

STATISTICAL EVALUATION OF PCDD/Fs LEVELS AND PROFILES IN MILK AND FEEDINGSTUFFS FROM CAMPANIA REGION - ITALY

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Introduction

PCDD/Fs levels exceeding the European Union (EU) tolerance limit were detected in milk and animal feed samples collected in Campania region in the years 2001-2003, as reported in a previous paper¹. The analyses were performed on milk samples from different animal species (cow, sheep, goat and buffalo) and on animal feed samples (silage, hay, grass, cereals, premixes and mixed feeds) permitting to assess the levels and the geographical extension of the contamination. The preliminary results of this survey had given clear indications of the dioxin contamination of feedingstuffs and their contribution to the high PCDD/Fs levels recorded in milk but a more detailed analysis was needed in order to confirm the previous observations¹. Aim of this work is the evaluation of the correlation between the PCDD/Fs levels and patterns found in milk and animal feed samples through the statistical analysis of the congeners profiles and concentrations. Moreover, the typical congeners profiles of milk samples taken in the area under investigation were compared to those obtained from samples collected in the framework of the National Residues Surveillance Plan (NRSP) in 2003. The contamination phenomenon was also studied by means of the spatial correlation analysis.

Methods and Materials

PCDD/Fs analysis

Samples were analysed by applying a validated procedure¹ routinely used for dioxins monitoring in food and animal feed based on EPA Method 1613 Revision B² and successfully tested in a number of inter-laboratory studies and quality control programmes.

Milk samples vs animal feed

The correlation between PCDD/Fs WHO-TEQ values in milk and feedingstuffs was evaluated by the calculation of the Spearman correlation coefficient on 197 paired samples collected in the same farms. Milk samples from cow, sheep, goat and buffalo were included in the statistical analysis as well as forages and cereals samples (fodder likely grown in the area of study).

NRSP vs Campania milk samples

The differences in PCDD/Fs congeners profiles between bovine milk from Campania (15 random samples, PCDD/Fs > 3 pg WHO-TEQ/g fat) and bovine milk collected in the framework of the NRSP 2003 (15 random samples, average PCDD/Fs = 0.33 pg WHO-TEQ/g fat) were investigated. For each PCDD/Fs congener (normalised concentration) the Mann Whitney³ (M-W) test was applied in the two groups of samples in order to verify if a significant difference in the congeners levels existed.

Cluster analysis

To identify similar congeners profiles, a hierarchical cluster analysis⁴ based on Euclidean distance and between groups linkage, was applied on 49 milk samples (cow, sheep and buffalo milk) and 49 animal feed samples (grass, hay and silage probably grown in the area of sampling) taken in the same farms. The samples were selected on the basis of the following criteria: milk containing PCDD/Fs > 2 pg WHO-TEQ/g fat, feedingstuffs with PCDD/Fs > 0.30 ng WHO-TEQ/kg (12% moisture). Milk samples above 2 pg WHO-TEQ/g fat were chosen since it is the action level for dioxins established by the EU Commission Recommendation 2002/201/EC⁵. The feedingstuffs PCDD/Fs levels were above the mean PCDD/Fs concentration normally present in roughages⁶. Each congener was considered as a variable. The analysis was carried out with the SPSS[®] Version 11.0 software.

Spatial autocorrelation analysis

In order to verify the presence of spatial autocorrelation in the PCDD/Fs concentration in milk and animal feed samples, the I-Moran index⁷ was calculated. All the municipalities of sample collection were included in the analysis.

Results and DiscussionMilk samples vs animal feed

The Spearman coefficient was 0.670 ($p < 0.01$), thus showing a positive correlation between PCDD/Fs WHO-TEQ values in milk and feedingstuffs. The obtained result was a statistical confirmation of the evidence observed in a previous research¹.

NRSP vs Campania milk samples

The Mann Whitney test results are reported in Table 1. A statistical significance ($p < 0.05$) was found in the difference of the normalised concentration for 12 out of the 17 PCDD/Fs congeners in the two groups of samples. The M-W values for the highest toxic congeners (2,3,7,8-TCDD and 1,2,3,7,8-PeCDD, WHO-TEF = 1) were among those more statistically significant. The difference in the 2,3,4,7,8-PeCDF (WHO-TEF = 0.5) relative abundance in the two groups was not significant even though its analytical level was very different (5.4 pg/g fat in Campania milk and 0.30 in NRSP milk, M-W value = 0, $p > 0.05$). This is due to the fact that 2,3,4,7,8-PeCDF is one of the most abundant congeners both in contaminated and background milk samples. The average PCDFs/PCDDs ratio was 1.7 for the milk samples from Campania and 1.1 for the NRSP milk samples. The major relative abundance of PCDFs recorded in the contaminated samples compared to the background level was also observed in most of the 49 milk samples selected for the cluster analysis.

Cluster analysis

The cluster analysis results identified two main congeners profiles both for milk and feedingstuffs as shown in Figure 1 and 2. The majority of milk samples (44 out of 49) belonged to the group 2 in which the PCDF congeners were predominant. This finding seems to be related to the uncontrolled combustion of waste materials of urban/industrial origin, widespread in the area of study. The prevalence of PCDDs in the group 1 milk samples could be due to different emission sources that have not been identified yet. Concerning feedingstuffs, samples belonging to the group 2 (21 samples) showed a mean profile characterised by a prevalent abundance of PCDF congeners (in particular 1,2,3,4,6,7,8-HpCDF) while OCDD dominated the group 1 profile (mean of 28 samples).

Spatial autocorrelation analysis

The I–Moran index was significant both for milk and animal feed (milk I-Moran = 0.5301 $p < 0.01$, feedingstuffs I-Moran = 0.196 $p < 0.01$). The PCDD/Fs levels in milk and animal feed samples from Campania are presented in Figure 3 and 4. The presence of spatial autocorrelation of the contamination phenomenon was clear but a wider collection of samples should be performed to better verify this finding.

References

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Table 1 Mann-Whitney test results (Campania milk vs NRSP milk)

Congener	Mann-Whitney value	P
2,3,7,8-TCDD	36	<0.05
1,2,3,7,8-PeCDD	41	<0.05
1,2,3,4,7,8-HxCDD	61	<0.05
1,2,3,6,7,8-HxCDD	25	<0.05
1,2,3,7,8,9-HxCDD	56	<0.05
1,2,3,4,6,7,8-HpCDD	65	<0.05
OCDD	19	<0.05
2,3,7,8-TCDF	90	>0.05
1,2,3,7,8-PeCDF	70	>0.05
2,3,4,7,8-PeCDF	74	>0.05
1,2,3,4,7,8-HxCDF	22	<0.05
1,2,3,6,7,8-HxCDF	53	<0.05
2,3,4,6,7,8-HxCDF	70	>0.05
1,2,3,7,8,9-HxCDF	56	<0.05
1,2,3,4,6,7,8-HpCDF	90	>0.05
1,2,3,4,7,8,9-HpCDF	63	<0.05
OCDF	38	<0.05

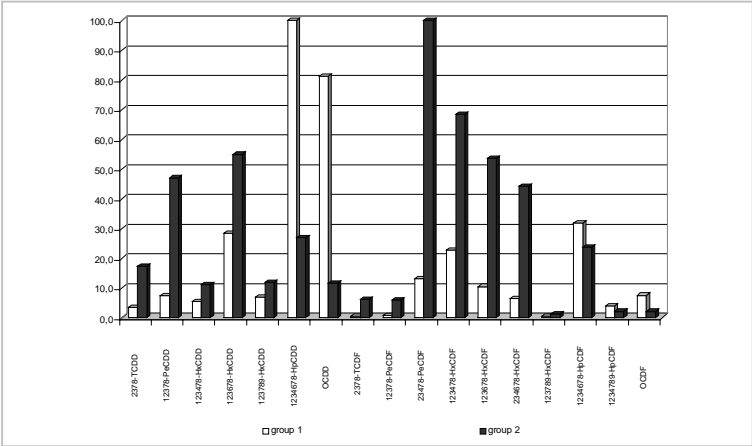


Figure 1. PCDD/Fs normalised congeners profiles in the two clusters identified - MILK

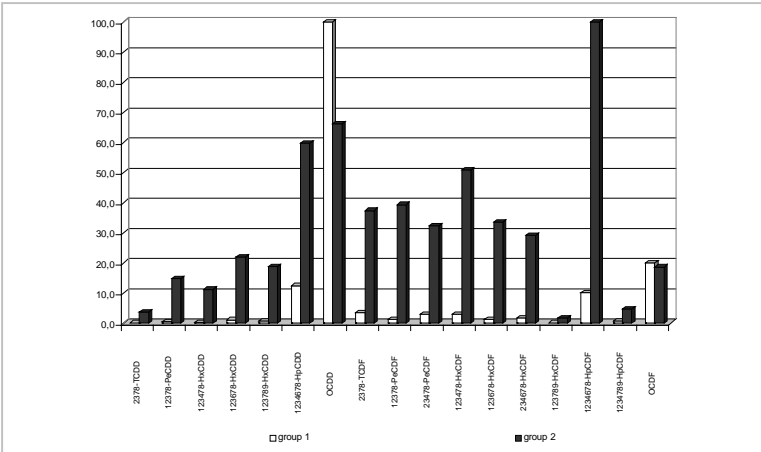


Figure 2. PCDD/Fs normalised congeners profiles in the two clusters identified - FEEDINGSTUFFS

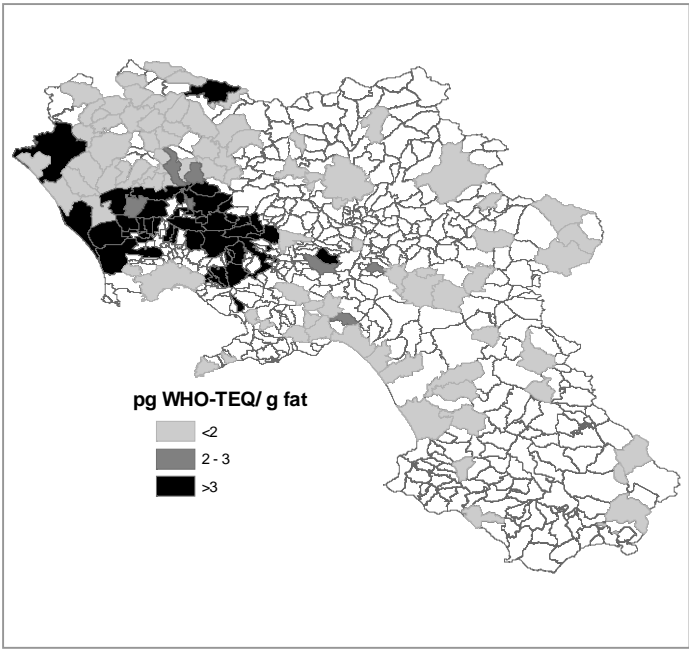


Figure 3. PCDD/Fs levels in milk from Campania region

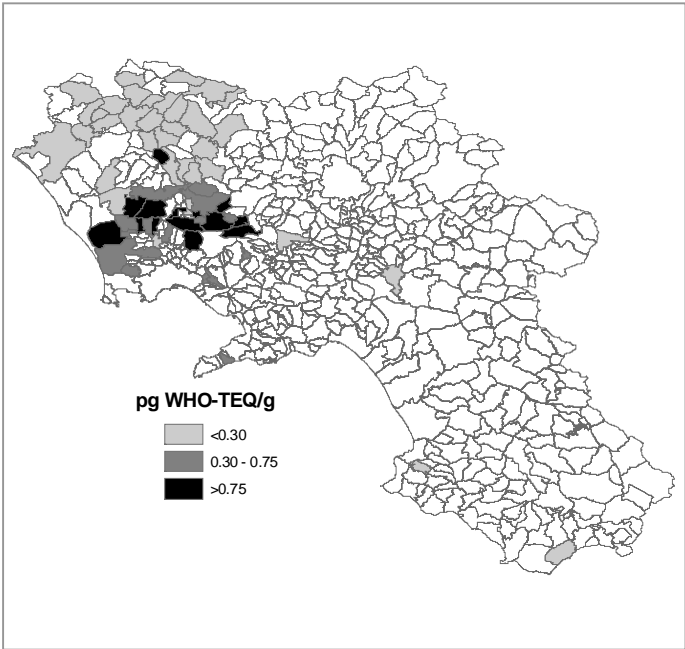


Figure 4. PCDD/Fs levels in feedingstuffs from Campania region