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Dioxins and PCBs contamination in milk and dairy products from Province of Taranto (Puglia Region, Southern Italy): a six years spatio-temporal monitoring study

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Abstract
Introduction. Taranto Province (Puglia Region, Southern Italy) is of particular Public Health relevance due to the presence of industrial sources of dioxins and PCBs. The aim of this study was to analyze the spatio-temporal distribution of these pollutants in milk and cheese produced from 2013 to 2018.
Materials and methods. Raw milk and dairy products were sampled in the farms located within 20 km from the industrial area.
Results. 1005 milk samples were collected. Median (IQR) concentrations were: dioxins 0.21 (0.21) pg WHO-TEQ/g fat; dioxins+DL-PCBs 0.83 (0.71) pg WHO-TEQ/g fat; NDL-PCBs 1.92 (1.56) ng/g fat. Overall, only 6 (0.6%) samples were found to be non-compliant for at least one pollutants group. Temporal analysis showed a decreasing trend in dioxins and PCBs concentrations over the observed years and higher values in the first trimester. Spatial analysis showed higher levels of PCBs in areas closest to the industrial pole. 70 dairy products samples were collected. Median pollutants concentrations were far below the EU limits and no exceedances were observed.
Conclusions. The extremely low number of exceedances appeared as an encouraging result and supported the validity of the Public Health measures adopted by the Department of Prevention of Taranto.

Key words
- dioxins
- PCBs
- environmental contamination
- Taranto
- milk
- dairy products

INTRODUCTION

Polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and polychlorinated biphenyls (PCBs) are classified by World Health Organization (WHO) as environmental pollutants with a global distribution and high resistance to degradation [1-6].

Long-term exposure to Dioxins (PCDD/Fs) and some PCBs, referred to as dioxin-like PCBs (DL-PCBs) due to their similar toxicological properties, has been shown to cause a range of adverse effects on the nervous, immune and endocrine systems, to impair reproductive function and to cause cancer. Other PCBs referred to as non-dioxin-like PCBs (NDL-PCBs) have a different mechanism of toxicity, but they too can damage human health [1, 3, 5-9].

More than 80% of total exposure is attributable to dietary intake, that represents the main route of PCDD/Fs and PCBs exposure for humans [1, 5, 7]. In particular, the consumption of animal origin foods, like milk and eggs, leads to a greater risk of bioaccumulation due to the lipophilic properties of these pollutants [1, 5].

Taranto, a coastal city in the South of Italy (Ionian Sea, Puglia Region), is of particular relevance in this context due to the type of industrial settlements accounting for known potential sources of PCDD/Fs and PCBs (the most important steel plant in Europe, an oil refinery, a cement works, thermoelectric plants, waste

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incinerators, discharges and military harbours) and to the environmental contamination present in different matrices, including soil [10-13].

In this regard, in the Province of Taranto several farms account for a significant production of different foods of animal origin, including milk and dairy products. Here because, since 2008, the Department of Prevention of the Local Health Authority of Taranto has carried out an extraordinary monitoring plan in order to assess PCDD/Fs and PCBs contamination in different food matrices produced in the farms adjacent to the industrial area of Taranto.

The aim of this study was to analyze the spatio-temporal distribution of PCDD/Fs, DL-PCBs and NDL-PCBs values and EU limit [14] exceedances in raw milk and dairy products collected between January 2013 and December 2018 from the farms located within a radius of 20 km from the industrial area of Taranto, in order to guarantee the healthiness of the product placed on the market, identify critical seasons for food contamination, and verify and develop effective public health strategies to protect the health of consumers together with the production chain of the territory.

MATERIALS AND METHODS

Sampling

We included in this study all the raw milk and dairy products samples collected by the staff of the Department of Prevention of Local Health Authority of Taranto between January 2013 and December 2018 from the monitored farms of Province of Taranto. The extraordinary monitoring plan activities included inspections and georeferenced samplings in farms located within a radius of 20 km from the industrial area of Taranto. All distances from the industrial area were measured from the reference center of the steel plant (40.505647, 17.210601). Raw milk sampling was carried out at the time of milking in farms within the study area and preferably from animals raised outdoors and fed on pasture. Dairy products sampling was carried out in dairies that produced milk-based products using raw milk from farms within the study area. The samples were sent for chemical analysis to the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (National Reference Laboratory for Halogenated POPs in food and feed).

Chemical analysis

Chemicals. Solvents such as absolute ethanol, ethyl ether, petroleum ether, n-hexane, dichloromethane, acetone, toluene and isooctane were analytical grade (Honeywell Burdick & Jackson, Seezle, Germany). Ultra-pure water was generated within the laboratory by means Purelab option-Q system (ELGA LabWater, High Wycombe, United Kingdom). Other reagents included anhydrous sodium sulphate, concentrated sulphuric acid, ammonium hydroxide solution and sodium chloride, all at reagent grade (Honeywell Burdick & Jackson, Seezle, Germany).

Prepacked multilayer silica, alumina, and carbon columns were obtained from Fluid Management Systems (Massachussetts, USA).

All standard solutions for PCDD/Fs, DL-PCBs and NDL-PCBs were supplied by Wellington Laboratories (Guelph, Ontario, Canada).

Analytical methodology. Samples were tested by validated and accredited methods (EN ISO/IEC 17025) routinely used for PCDD/F and PCB analysis in food; these methods have successfully been tested in a number of inter-laboratory studies.

The 17 PCDD/Fs, the 12 DL-PCBs and the 6 NDL-PCBs [14] were determined through methods based on US EPA (1994) Method 1613 B for PCDD/Fs and US EPA (2008) Method 1668 B for PCBs. Both methods are based on isotopic dilution and high resolution mass spectrometry (HRMS) detection. In order to adapt the analytical procedures to the matrix under examination, variations have been made in the extraction and purification phases of the sample.

All the samples under examination were homogenized and a representative amount of raw milk (100-200 g) and dairy products (10-30 g) reconstituted with water, was taken. Before extraction, samples were fortified with a mixture of the internal standards containing: 17 PCDD/Fs $^{13}C_{12}$-labeled (0.2-0.4 ng); 12 DL-PCBs $^{13}C_{12}$-labeled (1.0 ng); 6 NDL-PCBs $^{13}C_{12}$-labeled (1.0 ng).

Samples were mixed with ethyl alcohol and ammonia solution and then fat was extracted by a mixture of diethyl ether and petroleum ether 1:1 (v/v). Lipid content was determined gravimetrically for all samples after evaporation of the solvent. The extract fat was dissolved in hexane and a liquid-liquid partitioning process was performed to exclude the lipid component using concentrated sulfuric acid, 20% aqueous potassium hydroxide, and saturated aqueous sodium chloride. It was then purified on an automated Power-Prep™ system (Fluid Management System Massachusetts, USA) using multilayer silica, activated carbon and alumina columns. The two eluates containing PCDD/Fs and PCBs, were concentrated by evaporation under nitrogen stream and dissolved in the corresponding recovery standards solutions ($^{13}C_{12}$-labeled PCDD/Fs and PCBs different from the previous ones).

The instrumental analysis was performed using high resolution gas chromatography - high resolution mass spectrometry (HRGC-HRMS), using GC Trace Series 2000 coupled to a MAT 95 XL (Thermo Fisher Scientific, USA) and a Trace Series 1310 GC, coupled to a DFS (Thermo Fisher Scientific, USA). PCDD/F congeners were separated on a DB-5 MS capillary column 60 m × 0.25 mm × 0.10 µm (J&W Scientific, California, USA) while the chromatographic separation of DL-PCBs and NDL-PCBs was carried out on HT8-PCB capillary column 60 m × 0.25 mm × 0.25 µm (SGE Analytical science, Melbourne, Australia). The acquisition of the masses was performed in Single Ion Monitoring (SIM) mode at a resolution of 10,000.

Toxic equivalents (TEQs) for PCDD/Fs and DL-PCBs were calculated using the World Health Organization Toxic Equivalency Factors (WHO-TEFs) [9]. TEQ concentrations were determined multiplying the analytical result of each congener by the corresponding WHO TEF, while for NDL-PCBs, the result was reported as the sum of the 6 indicator congeners.

According to the European legislation [14], all reported sum concentrations and TEQ values were expressed in "upper bound" terms (not detects posed equal to the LOQ – limit of quantification) and the contamination levels were expressed on fat basis.

A laboratory blank and a control sample were analyzed for each batch of 10 and 20 samples, respectively. Recovery rates of labeled congeners ranged from 60% to 90%, and the analytical uncertainty was in the order of ± 18% for WHO-TEQs and the sum of six NDL-PCBs. Method performance was in agreement with the requirements for method of analysis used in official control of the levels of PCDD/Fs and PCBs in foodstuff [15].

Statistical analysis

Statistical analysis was performed using R version 3.6.2 (released on 2019-12-12). Statistical significance α was fixed to 0.05.

In order to account for non-normality, evaluated through Shapiro-Wilk test, numerical variables (means of the measured values of the pollutants concentrations) [14, 15] were reported as range, IQR, median and mean and compared through Kruskal Wallis rank sum test. Comparisons were carried out between years and trimesters.

Categorical variables (means of the measured values of the pollutants concentrations minus the associated expanded uncertainty that are above the established EU maximum level) [14, 15] were reported as absolute and relative frequencies and compared through Fisher Exact Test (Fisher-Freeman-Halton Exact Test for contingency tables larger than 2×2). Comparisons were carried out between years and trimesters.

Maps were created with Microsoft Excel version 2002 (Build 12527.20194).

In order to assess the correlations between the pollutants (values) non-normally distributed and the distance from the industrial area as well as within each combination of these pollutants, Spearman rank correlation coefficients ρ were calculated. P-values were computed via the asymptotic t approximation.

RESULTS

1005 raw milk samples were collected between 2013 and 2018 from the monitored farms of Province of Taranto: 359 bovine, 324 sheep, 320 goat, 1 equine and 1 buffalo milk samples. Results of overall pollutants values and exceedances were reported in *Table 1*. Overall, only 6 (0.6%) samples were found to be non-compliant for at least one pollutants group: 2 bovine (2013), 1 sheep (2014) and 3 goat (2017) milk samples.

Temporal trends of milk pollutant values over the observed years and trimesters were reported in *Figure 1*.

Kruskal Wallis rank sum test between years showed a significant difference for dioxins ($p < 0.0001$), dioxins+DL-PCBs ($p < 0.0001$) and NDL-PCBs ($p < 0.0001$), with a decreasing trend over the years.

Kruskal Wallis rank sum test between trimesters showed a significant difference for dioxins ($p < 0.0001$), dioxins+DL-PCBs ($p < 0.0001$) and NDL-PCBs ($p < 0.0001$), with higher values in the first trimester.

Milk pollutants exceedances (%) distribution between years were (p from Fisher Exact test):

- for dioxins >2.5 pg WHO-TEQ/g fat: 1 (33.3%) in 2014 and 2 (66.7%) in 2017 (p = 0.2636);
- for dioxins + DL-PCBs >5.5 pg WHO-TEQ/g fat: 2 (33.3%) in 2013, 1 (16.7%) in 2014 and 3 (50.0%) in 2017 (p = 0.2067);
- for NDL-PCBs >40 ng/g fat: 1 (100.0%) in 2013 (p = 0.5871).

Milk pollutants exceedances (%) distribution between trimesters were (p from Fisher Exact test):

- for dioxins >2.5 pg WHO-TEQ/g fat: 1 (33.3%) in II and 2 (66.7%) in III (p = 0.0398);
- for dioxins + DL-PCBs >5.5 pg WHO-TEQ/g fat: 1 (16.7%) in I, 2 (33.3%) in II, 2 (33.3%) in III, 1 (16.7%) in IV (p = 0.2919);
- for NDL-PCBs >40 ng/g fat: 1 (100%) in IV (p >0.9999).

Spatial distribution of milk pollutants values in the monitored farms of the Province of Taranto was shown in *Figure 2*.

Results of Spearman rank correlation ρ for milk pollutants were reported in *Table 2*.

Distance from the industrial area of Taranto showed significant ($p < 0.0001$) negative correlation with dioxins+DL-PCBs (ρ = -0.19) and NDL-PCBs (ρ = -0.29), while no significant correlation was found with dioxin (p = 0.2717).

All pairwise combinations of milk pollutants values

Table 1
Pollutants values in raw milk and dairy products (Taranto, 2013-18)

Milk (n = 1005)	**Min**	**1st Qt.**	**Median**	**Mean**	**3rd Qt.**	**Max**	**EU limit**	**N. exceedances (%)**
Dioxins (pg/g)	0.00	0.13	0.21	0.28	0.34	4.65	2.50	3 (0.3%)
Dioxins + DLPCBs (pg/g)	0.01	0.56	0.83	1.10	1.27	23.28	5.50	6 (0.6%)
NDLPCBs (ng/g)	0.03	1.30	1.92	2.53	2.86	57.32	40.00	1 (0.1%)
Dairy products (n = 70)	**Min**	**1st Qt.**	**Median**	**Mean**	**3rd Qt.**	**Max**	**EU limit**	**N. exceedances (%)**
Dioxins (pg/g)	0.04	0.10	0.17	0.21	0.26	0.63	2.50	0 (0.0%)
Dioxins+DLPCBs (pg/g)	0.25	0.43	0.62	0.75	0.85	2.96	5.50	0 (0.0%)
NDLPCBs (ng/g)	0.79	1.09	1.43	1.83	2.06	7.02	40.00	0 (0.0%)

Dioxins = sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs = sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins+DLPCBs = sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs = sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); pollutants values = mean of the measured values, fat basis; pollutants exceedances = mean of the measured values minus the expanded uncertainty of the mean, fat basis [14, 15].

Figure 1
Temporal trends of raw milk pollutants values over the observed years and trimesters (Taranto, 2013-18).
Dioxins = sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs = sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins+DLPCBs = sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs = sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); pollutants values = mean of the measured values, fat basis [14, 15].

showed significant correlation (p <0.0001): the lowest correlation was found between dioxins and NDL-PCBs (ρ = 0.53).

Finally, 70 dairy products samples were collected between 2013 and 2018 from dairies that produced milk-based products using raw milk from the monitored farms of Province of Taranto: 41 bovine, 20 sheep and 9 goat cheese samples. Results of overall pollutants values and exceedances were reported in *Table 1*. No exceedances were observed.

DISCUSSION

Since milk and cheese production represents an important cultural and economic heritage for the population of the Province of Taranto, the Department of Prevention of the Local Health Authority have long been engaged on the dual front of protecting consumers and safeguarding primary production.

Median PCDD/Fs and PCBs concentrations in milk were far below the EU limits and very few exceedanc-

Figure 2
Spatial distribution of raw milk pollutants values (Taranto, 2013-18).
Dioxins = sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs = sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins + DLPCBs = sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs = sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); pollutants values = mean of the measured values, fat basis [14, 15].

Table 2
Spearman rank correlation ρ for raw milk pollutants values (Taranto, 2013-18)

Milk (n = 1005)			
Pollutant	**Distance**	**ρ**	**p**
Dioxins (pg/g)	Distance (km)	-0.03	0.2717
Dioxins + DLPCBs (pg/g)	Distance (km)	-0.19	<0.0001
NDLPCBs (ng/g)	Distance (km)	-0.29	<0.0001
Poll. 1	**Poll. 2**	**ρ**	**p**
Dioxins (pg/g)	Dioxins + DLPCBs (pg/g)	0.78	<0.0001
Dioxins (pg/g)	NDLPCBs (ng/g)	0.53	<0.0001
Dioxins + DLPCBs (pg/g)	NDLPCBs (ng/g)	0.77	<0.0001

Dioxins = sum of dioxins (WHO-PCDD/F-TEQ); DLPCBs = sum of dioxin-like PCBs (WHO-PCB-TEQ); dioxins + DLPCBs = sum of dioxins and dioxin-like PCBSs (WHO-PCDD/F-PCB-TEQ); NDLPCBs = sum of non-dioxin like PCBs: PCB28, PCB52, PCB101, PCB138, PCB153 and PCB180 (ICES – 6); pollutants values = mean of the measured values, fat basis [14, 15].

es were observed: overall, only 6 (0.6%) samples were found to be non-compliant for at least one pollutants group. In farms with non-compliant results, accurate epidemiological investigations were carried out to identify the potential hazards and the subsequent analyzes for dioxins and PCBs showed compliance results.

Temporal analysis showed a decreasing trend in dioxins and PCBs concentrations in raw milk over the observed years, with higher values in the first trimester and, mainly for NDL-PCBs, in the fourth trimester. The seasonal concentrations pattern we observed could be linked or to cyclical variations of animal physiological state or to a difference in animal pollutant concentrations exposures between indoor and outdoor environments among different seasons. Whatever the causes of this seasonal fluctuation, these findings could be useful in orienting the timing of sampling in Taranto or in any other areas affected by halogenated POPs contamination, focusing on the periods with presumably highest risk.

As far as spatial analysis of milk pollutants concentrations is concerned, despite areas closest to the industrial pole showed, as expected, higher levels of PCBs, maps and correlation analysis didn't show a clear distribution for dioxins, whose concentrations did not appear to be related to the distance from the pole. Moreover, our results didn't show strong correlation between dioxins and PCBs, suggesting a complex contamination scenario with partially unrelated polluting sources. All the more reason, precisely in such a varied and partly unpredictable scenario, the vigilant and constant control of the Prevention Department plays a pivotal role in intercepting those few possible unsafe products before they are placed on the market. On the other hand, the possibility to identify an inverse relationship between distance from the industrial area and PCBs concentrations in milk provides us with a useful tool to guide sampling in farms exposed to a greater risk of contamination and could be of great Public Health importance in Taranto as well as in any site contaminated by halogenated POPs, in the light of the fact that there is sufficient evidence in humans for the carcinogenicity (Group 1) of polychlorinated biphenyls (PCBs): in particular, PCBs cause malignant melanoma and positive associations have been observed for non-Hodgkin lymphoma and cancer of the breast [16]. Our results regarding the possible association between the levels of PCBs in raw milk and the emissions of the industrial pole seem consistent with the 2009 ARPA (Agenzia Regionale per la Prevenzione e la Protezione Ambientale) Puglia wind-selective environmental monitoring campaigns for organic micropollutants, in which the downwind/upwind concentration ratios in 4 sites located 0.5 km (two sites), 3.5 km and 6 km from the industrial area showed a clear directional origin of PCDD/Fs and PCBs [17]. Moreover, this is in line with the findings of the 2012 ISS (Istituto Superiore di Sanità – Italian National Institute of Health) exploratory biomonitoring study in which the blood levels of dioxins and PCBs among livestock farms workers of the Taranto Province appeared to be strongly associated with the distance of the farm from the industrial site [18].

Finally, also median PCDD/Fs and PCBs concentrations in dairy products were far below the EU limits and no exceedances were observed. In conclusion, despite the environmental pressures, the low median dioxins and PCBs concentrations and the extremely low number of exceedances over such a long period and so many samplings appear as an encouraging result regarding concerns about the safety of milk and cheese produced in the Province of Taranto. These findings offer a Public Health analysis and control model that is potentially applicable to other areas contaminated by halogenated POPs. The model's risk assessment is based on the distance from the principal polluting sources, even if the partial unpredictability of the contamination scenario makes it necessary to monitor even the most distant farms. In such a complex context, the constant control of the Prevention Department makes it possible to block the food at risk, protecting the health of consumers together with the production chain of the territory.

Conflict of interest statement
None to declare.

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