DIOXINS LEVELS IN BREAST MILK OF WOMEN LIVING IN CAMPANIA REGION: ASSESSMENT OF ENVIRONMENTAL RISK FACTORS

Giovannini A[1], Rivezzi G[2], Ceci R[1], Diletti G[1], Ippoliti C[1], Migliorati G[1], Ripani A[1], Salini R[1], Scortichini G*[1]

[1]Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Via Campo Boario, 64100 Teramo, Italy; [2]St. Anna & St. Sebastian Hospital, Via F. Palasciano, 81100 Caserta, Italy

Introduction

A vast area of the provinces of Caserta and Naples in the Campania region of Italy has been extensively affected, since the eighties of the past century, by the illegal dumping of hazardous and urban wastes, which were periodically set to fire. In the last decades, several studies on the possible health impact of this illegal waste management and disposal were published[1-4]. All studies showed significant excess of cancer mortality. No specific factors were investigated in detail and no attempt was made to investigate the association of mortality with specific carcinogenic substances so far. In this area, dioxins levels exceeding the European Union maximum level were detected in sheep milk samples collected in 2001[5]. To investigate this phenomenon a survey was conducted in the period 2002 - 2004. Results showed the correlation between the PCDDs-PCDFs levels detected in milk and in animal feed[6]. In 2006, the Ministry of Health financed a study to assess dioxins exposure of human population and correlations with environmental and dietary factors in the provinces of Caserta and Naples. The study was performed by testing breast milk provided by donors from the two provinces, with a geographical distribution able to ensure the representativeness of the different local conditions. The aim of this paper is to present the results of this study and to assess the possible source of dioxins in the affected areas.

Materials and methods

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[2]. 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 of our study, the entire dataset of the WHO study was dichotomized 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.

Environmental information

Besides the classification of municipalities according to the WHO study, mainly based on the presence of dumping sites, additional environmental information was 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 of 291 buffalo milk samples from Campania region were analyzed between 2002 and 2004[6]. 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 was collected from the WHO study[2].

Collection of samples

A total of 95 volunteer mothers who have given birth to newborns at the St. Anna & St. Sebastian Hospital (Caserta) were enrolled from June 2007 to May 2008. To obtain reliability of exposure data and to allow comparison with previous exposure studies, donors were selected according to WHO protocol[7]. The study design also included a questionnaire to collect data on the exposure to possible risk factors. In detail, risk factors considered were as follows: 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. Data collected with the questionnaire and analytical results were used for statistical evaluation of which parameters influenced the body burden.

Chemical analysis

The analytical method has been already described elsewhere[8]. Toxic equivalent (WHO-TEQ_{98}-PCDDs-PCDFs and WHO-TEQ_{05}-PCDDs-PCDFs) values were calculated as upper bound concentrations using the toxic equivalent factor model proposed by World Health Organization in 1998[9] and then revised in 2005[10].

Statistical analysis

The environmental datasets analyzed in the study were not directly comparable each other. Most data was referred to a 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. To transform the point data on concentration of dioxins in buffalo milk into a municipal average, an interpolation based on Ordinary Kriging was used[11]. For each municipality, the average of the interpolation surface of this concentration was used as 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. The role of the possible factors affecting dioxins levels in breast milk was evaluated by analysis of covariance (ANCOVA). In the model, the age, the EDR, and the 5 classes of environmental exposure of the WHO study, smoking habit, cheese consumption, the occupational exposure of the mothers, the presence of facilities for the disposal of solid waste, and illegal burning of solid waste in the proximity of the residence of the donors were considered as risk factors.

Results and discussion

Comparison between EDR and the WHO study

The geographical distribution of EDR in the study area is shown in Figure 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 of 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 was considered the risk map for exposure to dioxins and used in all subsequent analysis.

Contamination levels in breast milk

A total of 95 breast milk samples were collected from 41 municipalities: 48 samples were taken from low-risk municipalities and 47 samples from high-risk municipalities. Descriptive statistical data on levels of dioxin congeners in breast milk are reported in Table 1. The results are expressed as pg/g milk fat, and the total equivalent toxicity as WHO-TEQ_{98}-PCDDs-PCDFs and WHO-TEQ_{05}-PCDDs-PCDFs pg/g milk fat. The geographical distribution of the mean concentration of dioxins in breast milk, standardized by age to the median age of the mothers, is shown in Figure 2. This geographical distribution overlapped the distribution of the most contaminated municipalities (Figures 1 and 2). This observation has been confirmed by the analysis of covariance. Previous surveys carried out in Italy showed contamination levels within the same range detected in this study. Pooled breast milk samples collected between 1998 and 2001 in Rome recorded total WHO-TEQ_{98}-PCDDs-PCDFs values of 9.40 pg/g fat, while in Venice concentrations were 11.6 pg/g fat in the sub-population with high fish consumption and 14.8 in the sub-population with low consumption of fish[12]. In our study, the average of WHO-TEQ_{98}-PCDDs-PCDFs value was 8.53 pg /g 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}-PCDDs-PCDFs levels of 12.66 pg/g fat[13]. In Italy, only another study was conducted based on individual samples of breast milk and, therefore, thoroughly comparable to our study[14]. 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 as WHO-TEQ_{05}-PCDDs-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). 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}-PCDDs-PCDFs. This information 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.[14].

Factors influencing dioxin levels in breast milk

The distribution of WHO-TEQ_{98}-PCDDs-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 PCDDs-PCDFs in breast milk are shown in Table 2. The parameters of the

model and the statistical significance of the variables analyzed are shown in Table 3. 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 burning of solid waste in the vicinity of the residence of the sampled women. Concerning the possible origin of dioxins, a significant correlation was observed between the percentage of municipal area impacted by dumping sites and the EDR (Spearman rank correlation coefficient ρ=0.344, 2-sided p<0.0001). This significant correlation might be the result of one of the possible sources of dioxins contamination, i.e. the illegal burning of solid waste disposed in the dumping sites.

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

References

1. Comba P et al. (2006) *Ann NY Acad Sci* 1076:449–461
2. Martuzzi M et al. (2007) http://www.protezionecivile.it/cms/attach/editor/rapportoRifiuti2/Studio_di_correlazione.pdf
3. Martuzzi M et al. (2009) *Occup Environ Med* 66:725-732
4. Fazzo L et al. (2011) *Ann Ist Super Sanita* 47:181-191
5. Diletti G et al. (2003) *Organohalogen Compd* 64:1-4
6. Diletti G et al. (2008) In: Mehmetli E, Koumanova B, eds. *The fate of persistent organic pollutants in the environment*. Dordrecht, The Netherlands: Springer, 301-314
7. WHO (2007) http://www.who.int/foodsafety/chem/POPprotocol.pdf)
8. Diletti G et al. (2008) *Organohalogen Compd* 70:1337-1340
9. Van den Berg M et al. (1998) *Environ Health Perspect* 106:775-792
10. Van den Berg M et al. (2005) *Toxicol Sci* 93:223-241.
11. Burrough PA (1986) *Systems for Land Resources Assessment*. New York, USA: Oxford University Press
12. Abballe A et al. (2008) *Chemosphere* 73:Suppl 1:S220-S227
13. Malisch R et al. (2003) *Organohalogen Compd* 64:140-143
14. Ulaszewska MM et al. (2011) *Chemosphere* 82:1-8

Figure 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 fat

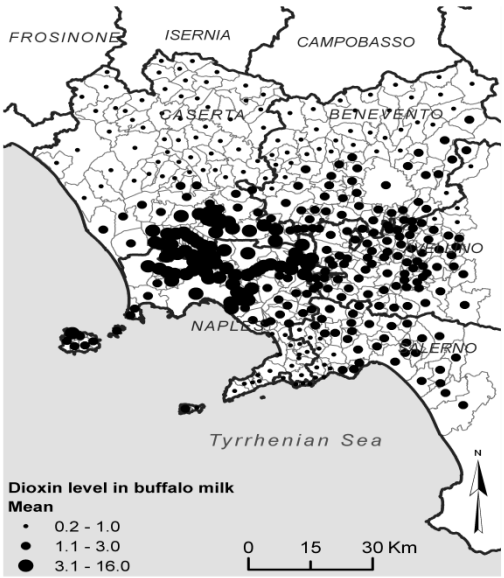

Figure 2. Mean concentration of dioxins in breast milk per municipalities, standardized by age to the median age of the mothers in the sample (29 years). Dioxin levels are expressed as pg WHO-TEQ_{98}/g fat

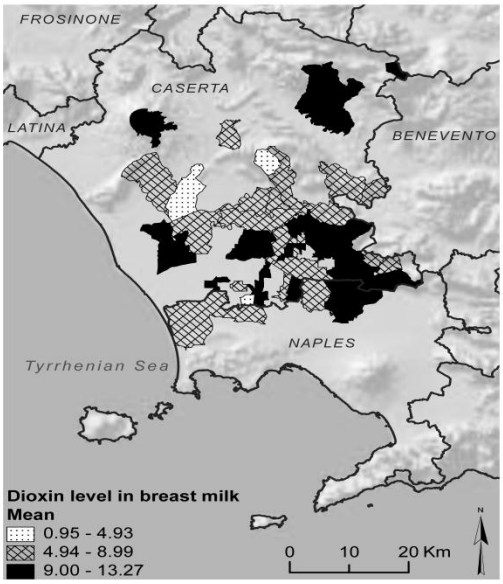

Table 1. PCDDs-PCDFs levels in breast milk, expressed as concentration in pg/g fat and as WHO-TEQ_{98} and WHO-TEQ_{05}

Dioxins and Furans	**Concentration of PCDDs-PCDFs (pg/g fat) Median (25^{th}-75^{th} 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 (1.848-3.00, 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 (0.00-0.05, 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, 0.00-5.29)
OCDF	0.31 (0.18-0.60, 0.00-14.76)
PCDDs-PCDFs (pg WHO-TEQ_{98}/g fat)	8.47 (6.64-9.81, 3.81-18.97)
PCDDs-PCDFs (pg WHO-TEQ_{05}/g fat)	6.46 (5.10-7.50, 2.89-14.64)

Table 2. Summary results of the analysis of covariance for the concentration of PCDDs-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			

Table 3. Parameters of the model for PCDDs-PCDFs and the statistical significance of the variables analyzed

Parameter	Value	Standard error	Student's t	Pr > t	95 % lower confidence limit	95 % upper confidence limit
Intercept*	-2.889	2.308	-1.252	0.214	-7.412	1.634
Age*	0.366	0.080	4.587	1.43e-05	0.210	0.523
EDR*	0.193	0.070	2.752	0.007	0.056	0.331
Illegal burning of solid waste – No*	0.000	-	-	-	-	-
Illegal burning of solid waste – Yes*	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						

* Variables significantly correlated to PCDDs-PCDFs levels in breast milk