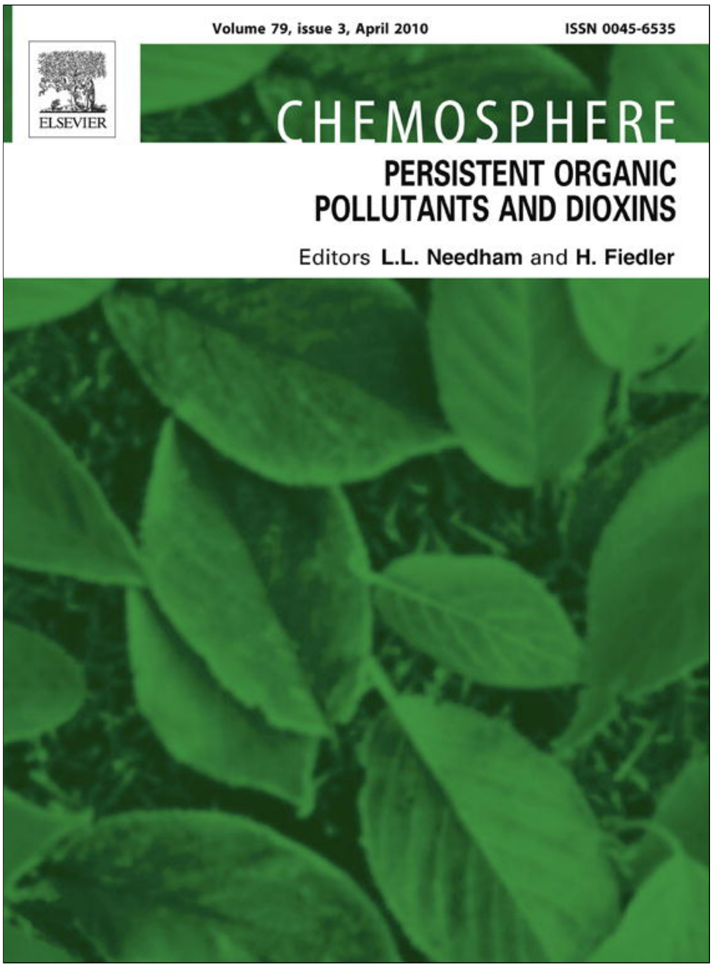
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# Contamination levels and congener distribution of PCDDs, PCDFs and dioxin-like PCBs in buffalo's milk from Caserta province (Italy)

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ABSTRACT

An extraordinary plan of official control was carried out in 2008 in Campania (Italy) with the aim to monitor polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (dl-PCBs) levels in buffalo milk and to detect the contaminated farms, most of which are located in Caserta province.

For these companies has been ordered seizure and execution of additional analyses has been requested in farms falling in the nearness, within a distance of 3 km, for a total of 304 farms examined. Moreover, all non-compliant farms were subjected to a periodic sampling in order to monitor trends in the levels of contamination.

In this paper the distribution and the concentrations of 17 PCDD/Fs and 12 dioxin-like PCBs in 460 samples of buffalo milk collected in the province of Caserta (Italy) are presented.

The range of WHO-TEQ values for the PCDD/Fs in milk was 0.17 pg TEQ $g^{-1}$ fat and 87.0 pg TEQ $g^{-1}$ fat with a mean value 3.63 pg TEQ $g^{-1}$ fat and medium value 2.25 pg TEQ $g^{-1}$ fat.

The concentrations of dioxin-like PCBs in the analysed samples ranged from 0.21 pg TEQ $g^{-1}$ fat to 15.9 pg TEQ $g^{-1}$ fat and the WHO-TEQ values of sum of PCDDs, PCDFs and dl-PCBs ranged from 0.45 pg TEQ $g^{-1}$ fat to 103.0 pg TEQ $g^{-1}$ fat.

The geo-referencing analysis allowed to individuate a restricted area of the region object of the present study where is located the majority of the non-compliant farms.

The study of the congeners distribution has finally suggested that the likely cause of contamination is to be attributed to the illegal burning of waste.



## 1. Introduction

The PCDD/Fs are a class of polychlorinated tricyclic compounds highly stable and extremely persistent; due to these properties they are ubiquitous environmental contaminants. Even though they can form through natural processes such as bush fires or volcanic processes, the main sources are anthropogenic processes as incineration of chlorine-containing wastes, industrial plants, cement factories and paper production, use and production of PVC and use and manufacturing of chlorinated aromatic chemicals, in which these compounds are formed as unwanted by-products.

These persistent organic pollutants are lipophilic and accumulate in fat tissues, then they enter the food chain and consequently can be found in humans in considerable concentrations. They are potent toxicant with a potential to produce a broad spectrum of adverse effects (Fry, 1995; Kerkvliet et al., 1996; Dienhart et al., 2000; Hassoun et al., 2000). Among all PCDD/Fs, the most toxic is the 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) which was included in the list of the human carcinogens from IARC (McGregor et al., 1998; Steenland et al., 2004) even if others congeners show similar features of toxicity, particularly those having chlorine atoms in all of the 2, 3, 7 and 8 positions.

The dioxin-like PCBs (dl-PCBs) are a group of 12 polychlorinated biphenyls, showing chemical and toxicological properties similar to those of PCDD/Fs.

Various cases of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and/or dioxin-like polychlorobiphenyls (dl-PCBs) contamination have been reported in Europe since 1997.

Recently in Italy, at the beginning of 2008, during the monitoring plans implementation (Residues National Plan, Regional Law 3/

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2005, Surveillance Plan) for the presence of PCDD/Fs in animals and food of animal origin made in Campania region, levels above the permitted maximum limit were detected in some samples of buffalo milk and *mozzarella* cheese.

In order to protect human health, the Italian Ministry of Health, in collaboration with Campania Region and in accordance with the provisions of the European Union, has developed an extraordinary monitoring plan (so-called EU plan) for a detailed analysis of the buffalo milk produced in the Campania region.

Applying this EU plan, through the analysis of buffalo milk samples, all the cheese factories for the production of *mozzarella* cheese have been reviewed in a few days.

The results of this monitoring plan in the Campania region have revealed a global situation of low contamination, very close to background levels, whereas contaminated milk samples were from a restricted area of the Caserta province. Therefore, samples from the majority of farms, where buffaloes are reared for milk production, were collected in this area.

In this paper we report the results of analysis of buffalo milk samples taken from farms located in the Caserta province between April 2008 and December 2008. The levels and the profile of PCDD/F and dl-PCB congeners are reported below to provide information about their distribution in this area and to hypothesize the sources of contamination.

The aim of this survey is also to fill the gap of data about the presence of PCDD/Fs and dl-PCBs in milk of buffaloes, an animal specie that is reared in this region of southern Italy.

## 2. Materials and methods

### 2.1. EU monitoring plan design

In the first two phases of the EU plan, during April 2008, 387 buffalo milk samples were collected in 239 cheese factories producing buffalo mozzarella, located in the five Campania provinces: Avellino, Caserta, Benevento, Salerno and Napoli.

Each sample of milk taken from the dairies was made of different milk samples constituting a pool ranging from one to a maximum of four samples.

The results of this first survey show that most of the contaminated buffalo milk came from the province of Caserta.

During the third phase, in case of non-compliant pool, samples were collected and analysed in all farms contributing milk to that specific pool sample. Once a non-compliant (positive) sample was identified, a buffer of 3 km around each contaminated farm was created and then milk samples were collected in all farms included in the area (Fig. 1).

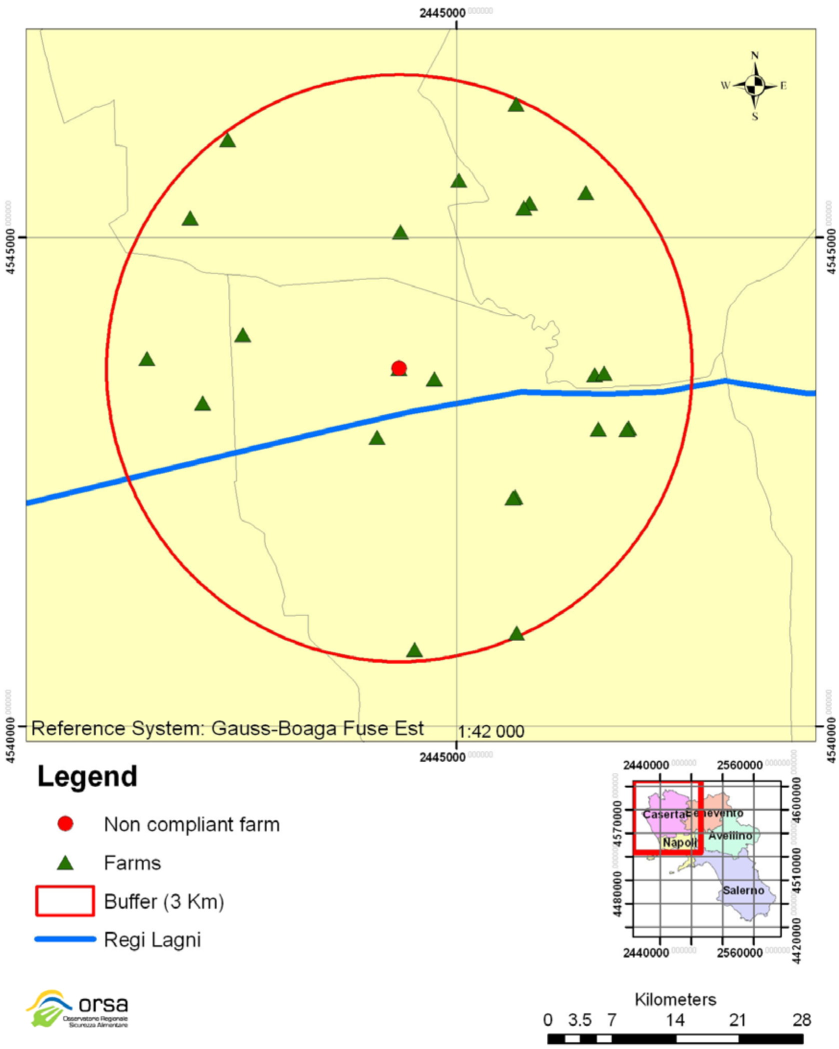


**Fig. 1.** Typical example of a buffer around a farm does not comply.

In addition, the application of other monitoring plans (Residue National Plan or Surveillance Regional Plan) made possible to perform more extensive control over all farms located in the area of interest.

### 2.2. Sampling

In carrying out all the monitoring plans to detect PCDD/Fs and dl-PCB levels, between April and December 2008, 460 buffalo's milk samples were collected by Veterinary Authorities in 304 farms located in Caserta province (Campania, Italy). The distribution of the sampling sites is shown in Fig. 2.

Milk was sampled according to the Commission Regulation (EC) 1883/2006 of 19 December 2006 laying down methods of sampling and analysis for the official control of levels of dioxins and dioxin-like PCBs in certain dietary components. Samples (about 1000 mL) were stored in glass recipients, frozen at −20 °C and then shipped on dry ice to the laboratory for the analysis by high-resolution gas chromatography/mass spectrometry (HRGC/HRMS) determination, according to the above regulation.

### 2.3. Analysis

The analyses on buffalo milk samples collected during the third phase and here reported, were conducted at the Eurofins|GfA GmbH laboratory Hamburg (Germany), using a high-resolution mass spectrometry (HRGC/HRMS) method according to EU legislation and in agreement with EPA method 1613.

Milk samples were processed by sodium oxalate assisted liquid/liquid extraction. Sample clean-up was carried out by a multi-column system using silica, basic alumina and others (e.g. florisil or carbon columns). Analysis was performed by HRMS/SIR on Waters AutoSpec mass spectrometers at a mass resolution of $R \geqslant 10\,000$ (10% valley) by isotope dilution with every analysed compound (exception: 1,2,3,7,8,9-HxCDD) having its own $^{13}$C-labelled internal quantification standard added to the sample before extraction. The criteria for ensuring the quality dioxin analysis include the application of quality control measures (QC) and quality assurance (QA) criteria such as sensitivity check of mass spectrometer (MS), check of resolution at 10 000, recovery, reference materials and participation in interlaboratory studies.

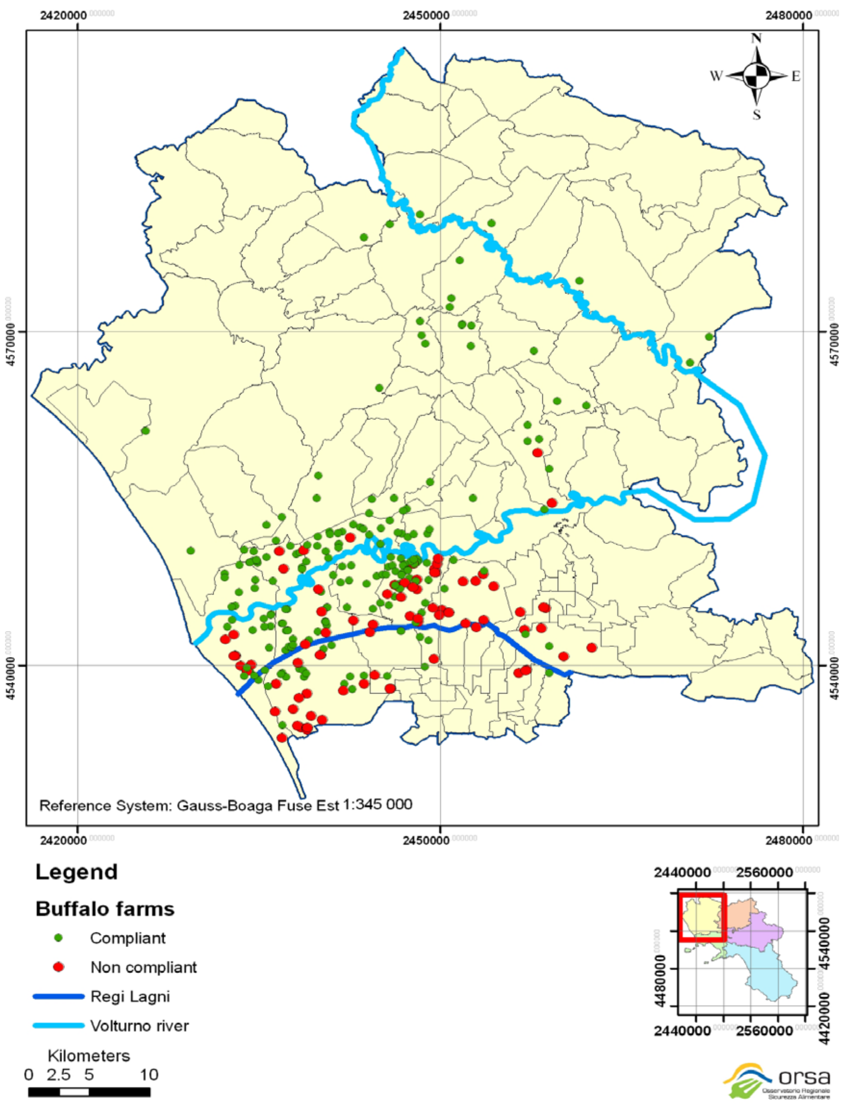


**Fig. 2.** Geographic distribution of sampling sites.

**Table 1**
Levels of PCDD/Fs (pg $g^{-1}$ fat) and WHO-TEQ PCDD/F (pg TEQ $g^{-1}$ fat) in buffalo milk.

| | Group 1 (54%) | | | | | Group 2 (15%) | | | | | Group 3 (31%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Median | sd | Min | Max | Mean | Median | sd | Min | Max | Mean | Median | sd | Min | Max |
| 2,3,7,8 TCDD | 0.08 | 0.08 | 0.03 | 0.0025 | 0.22 | 0.10 | 0.09 | 0.05 | 0.0025 | 0.30 | 0.16 | 0.12 | 0.11 | 0.0025 | 0.75 |
| 1,2,3,7,8 PCDD | 0.09 | 0.08 | 0.05 | 0.0025 | 0.67 | 0.16 | 0.12 | 0.19 | 0.0025 | 1.58 | 0.29 | 0.20 | 0.30 | 0.03 | 2.24 |
| 1,2,3,4,7,8 HCDD | 0.95 | 0.95 | 0.43 | 0.09 | 2.14 | 2.19 | 2.21 | 0.31 | 1.53 | 3.27 | 5.26 | 3.77 | 4.89 | 1.01 | 47.70 |
| 1,2,3,6,7,8 HCDD | 0.77 | 0.74 | 0.38 | 0.005 | 2.32 | 1.74 | 1.69 | 0.42 | 0.005 | 3.16 | 4.94 | 3.24 | 5.53 | 1.54 | 52.60 |
| 1,2,3,7,8,9 HCDD | 0.59 | 0.58 | 0.28 | 0.005 | 1.86 | 1.35 | 1.28 | 0.32 | 0.85 | 2.44 | 3.32 | 2.32 | 3.61 | 1.05 | 36.00 |
| 1,2,3,4,6,7,8 HPCDD | 0.08 | 0.07 | 0.06 | 0.005 | 0.63 | 0.09 | 0.08 | 0.08 | 0.005 | 0.69 | 0.09 | 0.08 | 0.04 | 0.01 | 0.43 |
| OCDD | 0.60 | 0.59 | 0.29 | 0.005 | 1.73 | 1.35 | 1.32 | 0.30 | 0.85 | 2.27 | 3.44 | 2.49 | 3.72 | 0.86 | 39.00 |
| 2,3,7,8 TCDF | 0.36 | 0.32 | 0.21 | 0.008 | 1.30 | 0.70 | 0.65 | 0.29 | 0.26 | 1.98 | 1.81 | 1.22 | 2.12 | 0.40 | 20.40 |
| 1,2,3,7,8 PCDF | 0.09 | 0.09 | 0.04 | 0.004 | 0.44 | 0.11 | 0.10 | 0.12 | 0.0075 | 0.98 | 0.16 | 0.12 | 0.14 | 0.05 | 1.12 |
| 2,3,4,7,8 PCDF | 0.21 | 0.19 | 0.11 | 0.001 | 1.18 | 0.26 | 0.21 | 0.38 | 0.01 | 3.31 | 0.26 | 0.23 | 0.13 | 0.01 | 1.17 |
| 1,2,3,4,7,8 HCDF | 0.12 | 0.10 | 0.07 | 0.0025 | 0.58 | 0.35 | 0.33 | 0.10 | 0.04 | 0.63 | 1.19 | 0.73 | 1.98 | 0.18 | 20.80 |
| 1,2,3,6,7,8 HCDF | 0.33 | 0.31 | 0.20 | 0.002 | 0.83 | 0.94 | 0.94 | 0.20 | 0.0025 | 1.35 | 2.49 | 1.75 | 2.42 | 1.01 | 23.80 |
| 1,2,3,7,8,9 HCDF | 0.13 | 0.12 | 0.06 | 0.005 | 0.33 | 0.31 | 0.29 | 0.12 | 0.012 | 0.90 | 0.70 | 0.53 | 0.51 | 0.21 | 4.33 |
| 2,3,4,6,7,8 HCDF | 0.55 | 0.49 | 0.31 | 0.005 | 1.83 | 1.41 | 1.36 | 0.41 | 0.52 | 2.48 | 3.58 | 2.61 | 3.96 | 1.06 | 41.50 |
| 1,2,3,4,6,7,8 HpCDF | 0.17 | 0.14 | 0.11 | 0.005 | 0.73 | 0.40 | 0.35 | 0.26 | 0.005 | 1.80 | 0.96 | 0.73 | 0.87 | 0.18 | 7.48 |
| 1,2,3,4,7,8,9 HpCDF | 0.42 | 0.34 | 0.38 | 0.0075 | 3.81 | 0.87 | 0.67 | 0.78 | 0.0075 | 5.38 | 1.54 | 1.17 | 1.08 | 0.38 | 7.53 |
| OCDF | 0.92 | 0.67 | 1.31 | 0.010 | 16.20 | 1.24 | 0.78 | 1.24 | 0.01 | 6.84 | 1.01 | 0.78 | 1.25 | 0.42 | 13.30 |
| WHO-TEQ PCDD/F | 1.23 | 1.18 | 0.59 | 0.17 | 2.46 | 3.08 | 3.09 | 0.5 | 2.51 | 3.74 | 8.09 | 5.73 | 8.56 | 3.77 | 87.0 |

Group 1: Milk samples below the action level for PCDD/F (<2.0 pg TEQ $g^{-1}$ fat).
Group 2: Milk samples above the action level for PCDD/F ($2.0 \leqslant$ pg TEQ $g^{-1}$ fat $\leqslant 3.0$).
Group 3: Milk samples exceeding the maximum level for PCDD/F (>3.0 pg TEQ $g^{-1}$ fat).

The fat percentage and the concentration of each congener, expressed as pg $g^{-1}$ of fat in agreement with the Regulation (EC) 1883/2006, were determined for each sample. As required in Regulation (EC) 1881/2006, the total PCDD/F concentration (pg TEQ $g^{-1}$ fat) is expressed as the sum of TEQs of 10 polychloro-substituted furans and seven polychloro-substituted dioxins congeners having chlorine atoms in 2,3,7,8-positions, the total dl-PCBs concentration (pg TEQ $g^{-1}$ fat) is expressed as the sum of TEQs of 12 non-ortho and mono-ortho PCB-congeners (PCB 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169 and 189).

The procedure used for the calculation of congener concentrations below the limit of quantification (LOQ) of the method was the "upper bound approach", based on the assumption that all the values minor than LOQ are equal to it.

According with Regulation (EC) 1881/2006 also the sum of WHO-TEQ PCDD/Fs and WHO-TEQ dl-PCBs was calculated.

## 3. Results

The analysis of the milk samples collected in Caserta province led to the identification of 94 contaminated farms, where PCDD/Fs levels in milk were above the maximum limit imposed by Regulation (EC) 1881/2006.

These farms were placed under seizure, produced milk was destroyed and the animal feed, the likely source of contamination, was removed and replaced. After this, the herds were sampled and analysed every 45 d to monitor the concentration of dioxins in milk, up to compliant values.

In 2008, among the 94 buffalo farms checked during the monitoring plans, 78 of them returned to acceptable levels of PCDD/Fs (WHO-TEQ < 3.0 pg TEQ $g^{-1}$ fat).

In 460 milk samples analysed between April and December 2008, the total concentrations of PCDD/Fs ranged between 0.17 pg TEQ $g^{-1}$ fat and 87.0 pg TEQ $g^{-1}$ fat, with an average value of 3.63 pg TEQ $g^{-1}$ fat and a median value of 2.25 pg TEQ $g^{-1}$ fat. The total concentrations of dl-PCBs ranged between 0.21 pg TEQ $g^{-1}$ fat and 15.9 pg TEQ $g^{-1}$ fat, with a mean value of 1.73 pg TEQ $g^{-1}$ fat and a median value of 1.19 pg TEQ $g^{-1}$ fat. The sum of PCDD/Fs and dl-PCBs concentrations was in the range 0.45 pg TEQ $g^{-1}$ fat and 103.0 pg TEQ $g^{-1}$ fat, with an average value of 5.36 pg TEQ $g^{-1}$ fat and a median value of 3.47 pg TEQ $g^{-1}$ fat.

On the basis of the contamination levels, the samples are divided in three groups.

The first one (Table 1) comprises 247 compliant samples (54%) that showed values of PCDD/F below the action level (<2.0 pg TEQ $g^{-1}$ fat) and these concentrations ranged between 0.17 pg TEQ $g^{-1}$ fat and 2.46 pg TEQ $g^{-1}$ fat (mean value = 1.23), whereas the total concentration of dl-PCB were between 0.21 pg TEQ $g^{-1}$ fat and 2.58 pg TEQ $g^{-1}$ fat with a mean value of 0.84 pg TEQ $g^{-1}$ fat (Table 2).

The second group involves 71 compliant samples (15%) that showed PCDD/F levels below the maximum level but above action level (2.0 pg TEQ $g^{-1}$ fat) established by Commission Recommendation 2006/88/EC. In these milk samples the WHO-TEQ PCDD/Fs mean value was 3.08 pg TEQ $g^{-1}$ fat and median value was 3.09 pg TEQ $g^{-1}$ fat (Table 1) whereas the WHO-TEQ dl-PCBs mean value was 1.61 pg TEQ $g^{-1}$ fat and median value 1.51 pg TEQ $g^{-1}$ fat (Table 2).

The third group involves 142 buffalo milks (31%) exceeding the maximum permitted limit set from EC legislation (WHO-TEQ = 3.0 pg TEQ $g^{-1}$ fat) taking into account the maximum uncertainly (±20%) as required by Regulation (EC) 1883/2006. In these samples the total concentrations of PCDD/Fs ranged between 3.77 pg TEQ $g^{-1}$ fat and 87.0 pg TEQ $g^{-1}$ fat (Table 1) and the WHO-TEQ dl-PCBs mean values were from 0.85 pg TEQ $g^{-1}$ fat to 15.9 pg TEQ $g^{-1}$ fat (Table 2).

## 4. Discussion

For identification of the sources of contamination it is important to know the concentration of the congeners and their relative contribution to the TEQ. The 2,3,7,8 congener profiles have been analysed in order to derive some further indications on the possible dioxin sources (Ramos et al., 1997; Alcock et al., 2002). The average profiles of PCDD/Fs are shown in Fig. 3 reporting the pattern for all congeners in the three groups of milk samples.

A first general observation is that the profile of PCDD/Fs seems to be homogeneous, giving an hypothesis of homogeneity on the total data. Generally PCDD/F accounts for the major part of the con-

**Table 2**
Levels of dl-PCBs (pg $g^{-1}$ fat) and WHO-TEQ dl-PCB (pg TEQ $g^{-1}$ fat) in buffalo milk.

| | Group 1 (54%) | | | | | Group 2 (15%) | | | | | Group 3 (31%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Median | sd | Min | Max | Mean | Median | sd | Min | Max | Mean | Median | sd | Min | Max |
| 3,3,4,4-TeCB (**77**) | 3.5 | 3.1 | 1.6 | 0.201 | 23.2 | 3.6 | 3.4 | 0.9 | 1.86 | 6.02 | 3.5 | 3.37 | 0.8 | 0.96 | 6.25 |
| 3,4,4,5-TeCB (**81**) | 1.2 | 0.7 | 1.4 | 0.28 | 9.83 | 1.8 | 1.2 | 1.7 | 0.45 | 9.45 | 3.0 | 2.18 | 2.9 | 0.52 | 20.5 |
| 2,3,3,4,4-PeCB (**105**) | 170.0 | 158.0 | 71.7 | 36.20 | 654 | 285.8 | 241.0 | 201.9 | 40.80 | 1450 | 418.5 | 371 | 284.0 | 130 | 2140 |
| 2,3,4,4,5-PeCB (**114**) | 17.1 | 15.9 | 7.7 | 4.68 | 52.2 | 35.2 | 30.3 | 22.0 | 6.08 | 180 | 86.3 | 57.5 | 145.2 | 20.2 | 1670 |
| 2,3,4,4,5-PeCB (**118**) | 572.4 | 544.0 | 259.1 | 86.7 | 2370 | 966.6 | 812.0 | 801.2 | 136.3 | 5950 | 1440.8 | 1230 | 1080.2 | 372 | 7880 |
| 2,3,4,4,5-PeCB (**123**) | 9.8 | 8.9 | 8.4 | 1.43 | 125.5 | 15.9 | 14.0 | 10.2 | 5.00 | 63.4 | 28.7 | 24.4 | 21.4 | 5 | 212 |
| 3,3,4,4,5-PeCB (**126**) | 6.7 | 6.4 | 3.0 | 1.35 | 19.5 | 13.2 | 12.5 | 4.7 | 4.15 | 26.2 | 28.0 | 23 | 16.1 | 1.84 | 112 |
| 2,3,3,4,4,5-HxCB (**156**) | 75.6 | 70.8 | 37.5 | 5 | 368 | 139.6 | 117.5 | 108.7 | 17.60 | 882 | 248.7 | 196 | 217.5 | 15 | 1840 |
| 2,3,3,4,4,5-HxCB (**157**) | 20.6 | 18.3 | 22.0 | 3.89 | 341 | 38.1 | 30.9 | 24.0 | 6.59 | 183 | 72.2 | 56.8 | 61.4 | 22 | 616 |
| 2,3,4,4,5,5-HxCB (**167**) | 26.5 | 24.1 | 11.9 | 5 | 71.5 | 50.5 | 42.3 | 37.8 | 6.69 | 291 | 91.4 | 75.9 | 70.6 | 21 | 616 |
| 3,3,4,4,5,5-HxCB (**169**) | 3.5 | 3.1 | 1.3 | 0.90 | 12.4 | 5.9 | 5.4 | 2.6 | 2.93 | 21.7 | 14.9 | 11.2 | 16.8 | 3.4 | 183 |
| 2,3,3,4,4,5,5-HpCB (**189**) | 11.3 | 10.6 | 5.2 | 3.42 | 34 | 26.2 | 21.9 | 17.8 | 5.00 | 135 | 55.7 | 40.7 | 57.0 | 6.26 | 577 |
| WHO-TEQ dl-PCB | 0.84 | 0.78 | 0.35 | 0.21 | 2.58 | 1.61 | 1.51 | 0.58 | 0.59 | 3.22 | 3.35 | 2.82 | 1.97 | 0.85 | 15.9 |

Group 1: Milk samples below the action level for PCDD/F (<2.0 pg TEQ $g^{-1}$ fat).
Group 2: Milk samples above the action level for PCDD/F ($2.0 \leqslant$ pg TEQ $g^{-1}$ fat $\leqslant 3.0$).
Group 3: Milk samples exceeding the maximum level for PCDD/F (>3.0 pg TEQ $g^{-1}$ fat).

tamination and the PCDF are present to a higher extent than the PCDD which might indicate influences from combustion of urban wastes (Brambilla et al., 2006).

In all samples the main contribution to the WHO-TEQ was from PCDF congeners, in particular 2,3,4,7,8 PCDF and 1,2,3,4,7,8 HxCDF were dominant. The PCDF/PCDD mean ratio was 0.66 in compliant samples and 0.62 in non-compliant (WHO-TEQ PCDD/F > 3.0 pg TEQ $g^{-1}$ fat) samples. In the compliant buffalo milk samples, the congener profile revealed that the most dominant congeners were 2,3,4,7,8 PCDF and OCDD.

Presence of 2,3,7,8 TCDF was found in the most part of samples while the congener 1,2,3,7,8,9 HCDF was found in very few samples at levels above the LOQ.

Pattern of PCDFs and PCDDs confirms the results reported by other studies (Rappolder et al., 2005); in fact, in literature, some cases, in which the analysis of the congener profiles can help in establishing the source of pollution, are described. In particular, it was demonstrated that, in a situation of presence of pollutants coming from processes of combustion (e.g. urban wastes incinerator), milk samples are characterized by a prevalence of PCDF congeners if compared to PCDD, that instead prevail in samples collected in rural areas hardly exposed to sources of contamination.

Among the milk samples collected in farms located in Caserta province, the PCDDs contribution to the total dioxin concentration is less than the PCDF's. This prevalence of PCDFs, with respect to PCDDs, was observed for more than 95% of the farms.

As shown in Fig. 3, chlorine fingerprinting analysis showed that PCDD/F profiles are similar in non-compliant samples and in samples above the action level, and also for the compliant samples with one exception. The pattern appears significantly different in the compliant samples for OCDD being one of the most abundant congeners.

The congener profiles are dominated by the same congeners, especially OCDD, 1,2,3,4,6,7,8-HpCDD, 2,3,4,7,8-PCDF, three of the HxCDF, and 1,2,3,6,7,8-HxCDD, suggesting that sources of contamination may be the same in different farms.

In literature there is a lack of information on PCDD/F levels in buffalo milk, therefore is not possible to compare these data with similar others.

However, these results agree with those found in some surveys conducted to evaluate PCDD/F contamination levels in cow milk from other European countries. In these studies OCDF and 1,2,3,4,6,7,8-HpCDF were predominant in the Spanish commercial pasteurised cow's milks (Ramos et al., 1997) whereas PCDFs and HxCDFs seemed to be the most abundant congeners in cow's milk from Sweden (Rappe et al., 1990), Catalonia (Abad et al., 2002) and Switzerland (Schmid et al., 2003).

Among the PCDFs, the lower chlorinated PCDFs (tetras and pentas) are in general more abundant than the higher chlorinated PCDFs (heptas and octa PCDFs). A similar pattern of a typical combustion process, where the congener profile is normally dominated by light furans such as PeCDFs and HxCDFs, was reported in literature (Hutzinger and Fiedler, 1993; Guerzoni and Raccanelli, 2004). Therefore, it seems to be apparent that combustion processes constitute the main contribution to the pollution and to the entry of PCDD/Fs in the food chain, from feed to milk.

Similarly to what has been discussed for sheep and cow (Furst et al., 1993; Alcock et al., 2002; Schulz et al., 2005), also for the bufalos it is probable that PCDD/F concentration in milk can increase if they graze on areas known for their high dioxin levels in the soil or that milk concentrations would directly reflect grass concentrations. At the same time, we cannot ignore some ethological characteristics of this animal species such as, for example, the buffalo wallowing in the mud or licking the contaminated fences and, then, the possibility that the contamination could also involve

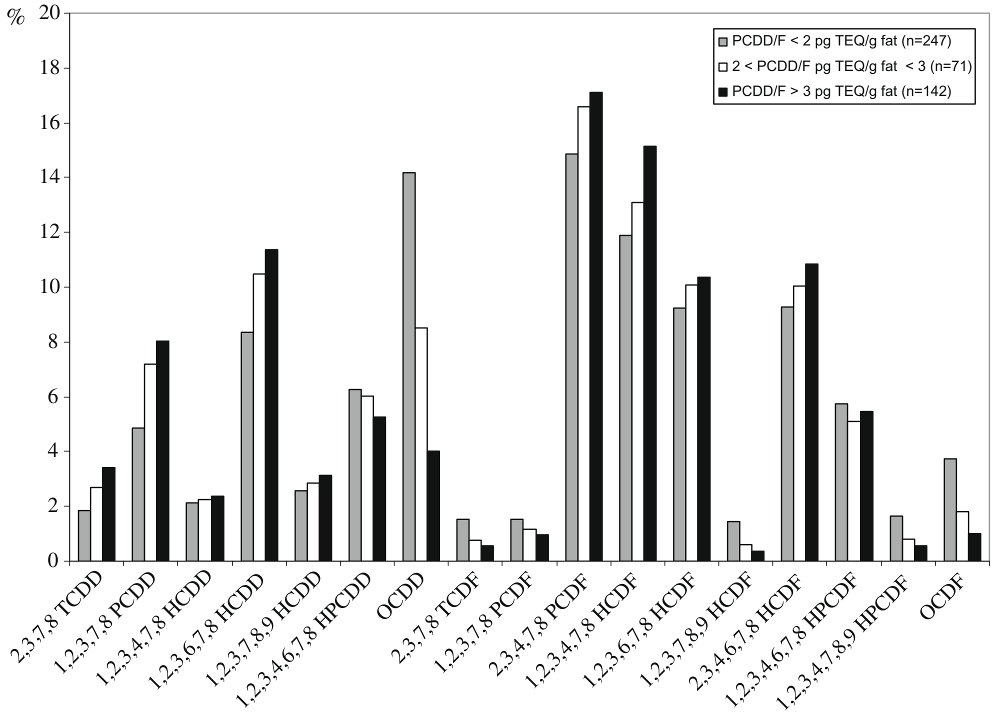


**Fig. 3.** The relative contribute to pattern of PCDD/F congeners in buffalo milk samples collected in Caserta province.

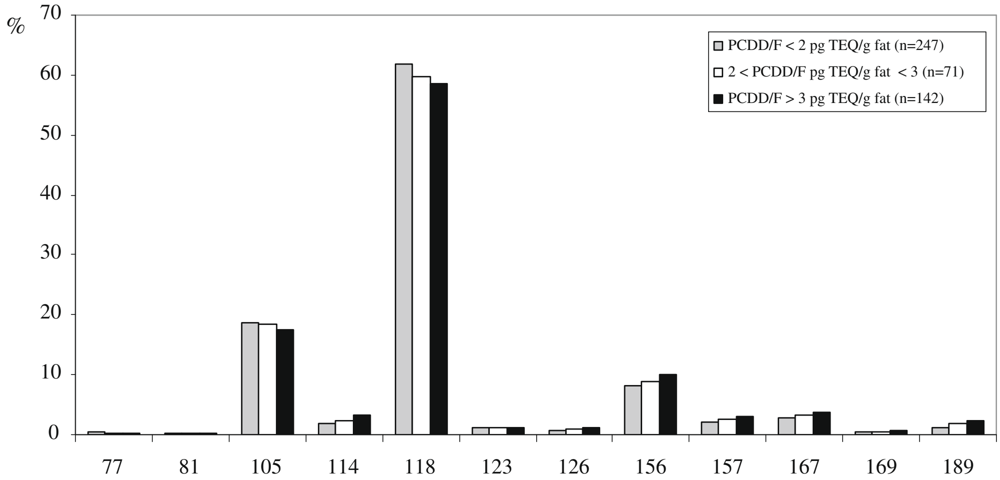


**Fig. 4.** The relative contribute to pattern of dl-PCB congeners in buffalo milk samples collected in Caserta province.

the drinking water or the organic wastes. Contamination levels of PCDD/Fs in feed collected in buffalo farms located in Caserta province (unpublished data), in fact, not always explain the level of contaminants present in milk fat, therefore there are probably other ways that could facilitate the increase of PCDD/F level in milk.

We analysed also the percentage contribution of dl-PCBs to the total WHO-TEQ in the non-compliant milk samples, it was about 29%, indicating that the contamination of buffalo milk is mainly caused by PCDD/F, whereas dl-PCB would contribute to only one third.

Nevertheless there is a significant contribution derived from dl-PCBs; their congener profile can be useful to identification of the pollution sources and estimation of their contribution can be useful in developing of good countermeasures.

As show in Fig. 4, there are no significant differences between the three groups of samples. The congener PCB-118 (2,3,4,4,5-PeCB) ranged from 59% to 62%, whereas congener PCB-105 (2,3,3,4,4-PeCB) ranged from 17% to 19%. Generally, in all samples congeners PCB-118, PCB-105 and PCB-156 prevail in all three groups and the contribution of others nine congeners is less than 5% for each of them. In all cases the statistical analysis proves that analysed congeners must be regarded as coming from the same population.

Even if technical PCB mixtures also could contain PCDD/F as unwanted by-products, dl-PCBs are not the main source of contamination. In fact, high PCBs levels in feed and soil were not detected, but, because of the close correlation between dl-PCBs e PCDD/Fs revealed in buffalo milk samples (Fig. 5), the likely hypothesis is that among the wastes burnt were materials containing PCBs.

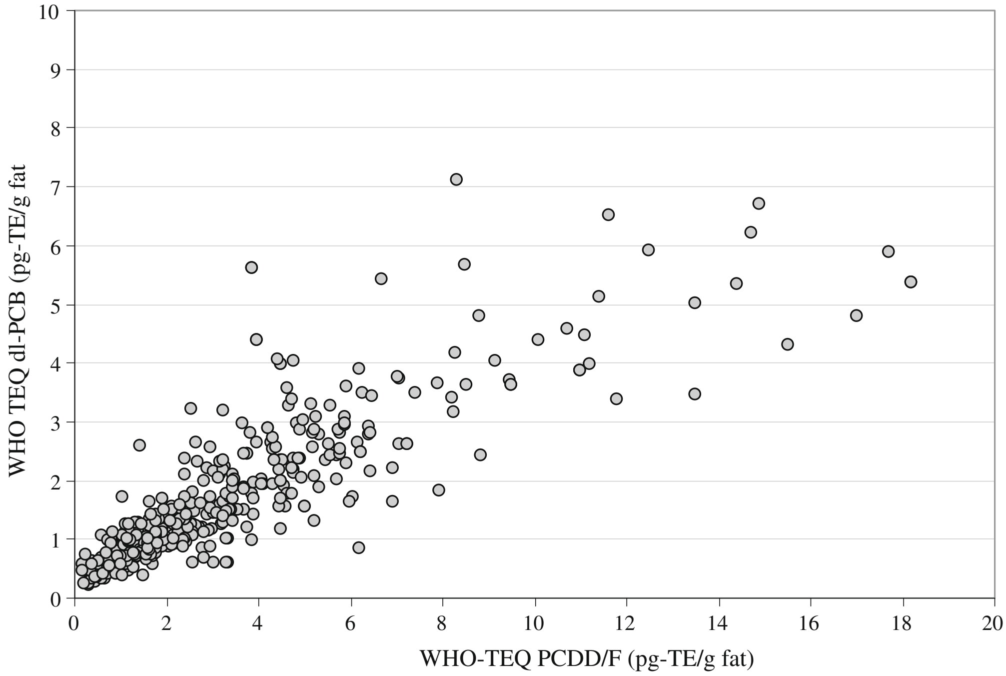


**Fig. 5.** Plot of WHO-TEQ dl-PCB against WHO-TEQ PCDD/F.

## 5. Conclusions

The monitoring plans on the buffalo milk produced in Campania led to identify all farms where the milk contamination with PCDD/F and PCB occurred. The activities of non-compliant farms were blocked and the feed identified as the main source of contamination. With the contribution of the geo-referencing analysis, it was possible to conclude that the most part of buffalo farms contaminated by dioxin are located in Caserta province, in a restricted area near the Volturno River and the Regi Lagni canal (Fig. 2).

These compounds, once introduced into the air, can deposit, in certain conditions, on feed such as vegetables cultivated on fields (corn, oat) or already manufactured and stored at the farms (silage). The analysis of congener profiles contributed to hypothesize that the likely source of contamination was the combustion of urban wastes chlorine-containing (Rapporto Istisan 06/5, 2006) producing the PCDD/Fs, but there is no hint for some of the specific PCDD/F-patterns originating from "chemical production sources", i.e. no indication for PCDD/F-patterns of Chlorophenol-(PCP-) or electrolysis type. This suggests the presence of highly heterogeneous urban wastes probably burned out illegally in the surroundings of the pastures. A comparison between soil/feed samples and milk samples is only partially possible because of the metabolisation effects.

The monitoring plan above described, was particularly useful to contain a situation of serious health emergency in a very limited period of time. However, a comprehensive monitoring of PCDD/Fs and dl-PCBs in the environment as well as in feed and food is still necessary in Campania to protect public health. Further actions to control the potential sources of environmental contamination by these pollutants, may need to be supplemented by measures to prevent direct contamination of feeding stuff or food to reduce general population exposure.

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