European Union tolerance limit were detected in sheep milk samples collected in November 2001 in Campania region (Diletti et al., 2003). To investigate this phenomenon, a survey was conducted from April 2002 to September 2004. The sampling area covered a wide area of the Naples and Caserta provinces, where samples of milk and dairy products were taken. Animal feed contamination levels were also determined by taking samples at the same farms where milk samples were collected. In particular, buffalos, the most valued dairy species in the area, were extensively sampled. Results showed that a correlation existed between the PCDD–PCDFs levels detected in buffalo milk and those detected in animal feed collected in the same farm (Diletti et al., 2008).

In addition, the analysis of congener profiles suggested a strong possibility that dioxin contamination in buffalo milk could be linked to uncontrolled urban and industrial waste incineration (Diletti et al., 2008). In contrast, no investigation was performed to assess the dioxin exposure of the population resident in the area of interest.

The aim of this paper is to assess dioxin exposure of human population, and its correlation with environmental and dietary factors in the provinces of Caserta and Naples. Data used for the assessment are the results of tests performed in 2006 on 100 breast milk samples, provided by donors from the two provinces. The geographical distribution of sampling was able to ensure representativeness of the different local conditions. The study was financed by the Ministry of Health.

2. Materials and methods

2.1. Sampling area

The study area encompassed Naples and Caserta provinces, where the practice of illegal waste dumping is widespread. To obtain a representative geographical distribution of samples from the study area, the total number of volunteer mothers to be recruited was stratified according to a previous investigation carried out in the area of interest by the World Health Organization (WHO), in cooperation with several Italian public scientific institutions (Martuzzi et al., 2007). In this study 196 municipalities in Naples and Caserta provinces were sorted into five groups of increasing environmental exposure to the presence of illegal dumping. To design the breast milk sampling scheme of our study, the entire dataset of the WHO study was dichotomised by grouping the five classes of environmental exposure into two classes: low-risk municipalities (groups 1–2 of the cited study) and high-risk municipalities (groups 3–5). The sample was equally stratified in the two classes of municipalities.

2.2. Environmental information

Besides the classification of municipalities according to the WHO study, which was mainly based on the presence of dumping sites, additional environmental information was also collected to assess the level of risk to which the recruited mothers were exposed.

Data issued from a previous study published by the authors on dioxin levels in buffalo milk from different herds fed with feed of local origin were used as indicators of environmental contamination due to dioxins. A total number of 291 buffalo milk samples from the Campania region were analysed between 2002 and 2004 (Diletti et al., 2008).

Data on the presence of dumping sites and on the fraction of population in each municipality living in the impact area of the dumping sites were collected from the WHO study (Martuzzi et al., 2007).

Data on the practice of solid waste illegally burned in the proximity of the residence of sampled volunteers were collected through a direct interview of the mothers themselves.

2.3. Collection of samples

From the study area, a total of 100 volunteer mothers who had given birth to newborns at the St. Anna & St. Sebastian Hospital

Table 1
Summary of the main studies published worldwide since 2002 on PCDD–PCDFs levels in breast milk samples.

References	Country	Period of sampling	Donors selection and origin	PCDD/F TEQ_{98} pg g^{-1} fat Mean and range	PCDD/F TEQ_{05} pg g^{-1} fat Mean and range	Pooled/individual samples
This study	Italy	2007–2008	95 primiparae, under 32 years of age From provinces of Naples and Caserta in southern Italy Body burdens were compared to environmental risk based on a continuous scale, without dichotomization	8.47 (3.81–18.97)[b]	6.46 (2.89–14.64)[b]	Individual samples
Sun et al. (2010)	China	2006–2007	60 primiparae, under 30 years of age From northern Chinese cities of Shijiazhuang, Tiantin and Yantai	ND	4.2 (1.4–7.13)	Individual samples
Schuhmacher et al. (2009)	Spain	2007	15 primiparae, 25–35 years 9 living in urban zones + 6 women living near an industrial area	ND	7.6 (2.8–11.2)	Individual samples
Mannetje et al. (2013)	New Zealand	2007–2010	39 primiparae, 20–30 years From rural and urban areas	ND	3.54 (1.39–10.83)	Individual samples
Chao et al. (2005)	Taiwan	2001	30 primiparae, 20–35 years Central Taiwan	7,37 (4,03–13,04)	ND	individual samples
Chovancová et al. (2011)	Slovakia	2006–2007	33 primiparae, 17–36 years Living close to industrial areas and incinerators	9.1 (2.3–39.1)	ND	Individual samples
Kamińska et al. (2013)	Poland	2008–2010	40 primiparae, 22–38 years From urban and rural areas	5.053 (0.261–11.01)	ND	Individual samples
Lignell et al. (2009)	Sweden	1996–2006	183 primiparae, 19–41 years From Uppsala County	8.2 (2.8–23.0)	7.0 (2.3–19.0)	Individual samples
Raab et al. (2008)	Germany	2005	42 primiparae, 22–46 years From Bavaria (samples collected in Munich Hospital)	9.92	8.17	Individual samples
Schecter et al. (2002)	Russia	1998	18 primiparae, age range not specified Living in industrialized cities near Lake Baikal	23.76 (10.10–54.59)	ND	Individual samples
Guan et al. (2006)	Japan	1999–2000	120 primiparae + 120 secundiparae, 25–34 years From Tokyo Reported values refer to primiparae only[a]	17.02	ND	Individual samples
Reis et al. (2007)	Portugal	1999–2003	Primiparae and multiparae, 18–52 years From Lisbon Community and Madeira Community Reported values refer to primiparae only (98 donors, 18–40-year old)[a]	9.2 (2.8–20.9)	ND	Individual samples
Todaka et al. (2008)	Japan	2002–2004	30 primiparae, 21–40 years; 30 multiparae, 21–47 years Living in Sapporo Reported values refer to primiparae only[a]	7.4 (4.0–22.0)	ND	Individual samples
Shen et al. (2012)	China	2008	74 primiparae and multiparae, 19–29 years Living in Zhejian province: 23 urban area + 51 rural area	ND	2.78 (0.66–12.30)	Individual samples
Wittsiepe et al. (2007)	Germany	2000–2003	169 mothers, parity not specified, 19–42 years Living in an industrialized area of Germany	13.84 (1.80–34.70)	ND	Individual samples
Çok et al. (2009)	Turkey	2007	51 primiparae and multiparae, 20–40 years From five different cities throughout Turkey	7.18	ND	Individual samples

Costopoulou et al. (2006)	Greece	2003	8 mothers, parity not specified, 28–44 years Living in different areas in Athens	7.27 (3.43–11.28)	ND	Individual samples
Focant et al. (2013)	France	2007	44 primiparae and multiparae, 24–41 years From Ile-de-France and from the region Rhône-Alpes	13.36 (0.86–42.22)	ND	Individual samples
Focant et al. (2002)	Belgium	2000–2001	20 primiparae and multiparae, 26–38 years Living in or close to the industrial area of Liege (Wallonia)	29.4 (16.0–52.11)	ND	Individual samples
Ulaszewska et al. (2011)	Italy	2008–2009	59 primiparae and multiparae, 20–40 years From Milan, Piacenza and Giugliano in Campania (province of Naples)	ND	4.37 (1.26–9.55)	Individual samples
Bake et al. (2007)	Latvia	2002–2004	30 primiparae, age not specified 15 from industrial area + 15 not exposed	7.66	ND	Pooled samples
Abballe et al. (2008)	Italy	1998–2001	39 primiparae, age range not specified From Venice and Rome Comparison based on food habits	12.56	ND	Pooled samples
Li et al. (2009)	China	2007	24 pooled samples from 1237 primiparae, 18–35 years From 12 provinces. In each province, one urban site and two rural sites were sampled	3.73 (1.66–6.67)	3.12 (1.38–5.82)	Pooled samples
Pratt et al. (2012)	Ireland	2010	11 pooled samples from 109 primiparae, 20–41 years Comparison of data from Dublin, Cork, Wicklow and Donegal in 2002, and Dublin and Galway in 2010 Reported values refer to 2010 only[a]	6.32	ND	Pooled samples

[a] Reported values = values in columns PCDD/F TEQ_{98} and PCDD/F TEQ_{05}.
[b] The median is reported instead of the mean.

(Caserta) were enrolled. To obtain reliability of exposure data, and to allow comparison with previous exposure studies, donors were selected according to WHO protocol (WHO, 2007), using the following criteria: primiparous mothers below 32 years of age, who have been residing at least for the previous 10 years in the sampling area, exclusively breastfeeding. Both mother and child were apparently healthy and pregnancy was without complications. All participants gave written informed consent.

Enrolled women were provided with accurate instructions on how to collect and temporarily store breast milk samples. Sampling occurred between the second and eighth week after delivery. At least 50 mL of milk were collected during or immediately after the breastfeeding, within a 48-h interval. Each mother collected the sample in glass containers, either using a human milk pump or manually. Subsequently, all the individual samples were stored at the St. Anna & St. Sebastian Hospital, and separately kept at +4 °C for a maximum of 72 h, and for longer time at −20 °C. Finally, samples were sent to the Italian National Reference Laboratory for Dioxins and PCBs, where the laboratory analyses were performed.

The study design also included a questionnaire to collect data on the exposure to possible risk factors. In detail, the risk factors and type of data considered were as follows: age of the mother, smoking habits, frequency of cheese consumption and origin (local, national or both) of the consumed cheese, occupation in activities at risk, presence of toxic waste disposal plants or illegal burning of solid waste near the residence of the donor.

Data collected with the questionnaire, and the analytical results were used for statistical evaluation of the parameters that influenced the body burden.

2.4. Chemical analysis

Milk from individual mothers (50–100 mL) was spiked with the specific PCDD–PCDFs standard solution, a mixture of $^{13}C_{12}$-labelled congeners.

Fat was extracted from samples by a liquid–liquid extraction procedure. After solvent evaporation, gravimetric lipid determination was performed.

The resulting extract was cleaned up using sulphuric acid and potassium hydroxide, followed by an automated cleanup process with silica gel, alumina and activated carbon columns.

Each batch of seven samples was accompanied by a laboratory blank and a control sample.

PCDD–PCDFs were separated by high resolution gas chromatography (HRGC) on a DB5 MS capillary column (60 m × 0.25 mm, 0.10 μm film thickness), and determined by high-resolution mass spectrometry (HRMS), at a resolution of 10000 in the selected ion monitoring mode (SIM). The HRGC–HRMS system consisted of a GC Trace Series 2000 coupled with a MAT 95 XP (Thermo Fischer, Bremen, Germany).

Toxic equivalent (WHO-TEQ_{98}-PCDD–PCDFs and WHO-TEQ_{05}-PCDD–PCDFs) values were calculated using the toxic equivalent factor model proposed by World Health Organization in 1997 (Van den Berg et al., 1998), and then revised 2005 (Van den Berg et al., 2006). Both calculation methods were used for the sake of comparison of our results with those in the published literature, where, in many cases, only one of the methods is reported. The statistical analyses performed in this study are based on the values of WHO-TEQ_{98}-PCDD–PCDFs.

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.

For the PCDD–PCDFs analysed, $^{13}C_{12}$-labelled congeners recovery rates ranged from 60% to 90%. Analytical uncertainty was in the order of ±15% for WHO-TEQs.

2.5. Statistical analysis

The environmental datasets analysed in the study were not directly comparable to each other. Most data were referred to the municipal basis, while the measurements of dioxins in buffalo milk samples were individually geo-referenced at the geographical coordinates of the holding where buffaloes were kept. For the statistical analysis, it was necessary to degrade the geographical information available to a common degree of detail. Therefore, all data were assigned to the relevant municipality.

To transform the point data on concentration of dioxins in buffalo milk into a municipal average, an interpolation based on Ordinary Kriging was used (Burrough, 1986).

For each municipality, interpolation surface average of this concentration was used as an indicator of the risk level for the entire municipality. For simplicity, this indicator is referred to as Environmental Dioxin Risk (EDR) by the authors hereafter.

To choose the proper statistical analysis to apply normality of the distributions used in the analysis was evaluated (Siegel and Castellan, 1988).

The role of the possible factors affecting dioxins levels in breast milk was evaluated by analysis of covariance (ANCOVA). In the model, age, EDR, the 5 classes of environmental exposure of the WHO study, smoking habits, cheese consumption, the mothers' occupational exposure, presence of solid waste disposal facilities, and illegal solid waste burning in the proximity of the donors' residence were considered as risk factors. Concerning the exposure to cheese, only the frequency of its consumption was considered, because only 7% of the donors declared not to consume cheese of local origin. All donors who consume cheese at least twice a week were considered exposed.

Finally, the correlation between the percentage of the dumping sites' impact area in the total municipality area and the EDR was investigated using the Spearman rank correlation coefficient.

3. Results

3.1. Comparison between EDR and the WHO study

The geographical distribution of EDR in the study area and in the neighbouring province of Benevento is shown in Fig. 1. The highest values of EDR are located in the urban areas along the border between Caserta and Naples provinces.

The municipalities characterized by the high values of EDR are those with the highest scores on the index of exposure in the WHO study. This relation is statistically significant, since the Spearman rank correlation coefficient between the index of exposure and the EDR in the municipalities was $\rho = 0.275$ ($p < 0.0001$).

Therefore, the geographical distribution of the EDR illustrated in Fig. 1 was considered as a risk map for exposure to dioxins, and used in all subsequent analyses.

3.2. Contamination levels in breast milk

The sampling involved 45 municipalities located in Caserta, Naples and Benevento provinces. A total of 100 breast milk samples were collected between June 2007 and May 2008.

After preliminary analysis, a total of five samples were excluded: for two of the five were collected from donors over 32 years of age, and the other three were collected in the Benevento province, which was not included in the study area.

Of the remaining 95 samples, a total of 48 samples were taken from low-risk municipalities, and 47 samples from high-risk municipalities. A total of 41 municipalities in the study area were sampled.

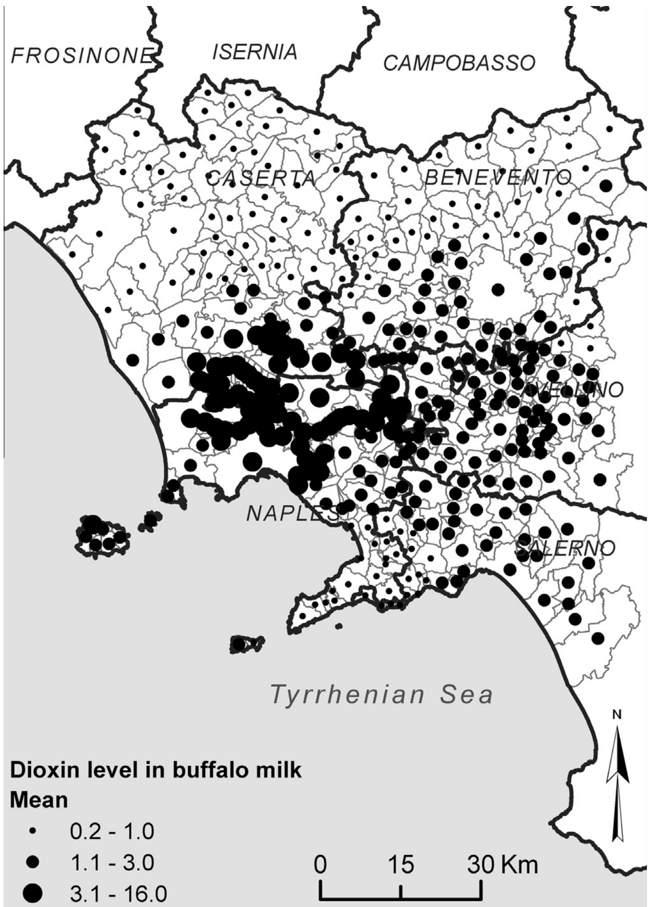

Fig. 1. Geographical distribution of the Environmental Dioxin Risk (EDR) derived from 291 buffalo milk samples. Dioxin levels are expressed as pg WHO-TEQ_{98} g^{-1} fat.

Descriptive statistical data on the levels of PCDD–PCDFs congeners in breast milk are reported in Table 2. The results are expressed as pg g^{-1} milk fat, and the total equivalent toxicity as WHO-TEQ_{98}-PCDD–PCDFs and WHO-TEQ_{05}-PCDD–PCDFs pg g^{-1} milk fat.

The median concentration of WHO-TEQ_{98}-PCDD–PCDFs was 8.47 pg g^{-1} fat. The pattern of congeners is very similar in all samples, although the total concentrations of PCDD–PCDFs are different among mothers from different municipalities. The contamination profiles were not significantly grouped in different clusters, indicating a possible common source for the contamination.

The contamination profile is in line with those reported in other biomonitoring surveys conducted in Europe (Focant et al., 2002; Wittsiepe et al., 2007).

The geographical distribution of the mean concentration of PCDD–PCDFs in breast milk, standardized by age to the median age of the mothers in the sample, is shown in Fig. 2.

This geographical distribution overlapped the distribution of the most contaminated municipalities (Figs. 1 and 2). This observation has been confirmed by the analysis of covariance.

3.3. Factors influencing PCDD–PCDFs levels in breast milk

The distribution of WHO-TEQ_{98}-PCDD–PCDFs values in breast milk was not significantly different from a normal distribution, and did not require transformations to ensure normality.

The summary results of the analysis of covariance for the concentration of PCDD–PCDFs in breast milk are shown in Table 3. The parameters of the model and the statistical significance of the variables analysed are shown in Table 4.

The concentration of PCDD–PCDFs in breast milk is significantly correlated to the EDR and the age of the sampled women. A significant association was also found with the presence of illegal burning of solid waste in the vicinity of the residence of the sampled women.

Finally, a significant correlation was observed between the percentage of municipal area impacted by dumping sites and the EDR (Spearman rank correlation coefficient $\rho = 0.344$, $p < 0.0001$). This significant correlation might be due to one of the possible sources of dioxins contamination, such as the illegal burning of solid waste disposed in the dumping sites.

4. Discussion

4.1. Comparison between the present study and the published literature

A difficulty in comparing studies performed in different years is the use of two different sets of TEQ (WHO-TEQ_{98}-PCDD–PCDFs and WHO-TEQ_{05}-PCDD–PCDFs). Studies published before 2006 use

Table 2
PCDD–PCDFs levels in breast milk samples collected between 2007 and 2008 from 95 primiparae donors resident in Caserta and Naples provinces, expressed as concentration in pg g^{-1} fat and as WHO-TEQ_{98}-PCDD–PCDFs and WHO-TEQ_{05}-PCDD–PCDFs (pg WHO-TEQ g^{-1} fat).

Contaminant	Concentration of the contaminant		
Dioxins and furans (pg g^{-1} fat)	Median	25th–75th percentile	Range
2,3,7,8-TCDD	0.70	0.57–0.93	0.30–2.19
1,2,3,7,8-PeCDD	2.57	1.85–3.00	1.13–6.65
1,2,3,4,7,8-HxCDD	0.98	0.78–1.27	0.30–3.29
1,2,3,6,7,8-HxCDD	5.55	4.13–6.71	2.35–20.51
1,2,3,7,8,9-HxCDD	0.96	0.72–1.27	0.05–3.45
1,2,3,4,6,7,8-HpCDD	5.12	4.15–6.99	1.20–20.95
OCDD	32.34	26.37–51.30	12.43–126.38
2,3,7,8-TCDF	0.41	0.31–0.61	0.03–1.30
1,2,3,7,8-PeCDF	0.31	0.18–0.43	0.01–1.11
2,3,4,7,8-PeCDF	7.48	5.53–8.83	3.38–15.89
1,2,3,4,7,8-HxCDF	2.26	1.78–2.81	1.28–5.26
1,2,3,6,7,8-HxCDF	2.12	1.62–2.64	1.00–4.88
2,3,4,6,7,8-HxCDF	1.00	0.71–1.34	0.03–2.43
1,2,3,7,8,9-HxCDF	0.01	0–0.05	0.00–0.25
1,2,3,4,6,7,8-HpCDF	1.54	1.27–2.56	0.24–66.56
1,2,3,4,7,8,9-HpCDF	0.04	0.01–0.10	0–5.29
OCDF	0.31	0.18–0.60	0–14.76
WHO-TEQ_{98}-PCDD–PCDF (pg WHO-TEQ g^{-1} fat)	8.47	6.64–9.81	3.81–18.97
WHO-TEQ_{05}-PCDD–PCDF (pg WHO-TEQ g^{-1} fat)	6.46	5.10–7.50	2.89–14.64

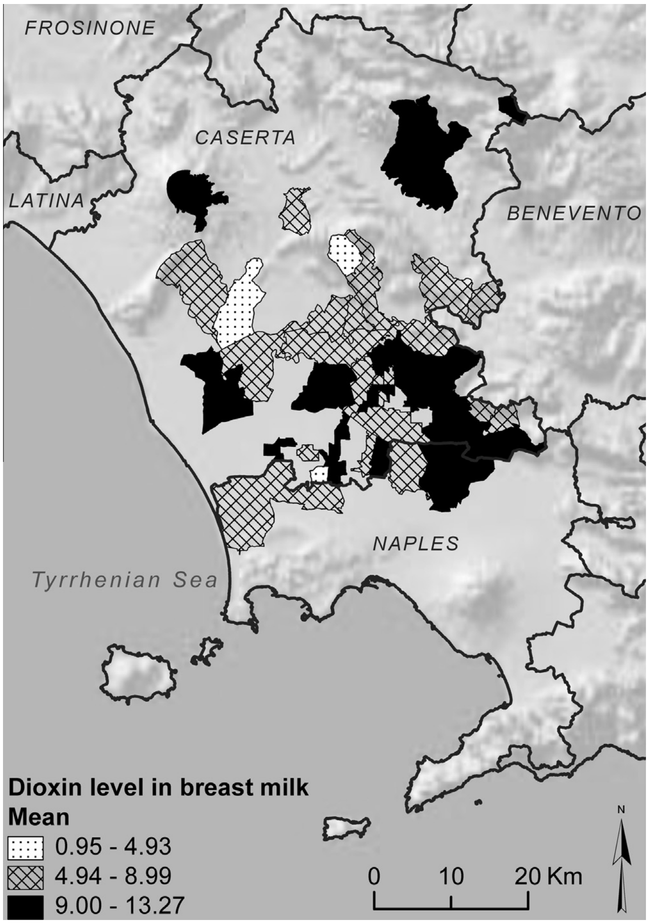

Fig. 2. Mean value of dioxins level reported in breast milk per municipalities. The concentration of dioxins in milk was standardized according to the age of the mother to the value expected for the median age of all donors participating in the study (29 years), based on the regression line obtained in the Analysis of covariance. Dioxin levels are expressed as pg WHO-TEQ$_{98}$ g^{-1} fat.

Table 3
Summary results of the analysis of covariance for the concentration of PCDD–PCDFs in breast milk.

Source	DF	Sum of squares	Mean of squares	Fisher's *F*	*Pr > F*
Model	3	191.84	63.95	12.12	9.612e−07
Residues	91	480.26	5.28		
Total	94	672.10			

WHO-TEQ$_{98}$-PCDD–PCDFs, while in subsequent studies WHO-TEQ$_{98}$-PCDD–PCDFs or WHO-TEQ$_{05}$-PCDD–PCDFs or both can be found. This complicates the comparisons along time. For the sake of comparability, in our study we expressed the results using both methods.

Considering the WHO-TEQ$_{98}$-PCDD–PCDFs, in which most studies express their results, a general remark that can be drawn from the exam of Table 1 is that the upper limits of the range for industrial areas are between 20 an over 50 pg g^{-1} fat, while elsewhere the upper limits are generally below 20 pg g^{-1} fat. The average burden is within narrower bounds than the upper limit (3.73–23.76 pg g^{-1} fat) for all studies. Our study involved rural and urban, but not industrial areas, and according to the previous remark, the upper limit of the range was 18.97 pg g^{-1} fat.

Finally, it can be noticed from Table 1 that (within the same set of TEQ and under similar environmental conditions) older studies have higher values of PCDD/Fs concentrations then more recent ones.

4.2. Comparison between the EDR and the WHO study

On the basis of our analysis, the municipalities with high values of EDR coincided with those that had received the highest score of the index of exposure in the WHO study. Nevertheless, the EDR, as calculated in our study, could probably provide a better indicator of the contamination level of dioxins compared with the index of exposure in the WHO study. The score in the WHO study was based on the presence of dumping sites, which are not the sole source of dioxins. The significant correlation between the concentrations of dioxins in breast milk and the EDR would support our assertion.

4.3. Contamination levels in breast milk

Previous surveys carried out in Italy and globally showed contamination levels within the same range detected in this study, with average values slightly higher: pooled breast milk samples collected between 1998 and 2001 in Rome recorded total WHO-TEQ$_{98}$-PCDD–PCDFs values of 9.40 pg g^{-1} fat, while in Venice concentrations were 11.6 pg g^{-1} fat in the sub-population with high fish consumption, and 14.8 in the sub-population with low consumption of fish (Abballe et al., 2008). In our study, the range of WHO-TEQ$_{98}$-PCDD–PCDFs values was 3.81–18.97, and the average was 8.53 pg g^{-1} fat.

In the frame of the third WHO Human Milk Field Study conducted in 2000–2002, pooled milk samples from Italy reported WHO-TEQ$_{98}$-PCDD–PCDFs levels of 12.66 pg g^{-1} fat. These data, together with those of a few other countries, represented the highest

Table 4
Parameters of the model for PCDD–PCDFs and the statistical significance of the variables analysed.

Parameter	Value	Standard error	Student's *t*	*Pr > t*	95% Lower confidence limit	95% Upper confidence limit
Intercept[a]	−2.889	2.308	−1.252	0.214	−7.412	1.634
Age[a]	0.366	0.080	4.587	1.43e−05	0.210	0.523
EDR[a]	0.193	0.070	2.752	0.007	0.056	0.331
Illegal burning of solid waste – No[a]	0.000	–	–	–	–	–
Illegal burning of solid waste – Yes[a]	1.840	0.691	2.662	0.009	0.485	3.195
Work in industry at risk	Variables not in the final model					
Presence of plants for disposal of solid waste near the residence						
Smoker						
Consumption of cheese						
Classification of municipalities according to Martuzzi et al. (2007)						

[a] Variables significantly correlated to PCDD–PCDFs levels in breast milk.

contamination levels recorded among all participating countries (Malisch and van Leeuwen, 2003).

In Italy, only another study was conducted based on individual samples of breast milk, therefore thoroughly comparable to our study (Ulaszewska et al., 2011). The authors compared the levels of contamination by dioxins in breast milk of women from Milan (Lombardy region), Piacenza (Emilia Romagna region) and Giugliano (near Naples in Campania region). The results from their study were expressed in the new format of WHO toxicity equivalent factors (WHO-TEQ_{05}-PCDD–PCDFs). The contamination levels in breast milk in their study were in the same range in the three cities, but with a higher mean in Milan and Piacenza than in Giugliano (mean and range: Milan: 4.70, 2.42–9.55; Piacenza: 4.67, 2.43–7.70; Giugliano: 3.78, 1.26–9.44). Our study included six mothers from Giugliano in Campania and their mean and range of contamination level, expressed in WHO-TEQ_{05}-PCDD–PCDFs, were 6.40 and 4.07–8.43 respectively. Our study also included other municipalities of Caserta and Naples provinces, in both high and low contamination areas. Overall, the contamination detected in our study had a mean and range of 6.53 and 2.89–14.64 respectively, when expressed as WHO-TEQ_{05}-PCDD–PCDFs. This information clearly indicated that the average contamination of the study area of Caserta and Naples is similar to that of the cities of Milan and Piacenza, but the highly contaminated municipalities of Caserta and Naples have dioxins concentrations that are up to 1.5 times the maximum contamination recorded in Milan by Ulaszewska et al. (2011).

In comparing data from studies performed over the past years, it must, however, be considered that a trend of declining concentrations of PCDD–PCDFs has been observed in many industrialized countries. A possible explanation is the reduction in the emission of these contaminants in the environments (Norén and Meironyté, 2000; La Kind et al., 2001; Stigum et al., 2005; Wittsiepe et al., 2007; Todaka et al., 2008).

4.4. Factors influencing dioxin levels in breast milk

The analysis of covariance detected an association between the level of contamination in breast milk and the age of the mother, the level of EDR and the presence of illegal burning of solid waste in the vicinity of the residence of the sampled woman.

The association between the age of the woman and the concentration of dioxins in the milk has been already reported in the scientific literature (Wittsiepe et al., 2007; Todaka et al., 2008).

The main source of dioxins for human beings is dietary. Within this context, foods of animal origin represent the predominant source, accounting for about 90%. The significant correlation between the EDR and the levels of contamination in breast milk is, in fact, an indication of the dietary importance of locally produced food despite the uniformity of diet fostered by modern distribution chains. Despite the contamination detected in buffalo milk and despite the spatial association observed between the contamination of buffalo milk and the levels of dioxin in human breast milk, the results obtained in the analysis of covariance, however, suggest that locally produced cheese was not the main source of exposure to dioxins.

The association between the level of contamination of breast milk and the presence of illegal burning of solid waste in the vicinity of the sampled mothers' residence would indicate that the local foods' source of dioxin contamination could be the illegal burning of solid waste. This indication is further confirmed by the significant Spearman rank correlation coefficient between the percentages of municipal area impacted by dumping sites and the concentration of dioxins in buffalo milk in the municipalities (EDR). The source of contamination, therefore, could be the burning of solid waste at the illegal dumping sites.

5. Conclusions

On the basis of our analysis, the municipalities with high values of EDR coincided with those that had received the highest score on the index of exposure in the WHO study.

Previous surveys carried out in Italy showed contamination levels within the same range detected in this study, and the contamination profile is in line with those reported in other biomonitoring surveys conducted in Europe.

The concentration of dioxins in breast milk is significantly correlated to the EDR, and with the age of the sampled women. A significant association was also found with the presence of illegal solid waste burning in the vicinity of the sampled women's residence.

Throughout our study, the level of environmental exposure of the sampled subjects is measured following continuous scales: the EDR is a continuous variable, as is the percentages of municipal area impacted by dumping sites. The use of a continuous scale instead of a dichotomous or polytomous classification allows the use of a statistical analysis with a higher potency than that allowed by dichotomous comparisons. It is, therefore, particularly suitable for detecting fine differences and general patterns.

The results obtained by the various phases and components of the statistical analysis performed confirm each other, and the general hypothesis of the study, in a consistent way.

In conclusion, the approach of this study would represent a useful model for the assessment of the exposure of human beings to dioxins.

Acknowledgement

The investigation was financially supported by the Italian Ministry of Health (Grant: IZSAM 11/06 RC).

References

Abballe, A., Ballard, T.J., Dellatte, E., di Domenico, A., Ferri, F., Fulgenzi, A.R., Grisanti, G., Iacovella, N., Ingelido, A.M., Malisch, R., Miniero, R., Porpora, M.G., Risica, S., Ziemacki, G., De Felip, E., 2008. Persistent environmental contaminants in human milk: concentrations and time trends in Italy. Chemosphere 73 (Suppl. 1), S220–S227.

Bake, M.A., Linnika, Z., Sudmalis, P., Kočan, A., Jursa, S., Pike, A., Ruce, M., 2007. Assessment of the exposure of breast milk to persistent organic pollutants in Latvia. Int. J. Hyg. Environ. Health 210, 483–489.

Burrough, P.A., 1986. Principles of Geographical Information Systems for Land Resources Assessment. Oxford University Press, New York, USA.

Chao, H.-R., Wang, S.-L., Su, P.-H., Yu, H.-Y., Yu, S.-T., Päpke, O., 2005. Levels of polychlorinated dibenzo-p-dioxins and dibenzofurans in primipara breast milk from Taiwan: estimation of dioxins and furans intake for breastfed infants. Hazard. Mater. A121, 1–10.

Chovancová, J., Ĉonka, K., Kocăn, A., Stacová Sejáková, S., 2011. PCDD, PCDF, PCB and PBDE concentrations in breast milk of mothers residing in selected areas of Slovakia. Chemosphere 83, 1383–1390.

Çok, I., Donmez, M.K., Uner, M., Demirkaya, E., Henkelmann, B., Shen, H., Kotalik, J., Schramm, K.-W., 2009. Polychlorinated dibenzo-p-dioxins, dibenzofurans and polychlorinated biphenyls levels in human milk from different regions of Turkey. Chemosphere 76, 1563–1571.

Consonni, D., Pesatori, A.C., Zocchetti, C., Sindaco, R., Cavalieri D'Oro, L., Rubagotti, M., Bertazzi, P.A., 2008. Mortality in a population exposed to dioxin after the Seveso, Italy, accident in 1976: 25 years of follow-up. Am. J. Epidemiol. 167, 847–858.

Cordier, S., Lehébel, A., Amar, E., Anzivino-Viricel, L., Hours, M., Monfort, C., Chevrier, C., Chiron, M., Robert-Gnansia, E., 2010. Maternal residence near municipal waste incinerators and the risk of urinary tract birth defects. Occup. Environ. Med. 67, 493–499.

Costopoulou, D., Vassiliadou, I., Papadopoulos, A., Makropoulos, V., Leondiadis, L., 2006. Levels of dioxins, furans and PCBs in human serum and milk of people living in Greece. Chemosphere 65, 1462–1469.

Diletti, G., Torreti, L., De Massis, M.R., Migliorati, G., Scortichini, G., 2003. A case of milk contamination by PCDD/Fs in Italy: analytical levels and contamination source identification. Organohalogen. Compd. 64, 1–4.

Diletti, G., Ceci, R., Conte, A., De Benedictis, A., Migliorati, G., Scortichini, G., 2008. Milk contamination from dioxins in Italy: source identification and intervention strategies. In: Mehmetli, E., Koumanova, B. (Eds.), The Fate of Persistent Organic

Pollutants in the Environment. Springer, Dordrecht, The Netherlands, pp. 301–314.
Focant, J.F., Pirard, C., Thielen, C., De Pauw, E., 2002. Levels and profiles of PCDDs, PCDFs and cPCBs in Belgian breast milk. Estimation of infant intake. Chemosphere 78, 763–770.
Focant, J.-F., Fréry, N., Bidondo, M.-L., Eppe, G., Scholl, G., Saoudi, A., Oleko, A., Vandentorren, S., 2013. Levels of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls in human milk from different regions of France. Sci. Total Environ. 452–453, 155–162.
Guan, P., Tajimi, M., Uehara, R., Watanabe, M., Oki, I., Ojima, T., Nakamura, Y., 2006. Congener profile of PCDDs, PCDFs, and dioxin-like PCBs in the breast milk samples in Tokio, Japan. Chemosphere 62, 1161–1166.
IARC, 1997. Polychlorinated dibenzo-para-dioxins and polychlorinated dibenzofurans. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 69. IARC Press, Geneva, Switzerland. <http://monographs.iarc.fr/ENG/Monographs/vol69/volume69.pdf> (accessed 31.10.11).
IARC, 2012. A review of human carcinogens—Part F: chemical agents and related occupations. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 100F. IARC Press, Geneva, Switzerland. <http://monographs.iarc.fr/ENG/Monographs/vol100F/index.php> (accessed 31.01.13).
Imura, H., Yamada, T., Mishima, K., Fujiwara, K., Kawaki, H., Hirata, A., Sogawa, N., Ueno, T., Sugahara, T., 2010. Effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin suggests abnormal palate development after palatal fusion. Congenit. Anom. 50, 77–84.
Kamińska, J. et al., 2013. The use of PowerPrep and HRGC/HRMS for biological monitoring of exposure to PCDD, PCDF and dl-PCB in Poland. Int. J. Hyg. Environ. Health. http://dx.doi.org/10.1016/j.ijheh.2013.02.009v.
Ketchum, N.S., Michalek, J.E., Burton, J.E., 1999. Serum dioxin and cancer in veterans of Operation Ranch Hand. Am. J. Epidemiol. 149, 630–639.
La Kind, J.S., Berlin, C.M., Naiman, D.Q., 2001. Infant exposure to chemicals in breast milk in the United States: what we need to learn from a breast milk monitoring program. Environ. Health Perspect. 109, 75–88.
Li, J., Zhang, L., Wu, Y., Liu, Y., Zhou, P., Weng, S., Liu, J., 2009. A national survey of polychlorinated dioxins, furans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) in human milk in China. Chemosphere 75, 1236–1242.
Lignell, S., Aune, M., Darnerud, P.O., Cnattingius, S., Glynn, A., 2009. Persistent organochlorine and organobromine compounds in mother's milk from Sweden 1996–2006: compound-specific temporal trends. Environ. Res. 109, 760–767.
Lundqvist, C., Zuurbier, M., Leijs, M., Johansson, C., Ceccatelli, S., Saunders, M., Schoeters, G., Ten Tusscher, G., Koppe, J.G., 2006. The effects of PCBs and dioxins on child health. Acta Paediatr. 95 (Suppl. 453), S55–S64.
Malisch, R., van Leeuwen, F.X.R., 2003. Results of the WHO-coordinated exposure study on the levels of PCBs, PCDDs and PCDFs in human milk. Organohalogen. Compd. 64, 140–143.
Mannetje, A., Coakley, J., Bridgen, P., Brooks, C., Harrad, S., Smith, A.H., Pearce, N., Douwes, J., 2013. Current concentrations, temporal trends and determinants of persistent organic pollutants in breast milk of New Zealand women. Sci. Total. Environ. 458–460, 399–407.
Martuzzi, M., Bianchi, F., Comba, P., Fazzo, L., Minichilli, F., Mitis, F., 2007. Trattamento dei rifiuti in Campania: impatto sulla salute umana. Studio di correlazione tra rischio ambientale da rifiuti, mortalità e malformazioni congenite. <http://www.protezionecivile.it/cms/attach/editor/rapportoRifiuti2/Studio_di_correlazione.pdf> (accessed 03.11.11).
Martuzzi, M., Mitis, F., Bianchi, F., Minichilli, F., Comba, P., Fazzo, L., 2009. Cancer mortality and congenital anomalies in a region of Italy with intense environmental pressure due to 15 waste. Occup. Environ. Med. 66, 725–732.
Michalek, J.E., Pavuk, M., 2008. Diabetes and cancer in veterans of Operation Ranch Hand after adjustment for calendar period, days of spraying, and time spent in Southeast Asia. J. Occup. Environ. Med. 50, 330–340.
Mocarelli, P., Gerthoux, P.M., Needham, L.L., Patterson Jr., D.G., Limonta, G., Falbo, R., Signorini, S., Bertona, M., Crespi, C., Sarto, C., Scott, P.K., Turner, W.E., Brambilla, P., 2011. Perinatal exposure to low doses of dioxin can permanently impair human semen quality. Environ. Health Perspect. 119, 713–718.
Norén, K., Meironyté, D., 2000. Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20–30 years. Chemosphere 40, 1111–1123.
Pavuk, M., Michalek, J.E., Schecter, A., Ketchum, N.S., Akhtar, F.Z., Fox, K.A., 2005. Did TCDD exposure or service in Southeast Asia increase the risk of cancer in air force Vietnam veterans who did not spray agent orange? J. Occup. Environ. Med. 47, 335–342.
Pesatori, A.C., Consonni, D., Rubagotti, M., Grillo, P., Bertazzi, P.A., 2009. Cancer incidence in the population exposed to dioxin after the "Seveso accident": twenty years of follow-up. Environ. Health 8, 39. http://dx.doi.org/10.1186/1476-069X-8-39 (Online 15 September 2009).
Pratt, I.S., Anderson, W.A., Crowley, D., Daly, S.F., Evans, R.I., Fernandes, A.R., Fitzgerald, M.P., Geary, M.P., Keane, D.P., Malisch, R., McBride, J., Morrison, J.J., Reilly, A., Tlustos, C., 2012. Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) in breast milk of first-time Irish mothers: impact of the 2008 dioxin incident in Ireland. Chemosphere 88, 865–872.
Puga, A., 2011. Perspectives on the potential involvement of the AH receptor–dioxin axis in cardiovascular disease. Toxicol. Sci. 120, 256–261.
Raab, U., Preiss, U., Albrecht, M., Shahin, N., Parlar, H., Fromme, H., 2008. Concentrations of polybrominated diphenyl ethers, organochlorine compounds and nitro musks in mother's milk from Germany (Bavaria). Chemosphere 72, 87–94.
Reis, F.M., Sampaio, C., Aguiar, P., Melim, J.M., Miguel, J.P., Päpke, O., 2007. Biomonitoring of PCDD/Fs in populations living near portuguese solid waste incinerators: levels in human milk. Chemosphere 67, S231–S237.
Schecter, A., Piskac, A.L., Grosheva, E.I., Matorova, N.I., Ryan, J.J., Fürst, P., Päpke, O., Abdibi, J., Silver, A., Ghaffar, S., 2002. Levels of dioxins and dibenzofurans in breast milk of women residing in two cities in the Irkutsk region of Russian Siberia compared with American levels. Chemosphere 47, 157–164.
Schecter, A., Birnbaum, L., Ryan, J.J., Constable, J.D., 2006. Dioxins: an overview. Environ. Res. 101, 419–428.
Schuhmacher, M., Kiviranta, H., Ruokojärvi, P., Nadal, M., Domingo, J.L., 2009. Concentrations of PCDD/Fs, PCBs and PBDEs in breast milk of women from Catalonia, Spain: a follow-up study. Environ. Int. 35, 607–613.
Shen, H., Ding, G., Wu, Y., Pan, G., Zhou, X., Han, J., Li, J., Wen, S., 2012. Polychlorinated dibenzo-p-dioxins/furans (PCDD/Fs), polychlorinated biphenyls (PCBs), and polybrominated diphenyl ethers (PBDEs) in breast milk from Zhejiang, China. Environ. Int. 42, 84–90.
Siegel, S., Castellan, N.J., 1988. Nonparametric Statistics for the Behavioral Sciences, second ed. McGraw-Hill, New York, USA.
Stigum, H., Eggesbø, M., Polder, A., Skaare, J.U., Becher, G., Nicolaysen, T., Thomsen, C., Magnus, P., 2005. Dioxin and dioxin-like compounds in breast milk from Norwegian mothers. Organohalogen. Compd. 67, 1560–1563.
Sun, S.J., Zhao, J., Leng, J., Wang, P., Wang, Y., Fukatsu, H., Liu, D., Liu, X., Kayama, F., 2010. Levels of dioxins and polybrominated diphenyl ethers in human milk from three regions of northern China and potential dietary risk factors. Chemosphere 80, 1151–1159.
Todaka, T., Hirakawa, H., Kajiwara, J., Hori, T., Tobiishi, K., Onozuka, D., Kato, S., Sasaki, S., Nakajima, S., Saijo, Y., Sata, F., Kishi, R., Iida, T., Furue, M., 2008. Concentrations of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and dioxin-like polychlorinated biphenyls in blood and breast milk collected from 60 mothers in Sapporo City, Japan. Chemosphere 72, 1152–1158.
Ulaszewska, M.M., Zuccato, E., Capri, E., Iovine, R., Colombo, A., Rotella, G., Generoso, C., Grassi, P., Melis, M., Fanelli, R., 2011. The effect of waste combustion on the occurrence of polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs) in breast milk in Italy. Chemosphere 82, 1–8.
Van den Berg, M., Birnbaum, L., Bosveld, A.T.C., Brunstrom, B., Cook, P., Feely, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., van Leeuwen, F.X.R., Liem, A.K.D., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillit, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Perspect. 106, 775–792.
Van den Berg, M., Birnbaum, L., Denison, M., De Vito, M., Farland, W., Feeley, M., Fiedler, H., Hakansson, H., Hanberg, A., Haws, L., Rose, M., Safe, S., Schrenk, D., Tohyama, C., Tritscher, A., Tuomisto, J., Tysklind, M., Walker, N., Peterson, R.E., 2006. The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol. Sci. 93, 223–241.
Warner, M., Mocarelli, P., Samuels, S., Needham, L., Brambilla, P., Eskenazi, B., 2011. Dioxin exposure and cancer risk in the Seveso Women's Health Study. Environ. Health Perspect. 119, 1700–1705.
WHO, 2007. Fourth WHO-Coordinated Survey of Human Milk for Persistent Organic Pollutants in Cooperation with UNEP—Guidelines for Developing a National Protocol, Geneva, Switzerland. <http://www.who.int/foodsafety/chem/POPprotocol.pdf> (accessed 11.11.11).
Wittsiepe, J., Furst, P., Schrey, P., Lemm, F., Kraft, M., Eberwein, G., Winneke, G., Wilhelm, M., 2007. PCDD/F and dioxin-like PCB in human blood and milk from German mothers. Chemosphere 67, 286–294.
Yamada, T., Mishima, K., Fujiwara, K., Imura, H., Sugahara, T., 2006. Cleft lip and palate in mice treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin: a morphological in vivo study. Congenit. Anom. 46, 21–25.