A CASE OF MILK CONTAMINATION BY PCDD/Fs IN ITALY: ANALYTICAL LEVELS AND CONTAMINATION SOURCE IDENTIFICATION

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Introduction

PCDD/Fs levels exceeding the European Union (EU) tolerance limit were detected in two sheep's milk samples from Campania region collected in November 2001[1]. Analyses were repeated on two samples from the same flocks with one result above and the other just below the maximum admissible level. In order to investigate the problem extension further milk samples were taken from April 2002, broadening the control to other milk-producing animal species existent in the territory (cows, goats and buffaloes). The sampling area has been progressively extended to a large part of the Campania regional territory. Animal feed samples were also taken at the same farms where milk was collected. The present study is still in progress and focused to define the phenomenon extension and to identify the potential contamination sources with the purpose of reducing the human exposure to dioxins through food consumption. This paper presents data on the levels of dioxins and furans in milk and animal feed samples and makes some hypothesis about the identification of contamination sources on the basis of milk and animal feed PCDD/Fs congeners profile comparison.

Materials and methods

Milk and feeding-stuff samples were taken at farms sited in rural areas and near urban and industrial zones in Campania region. Milk samples were stored in glass bottles and subsequently frozen. Animal feed samples were packaged in plastic bags. A total of 274 milk samples (82 from cows, 77 from buffaloes, 85 from sheep, 16 from goats and 14 from sheep/goats) and 152 feedstuffs samples including silage, hay, cereals and other farinaceous feed, premixes and mixed feeds were analysed for PCDDs and PCFDs. Samples were tested by a validated method routinely used for dioxins monitoring in food and animal feed and successfully verified in a number of inter-laboratory studies. Feeding-stuffs samples containing a high water amount were left to dry at room temperature before the analysis. Their moisture content was determined in drying oven at 103°C allowing the calculation of PCDD/Fs concentrations considering a 12% moisture content, as requested by the EU legislation[2]. After grinding feed-stuffs were extracted by accelerated solvent extraction (ASE) using an ASE 300 Dionex instrument with a mixture of n-hexane and acetone 80:20 (v/v)[3]. Milk samples were first added of ethyl alcohol in order to precipitate proteins and then fat was extracted by means of a mixture of diethyl ether and petroleum ether 50:50 (v/v)[4]. The clean-up procedure was based on an acid/base partitioning and a further purification was then performed according to EPA Method 1613 Rev. B by means of an automated clean-up process with Power-Prep system (Fluid Management System) using disposable columns (multilayer silica,

alumina and carbon)[5]. PCDD/Fs were separated by high resolution gas chromatography (HRGC) on a DB-5 MS capillary column (60 m x 0.25 mm, 0.1 μm) and determined by high resolution mass spectrometry (HRMS), at a resolution of 10000 in the selected ion monitoring (SIM) mode. The HRGC/HRMS system consisted of a GC Trace Series 2000 (ThermoQuest) coupled with a MAT 95 XL (ThermoFinnigan). The quantification of the seventeen 2,3,7,8 chlorine-substituted dioxins/furans was accomplished by the isotope dilution method. TEQ values were calculated using WHO-TEFs[6]. According to the European legislation, WHO-TEQs were calculated as upper bound concentrations assuming that all values of specific dioxins congeners below the limit of determination (LOD) are equal to the respective LOD[1,2].

Results and discussion

The analytical results are reported as pg WHO-TEQ/g fat for milk and pg WHO-TEQ/g product for feeding stuffs referring to a moisture content of 12%, in conformity with Council Regulation (EC) 2375/2001 and Council Directive 2001/102/EC, respectively[1,2].

The results for milk are presented in Table 1-2. Out of 274 samples, 99 (accounting for 36%) exceeded the EU maximum tolerance level of 3 pg WHO-TEQ/g fat[1]. Taking into account the different animal species the highest percentage of not compliant samples was for buffalo's milk (48%), followed by sheep's and goat's milk (43 and 44%, respectively), mixed sheep/goat's milk (29%) and cow's milk (17%).

Table 3-4 illustrate the results for animal feed samples, divided into two groups: the former embodies locally grown feed such as silage, hay and grass (95 samples), the latter includes all the other kinds of feed (57 samples), comprising cereals and other farinaceous feed, premixes and mixed feeds. This distinction was made by reason of a marked difference in the contamination levels between the two groups: the former showed a relatively high PCDD/Fs content (median 0.17 pg WHO-TEQ/g), the latter exhibited a PCDD/Fs presence at background level (median 0.017 pg WHO-TEQ/g). A percentage of 18.9% (18 out of 95 samples) of the first group exceeded the EU tolerance limit of 0.75 pg WHO-TEQ/g, while none of the second group was above this level[2].

All eighteen feed-stuffs with PCDD/Fs levels higher than 0.75 pg WHO-TEQ/g came from farms whose milk was contaminated above 3 pg WHO-TEQ/g fat, providing an evidence that PCDD/Fs presence in milk is directly related to feed contamination and in particular to locally grown feed. Unfortunately locally produced feeding stuffs were not always available for sampling in the same farms where milk was collected and an additional sampling has been undertaken in order to acquire more data concerning PCDD/Fs levels in these kinds of animal feed.

The PCDD/F congeners distribution seems very similar in all significantly contaminated milk samples with no influence from the sampling site and from the milk-producing animal species. As an example, congeners profile of a cow's milk is reported in Figure 1 in comparison with a grass sample profile taken in the same farm. It is remarkable the major relative abundance of PCDFs (65%) compared to PCDDs (35%) in milk that is typical in the vicinity of potential contamination sources, while PCDDs prevail in milk from rural areas where only background contamination is present[7]. In grass sample the relative percentage of PCDDs and PCDFs is almost inverted in comparison with milk (60% PCDDs and 40% PCDFs). These results are in agreement with those obtained for cow's milk and grass from locations near solid waste incinerators in a dioxin bioavailability study and it represents an important indication supporting the hypothesis of a thermal origin of the contamination[8].

Since this study is still ongoing it is not possible to reach definitive conclusions considering the large area interested by the contamination. Further investigations are needed to exactly identify the sources and geographic extension of the contamination. Epidemiological surveys and spatial analyses will allow to better identify potential risk factors linked to the contamination. Actions to reduce dioxin emissions should be then undertaken with consequent decreasing of PCDD/Fs levels in the feed and food chain.

References

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Table 1: number of milk samples of different animal origin from Campania region grouped for PCDD/Fs concentration intervals (years 2001-2003)

Parameter	Milk-producing animal species				
	Cow	Sheep	Goat	Sheep/Goat	Buffalo
N° of samples >3 pg WHO-TEQ/g fat	14	37	7	4	37
N° of samples 1-3 pg WHO-TEQ/g fat	19	17	6	3	20
N° of samples <1 pg WHO-TEQ/g fat	49	31	3	7	20

Table 2: PCDD/Fs levels (pg WHO-TEQ/g fat) in milk samples from Campania region exceeding the EU tolerance limit (years 2001-2003)

Parameter	Milk-producing animal species		
	Cow	Sheep, Goat and Sheep/Goat	Buffalo
Mean	6.56	6.07	6.19
Median	4.78	4.95	5.77
Minimum value	3.63	3.01	3.02
90 Percentile	9.47	10.83	9.12
95 Percentile	12.86	14.35	13.36
Maximum value	18.03	24.17	16.45

Table 3: number of feed samples from Campania region grouped for PCDD/Fs concentration intervals (years 2002-2003)

Parameter[a]	Silage, hay and grass	Cereals and other farinaceous feed, premixes and mixed feeds
N° of samples >0.75 pg WHO-TEQ/g	18	0
N° of samples 0.30-0.75 pg WHO-TEQ/g	21	2
N° of samples <0.30 pg WHO-TEQ/g	56	55

(a) pg WHO-TEQ/g referred to a 12% moisture content.

Table 4: PCDD/Fs levels (pg WHO-TEQ/g[a]) in animal feed samples from Campania region (years 2002-2003)

Parameter	Silage, hay and grass	Cereals and other farinaceous feed, premixes and mixed feeds
Mean	0.53	0.045
Median	0.17	0.017
Minimum value	0.02	0.005
90 Percentile	1.19	0.080
95 Percentile	1.99	0.136
Maximum value	8.95	0.565

(a) pg WHO-TEQ/g referred to a 12% moisture content.

Figure 1: PCDD/Fs profile comparison between cow's milk and grass samples