

PCDD/F AND PCB IN THE FOOD WEB: FOCUS ON FEED

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Introduction

Reducing dioxins (PCDD/Fs) and polychlorinated biphenyls (PCBs) human exposure is an important public health goal. A main step in this direction is the guarantee of food safety. Dietary intake is responsible of almost 90% of total human exposure to these organochlorinated compounds. In particular, food of animal origin is the main contributor to human body burden, being dioxins and PCBs lipophilic chemicals that accumulate in fatty tissues of animals¹. The presence of these chemical environmental pollutants in food derives mainly from the use of contaminated feed in animal diet². As a consequence, production of safe feed is the first important step in the management of risk of human exposure to chemical substances able to bioaccumulate along the trophic chain.

While the production and selling of industrial feeds is strictly ruled and controlled, local feeds, produced in farm and directly used, are unregulated, since they are not placed on the market³. However, in-farm produced fodder, mainly corn silage and hay, is often an ingredient in dairy cows diets, as it happens in Northern Italy. As a consequence, contamination problems are discovered too late, once food or humans are already involved.

In high risk area, such as agro-industrial territory, harvesting guidelines should be provided to farmers in order to obtain safe feed. In addition, many European countries lack dioxins and PCBs legislative limits for agricultural soils, making risk assessment in this field more difficult. In this study, we investigated the safety of feed produced in Brescia city (Northern Italy). Brescia is a high industrial city, characterized by many metallurgical plants and foundry activities and by one of the biggest European waste-to-energy plant. It was also the site of the only Italian PCB-producing plant. The level of contamination of an agricultural soil was investigated and compared to the levels found in corn silage, sorghum silage and hay, obtained from that field and intended for dairy cattle nutrition. Considerations about the safety of the produced feeds were made in relation to European and Italian legislative limits.

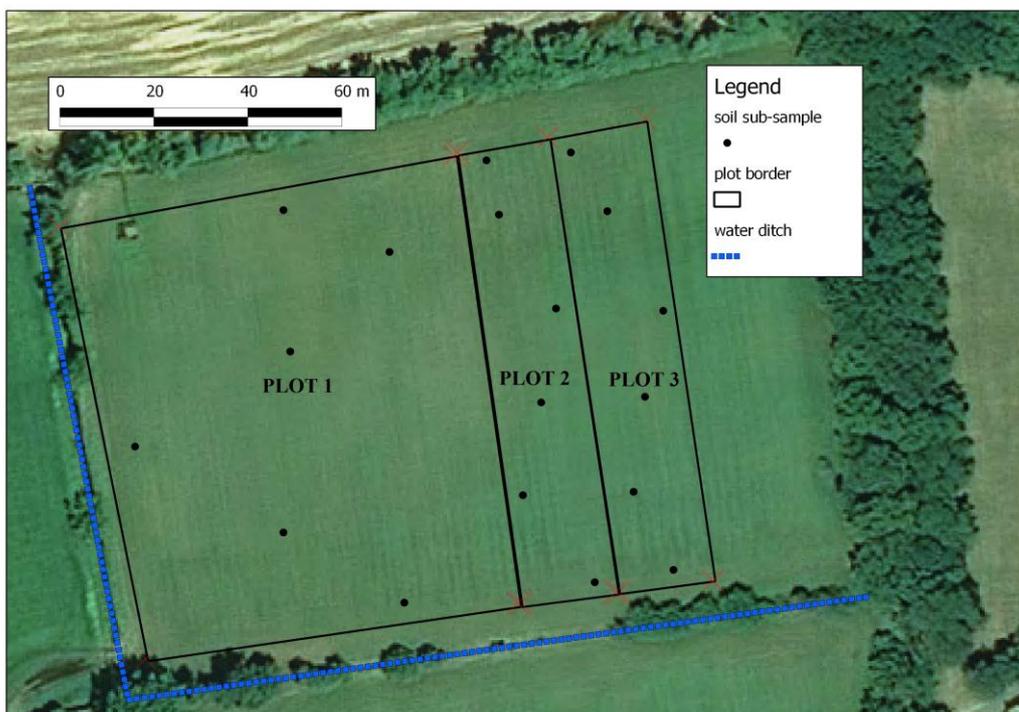


Figure 1. Sampling area

Materials and methods

Sampling

Soil samples were collected from an agricultural field of a dairy farm located in Brescia city. The farmland was located 1 km from a big steel plant, 2 km from the waste-to-energy plant and 5 km from the disused PCB-producing factory. The field (11,600 m²) was divided in 3 plots (plot 1, plot 2, plot 3). For each plot, 6 sub-samples of soil were collected at 0-30cm depth (Figure 1), using clean soil probes. Sub-samples of a same plot were carefully mixed together (composite soil sample), put in a chemically clean glass jar and transported to the laboratory. Sampling was carried out in agreement with the Italian law⁴.

After soil sampling, two plots were planted with corn (plot 1) and sorghum (plot 2); instead, on plot 3, grass was maintained. Soil was fertilized using a nitrogen-release fertilizer, weeding was not done.

Corn and sorghum were harvested for silage production and grass was cut in order to obtain hay. Harvesting operation were carried out by the farmer, as routinely done, and no guidelines were provided. Cutting heights for corn and sorghum were of 35 cm and 20 cm, respectively; while grass was cut at 5 cm. Corn and sorghum silages were packed in separated bunker silos. Grass was dried for 2 days on-field, then baled and wrapped. Samples of silages and hay were collected 45 days after the harvest. Feed sampling was carried out in agreement with the European Commission Regulation No 691/2013⁵.

Analytical methods

Soil and feed samples preparation and analysis were carried out by certified laboratories. Dioxins and PCB congeners were determined using high-resolution gas chromatography coupled with high-resolution mass spectrometry (HRGC-HRMS). For the analysis of PCDD/Fs and PCBs congeners, the US EPA Method 1613/B 1994⁶ and US EPA Method 1668/C 2010⁷ were applied. Quality assurance/quality control practices were regularly employed by the laboratories to ensure the accuracy of the analytical procedures. In both soil and feed samples, the seventeen 2,3,7,8-substituted PCDD/Fs, the twelve DL-PCBs congeners (PCB 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189) and the six PCB indicators (NDL-PCBs: 28, 52, 101, 138, 153, 180) were determined. In addition, for soil specimens other 10 NDL-PCB congeners were quantified. Toxic equivalency (TEQ) values for PCDD/Fs and DL-PCBs were calculated using WHO-TEFs 2005⁸ (toxic equivalency factors).

Results and discussion

PCDD/Fs and PCBs analytical levels and TEQ values are reported in Table 1 and Table 2, respectively. Results are expressed as upper-bound.

Matrix	Soil plot 1	Soil plot 2	Soil plot 3	Corn silage	Sorghum silage	Hay
∑ PCDFs	58.60	39.49	44.59	0.92	1.18	7.42
∑ PCDDs	154.06	101.90	96.47	0.87	1.17	20.22
∑ PCDFs, PCDDs	212.66	141.39	141.06	1.79	2.35	27.64
Ratio PCDFs/PCDDs	0.28	0.28	0.32	0.52	0.50	0.27
∑ Mono-ortho DL-PCBs	3690	3750	3790	350	481.85	874.26
∑ Non-ortho DL-PCBs	90	90	90	22	33.19	43.82
∑ 12 DL-PCBs	3780	3840	3880	372	515.04	918.08
∑ 6 indicator NDL-PCBs	24250	18140	17230	3000	3000	4730
∑ other NDL-PCBs	15257	10290	9614	n.a.	n.a.	n.a.

Table 1. PCDDs, PCDFs and PCBs concentrations. For soil samples the results are expressed as ng/kg dry weight; for feed samples the results are expressed as ng/kg 12% of moisture.

In soil of plot 1, the highest analytical levels of PCDD/Fs and PCBs were found. Plot 1 was the only part of the field subject to irrigation by overflowing of the close ditch. The stronger contamination could be link to the presence of sediments transported by the water. However, contamination profiles of the 3 plots overlap quite well as demonstrated by the Pearson correlation coefficients shown in Table 3. The use of Pearson coefficient has been already reported in literature for profile correlation⁹.

Since Italy lacks legislative values for agricultural soils, a comparison with National maximum levels established for green areas and residential areas is usually carried out¹⁰. For these kind of areas the Italian legislative limits

for PCBs sum (no specified congeners) and for the sum of the seventeen 2,3,7,8-substituted PCDD/Fs congeners are set at 60000 ng/kg and of 10 ng TEQ/kg, respectively¹¹. The soil investigated in our study showed contamination values under these thresholds.

Matrix	Soil plot 1	Soil plot 2	Soil plot 3	Corn silage	Sorghum silage	Hay
PCDFs	3.18	1.77	2.44	0.06	0.08	0.34
PCDDs	1.38	0.88	0.81	0.11	0.11	0.16
PCDFs + PCDDs	4.56	2.65	3.25	0.17	0.19	0.50
Ratio PCDFs/PCDDs	2.30	2.01	3.01	0.55	0.73	2.13
Mono-ortho DL-PCBs	0.11	0.11	0.11	0.01	0.03	0.03
Non-ortho DL-PCBs	2.58	2.16	2.02	0.13	0.33	0.52
DL-PCBs	2.69	2.27	2.13	0.14	0.36	0.55
PCDD/Fs + DL-PCBs	7.25	4.92	5.38	0.31	0.55	1.05
% PCDFs	43.9	36.0	45.4	19.4	14.5	32.4
% PCDDs	19.0	17.9	15.1	35.5	20.0	15.2
% PCDFs + PCDDs	62.9	53.9	60.4	54.8	34.5	47.6
% Mono-ortho DL-PCBs	1.5	2.2	2.0	3.2	5.5	2.9
% Non-ortho DL-PCBs	35.6	43.9	37.5	41.9	60	49.5
% DL-PCBs	37.1	46.1	39.6	45.2	65.5	52.4

Table 2. PCDDs, PCDFs and DL-PCBs toxic equivalent values in soil samples (ng WHO-TEQ/kg dry weight) and feed samples (ng WHO-TEQ/kg 12% of moisture).

Pearson correlation coefficient	Soil plot 1	Soil plot 2	Soil plot 3	Corn silage	Sorghum silage	Hay
Soil plot 1	1,00					
Soil plot 2	1,00	1,00				
Soil plot 3	0,99	1,00	1,00			
Corn silage	0,78	0,80	0,80	1,00		
Sorghum silage	0,83	0,85	0,85	0,98	1,00	
Hay	0,94	0,95	0,96	0,90	0,93	1,00

Table 3. Pearson correlation coefficients are reported. The coefficient was calculated including the molecules quantified in all the matrixes (17 PCDD/Fs, 12 DL-PCBs and 6 indicator NDL-PCBs).

Concerning feed, all the samples were found under the maximum limits set by the European Commission Regulation No 277/2012¹². On the other hand, hay specimen reached the action threshold¹² established for dioxins and exceeded the one set for DL-PCBs; moreover, sorghum silage showed a DL-PCB TEQ values slightly above the European action limit of 0.35 ng WHO-TEQ/kg 12% of moisture¹². Feed contamination profiles strongly correlate to each other and to plots profiles (Table 3), suggesting that fodder contamination is mainly due to soil. In particular, the highest correlation between feed and soil profiles was shown by the sample of hay. The hay was obtained cutting grass at 5 cm above the soil level, a procedure that could be responsible for the higher contamination of the final product, due to the presence of soil residues¹³. Cutting height of corn and sorghum were of 35 cm and 20 cm respectively. Sorghum silage resulted over the action limit for DL-PCBs, instead corn silage was found poorly contaminated probably due to the higher cut. In the corn silage sample, PCBs analytical levels were always under the limit of quantification (LOQ) and, among PCDD/Fs, only 4 molecules were quantified (2,3,4,7,8-PeCDF; 1,2,3,7,8-PeCDD; 1,2,3,4,6,7,8-HpCDD and 1,2,3,4,6,7,8,9-OCDD).

Cutting height has been demonstrated to influence the importance of feed contamination by soil during harvesting^{13, 14}. The fodder contamination found in this study seems to be strictly linked to soil pollution. Since plant radical absorption of these chemical compounds is considered to be minimal and volatilization from soil has been shown to be of minor importance under outdoor conditions¹⁵, the presence of ground residues in the feed could be the main cause of the obtained results.

Harvesting guidelines, in particular indication about the cutting height, should be provided to farmers to avoid production of contaminated feed in high risk areas. We demonstrated that in a city with a history of soil contamination⁹, due to the presence of a PCB-producing plant and other industrial activities, agricultural soil compliance based on National limits for green and residential areas does not guarantee the safety of the obtained feed. In fact, despite the supposed soundness of the soil, hay and sorghum silage, harvested by the farmer, reached, or even exceeded, the action limits set by the European legislation. A long-term intake of the obtained feeds by dairy cows may be able to determine a rise of the background contamination in milk and, as a consequence, it could increase the risk of human exposure to dioxin and PCBs, as already reported in literature¹⁶. Further study are necessary to clearly understand the risk of a significant dioxins and PCBs excretion in milk, following animal prolonged feeding with a fodder showing level of contamination under the European maximum limits but above the action thresholds. Other authors have already highlighted the weak consistence between feed and milk European regulatory levels¹⁷, and cases of milk non-compliance, despite the legislative soundness of feed, have been already reported¹⁶. Moreover, this study demonstrated the need of specific legislative limits for agricultural soils, not only at National level, but especially in Europe, in order to guarantee an uniform approach.

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References:

1. EFSA-European Food Safety Authority (2012); *EFSA Journal* 10(7): 2832.
2. Malisch R, Kotz A, Wahl K, Hädrich J (2012); *Organohalogen Compounds* 74: 1405-14077.
3. Brambilla G, Cherubini G, De Filippis SP, Magliuolo M, Di Domenico A (2004); *Anal. Chim. Acta.* 514: 1-7.
4. Decreto Ministeriale 13 September 1999 (1999); *Gazz. Uff. Suppl. Ordin.* 248.
5. Commission Regulation (EU) No 691/2013 (2013); *Official Journal of the European Union* L 197/1.
6. US EPA-United States Environmental Protection Agency (1994); Method 1613, Revision B.
7. US EPA-United States Environmental Protection Agency (2010); Method 1668, Revision C.
8. Van den Berg M, Birnbaum LS, 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 RE (2006); *Toxicological Sciences* 93(2): 223–241.
9. Turrio-Baldassarri L, Abate V, Alivernini S, Battistelli CL, Carasi S, Casella M, Iacovella N, Iamiceli AL, Indelicato A, Scarcella C, La Rocca C (2007); *Chemosphere* 67: 1822-1830.
10. Beccaloni E, Vanni F, Giovannangeli S, Beccaloni M, Carere M (2010); *Ann. Ist. Super. Sanità* 46(3): 303-308.
11. Decreto Legislativo n.152 (2006); *Gazz. Uff. n. 88 Suppl. Ordin.* 96.
12. Commission Regulation (EU) No 277/2012 (2012); *Official Journal of the European Union* L 91/1.
13. Pignedoli S, Assirelli A (2003); *Agricoltura* 4: 38-40.
14. Bertilsson J, Andersson I, Johanson KJ (1988); *Health Physics* 55(6): 855-862.
15. Lovett AA, Foxall CD, Creaser CS, Chewe D (1997); *Chemosphere* 34 (5-7): 1421-1436.
16. Diletti G, Ceci R, Scortichini G, Migliorati G (2009); *Organohalogen Compounds* 71: 2344-2348.
17. Brambilla G, Fochi I, Falce M, De Filippis SP, Ubaldi A, Di Domenico A (2008); *Chemosphere* 73: S216-S219