A STUDY ON PCB, PCDD/PCDF INDUSTRIAL CONTAMINATION IN AN URBAN/AGRICOLTURAL AREA. PART II: ANIMAL MATRICES

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Introduction

The Istituto Superiore di Sanità is involved in a project investigating the contamination of an area in the northern Italy (near Brescia). In this area an industrial plant produced PCBs for about 50 year. In its vicinity, during monitoring campaigns, high levels of PCBs were measured in soils. In the contaminated area there are several small farms, most of which constitute a sort of self contained food chain: animals feed on contaminated forage, farmers consume meat and milk from these animals.
The transfer from soil through animals to humans across the food chain will be evaluated. PCDD/PCDF and PCB levels in different matrices will be analyzed: soil, forage, food of animal origin, human blood and breast milk[1].
In this paper we report the results obtained on samples of bovine perirenal fat, liver and milk from cattle grazing in the contaminated area and belonging to four small farms located near the industrial area. The concentration of 17 dibenzo-*p*-dioxin (PCDDs) and dibenzofurans (PCDFs), of the four non-*ortho*-substituted polichlorinated biphenyls (PCB77, PCB126, PCB 169 and PCB 81), of other 60 PCB congeners (including 8 mono-*ortho* congeners) were determined. Levels, profiles and contribution to toxic equivalent concentration (TEQ) of PCDD/F and dioxin-like PCB (non-*ortho* and mono-*ortho*-substituted) are discussed.

Methods and Materials

Samples**.**
The samples from different animals of the same farm were pooled to make a farm-pool. In fact, the contamination of the animals is part of a food chain: it is caused by soil/forage contamination and in turn it causes exposure of the human consumers. In this respect we thought to keep separated different farms, as different contamination could be in their respective soils and forages; all the samples analyzed came from bovines belonging to four contaminated farms (A-D). Samples from the same farm were pooled. Pooled sample A was made up with samples from 18 different animals, B with 1, C with 5, D with 4, for both perirenal fat and liver. A further set of samples from A farm (A1) was analyzed singularly as it came from a cow whose milk was also singularly analyzed. The other milk samples were pooled milk from cows belonging to the same farm, but the number and identity of the animals that produced it is not known.
A blank sample was carried out for each batch of samples.
Extraction and clean-up**.**
Bovine perirenal fat. Bovine perirenal fat pooled samples were dissolved in *n*-hexane at 40°C and filtered. Lipid determination was performed gravimetrically. Two aliquots were obtained, the first for PCDD/PCDF and non-*ortho* PCB analysis (aliquot I) and the second (aliquot II) for the mono-*ortho* and the other PCB congeners. Both of them were spiked with the respective isotopically labelled standard and eluted on a column packed with concentrated sulfuric acid coated on an inert support (Extrelut, Merck) with *n*-hexane. Determination of the mono-*ortho* and of the other PCB congeners were performed on the eluate of aliquot II, directly analyzed by HRGC/LRMS. For PCDD/PCDF and non-*ortho* PCB analysis, the concentrated eluate of aliquot I was submitted to a further purification with the automated multi-column Power Prep system[1]. The *n*-hexane extracts were transferred to the multi-layer silica gel column and eluted to an alumina column with *n*-hexane-dichloromethane (98:2 v/v). The PCDDs/PCDFs and non-*ortho* PCBs were back-flushed from the carbon columns with toluene. Determination was performed by HRGC/HRMS.
Bovine liver. For PCDD/PCDF and non-*ortho* PCB analysis, pooled samples were homogenized, spiked with a mixture of isotopically labeled standards and lyophilized. They were extracted by means of a Dionex Accelerated Solvent Extractor (ASE) apparatus with a 1/1 *n*-hexane/acetone mixture. The extracts were concentrated and the lipid determination was performed gravimetrically. Clean-up and determination was carried out exactly as for perirenal fat samples. For analysis of the other PCB congeners the homogenized aliquots were extracted by ASE

apparatus with a 1/1 *n*-hexane/acetone mixture. The extracts were concentrated and eluted on a column packed with concentrated sulfuric acid coated on an inert support (Extrelut, Merck) with *n*-hexane. Determination was performed by HRGC/LRMS directly on the eluate.

Milk. Samples of milk, spiked with a mixture of isotopically labeled standard (PCDDs/PCDFs and non-*ortho* PCBs), were added of sodium oxalate, methanol and diethyl ether and extracted in a separatory funnel with *n*-hexane. The extracts were dried, concentrated, and the fat content of milk was calculated. Two aliquots were obtained from the extract (I and II). The aliquot I (for PCDD/PCDF and non-*ortho* PCB analysis) was treated as described for bovine perirenal fat. The aliquot II (for PCB analysis) was adsorbed on alumina, spiked with a mixture of isotopically labeled standards (other PCB congeners), purified by means of a supercritical CO_2 fluid extractor (SFE Hewlett-Packard 7680T) as elsewhere described[2] and directly analysed by HRGC/LRMS.

Determination.

The quantification of PCDDs/PCDFs and non-*ortho* PCBs was carried out on an Autospec HRGC-HRMS system (10,000 resolution) equipped with a BPX-5 column (50 m, 0.32mm i.d.).

The determination of the other PCB congeners was carried out on a HRGC-LRMS "Trace MS Finnigan" from Thermo Quest, equipped with a HT-5 SGE column (25m, 0.22mm i.d.), in Electron Ionization mode operating at 70eV.

Results and Discussion

For all the animal matrices a pooled sample, representative of each small farm, was prepared. As previously mentioned, pooled samples represent on one side the contamination level caused on the animals by the same forage and, on the other, the contamination level of food consumed by people eating food from the same farm.

The TEQ contribution of PCDD/F and of dioxin-like PCBs, together with the total TE (pgTE/g of lipid) measured in the three matrices obtained from each farm under study, are reported in Table 1. TEQ was calculated using WHO TEF values[3]. As shown in Table 1, PCDD and PCDF levels for all the matrices are significantly higher than the EU limits[4] of 3 pgTE/g of lipid for meat from ruminants, 6 pgTE/g of lipid for liver, and 3 pgTE/g of lipid for milk. Only B farm displays levels slightly lower than the EU regulation limits.

The overall congener PCDD and PCDF profiles for each matrix were generally constant over different farms. A typical congener profile for the three matrices analyzed in the small farm D is shown in Figure 1. In this figure the level of each

congener is normalized with respect to the most abundant one. For liver OCDD was the most abundant congener, followed by 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxCDF (from 40 to 60% with respect to the most abundant congener), and 1,2,3,4,6,7,8-HpCDD (25-30%). For both perirenal fat and milk 2,3,4,7,8-PeCDF is the most abundant congener, followed by 1,2,3,4,7,8-HxCDF (from 50 to 80% respect the most abundant congener), and 1,2,3,6,7,8-HxCDF and OCDD (about 20%).

Most of the farms display levels of dioxin-like PCBs 10-fold higher than the European limits for PCDDs and PCDFs (Table 1). Moreover the dioxin-like PCB contribution to toxic equivalent concentration is greatly prevailing over the dioxin one. The 126 congener is by far the major single contributor. These clues seem to confirm that PCBs are the source of the contamination.

Table 1. Total pgTE/g of lipid for PCDDs/PCDFs, non *ortho*-PCBs and mono-*ortho*-PCBs. Total TEQ value for dioxin and dioxin-like PCBs (pgTE/g of lipid).

farm		Cow perirenal fat (pgTE/g lipid)	Cow liver (pgTE/g lipid)	Cow milk (pgTE/g lipid)
A	**PCDDs+PCDFs**	8.35	44.5	11.7
	Non-*ortho* PCBs	43.0	99.1	-
	Mono-*ortho* PCBs	23.3	24.7	9.0
	Total	74.7	168.3	
A1	**PCDDs+PCDFs**	9.60	26.2	6.92
	Non-*ortho*	51.9	82.7	39.6
	Mono-*ortho*	24.2	18.9	18.5
	Total	85.7	127.8	65.0
B	**PCDDs+PCDFs**	2.84	21.4	-
	Non-*ortho*	24.7	92.4	-
	Mono-*ortho*	10.1	15.4	-
	Total	37.5	129.2	
C	**PCDDs+PCDFs**	10.1	26.8	9.4
	Non-*ortho*	60.1	84.0	-
	Mono-*ortho*	27.3	22.9	6.4
	Total	97.5	133.7	
D	**PCDDs+PCDFs**	10.9	30.2	6.31
	Non-*ortho*	65.3	99.9	36.3
	Mono-*ortho*	26.6	34.2	13.1
	Total	102.8	164.4	55.7

Figure 1. A typical PCDD and PCDF congener profile, normalized respect the most abundant one, for liver, perirenal fat and milk of cattle of farm D.

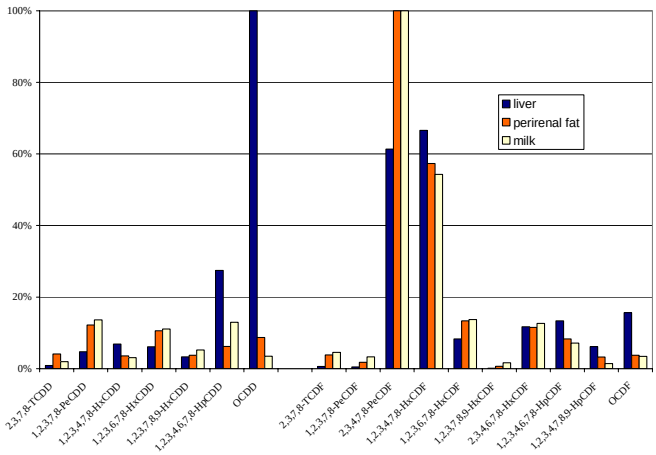

Table 2 reports total PCB concentration, expressed in ng/g of lipid; each result represents the sum of the concentration of 60 single congeners, including mono-*ortho* substituted. Levels observed in the present study were orders of magnitude higher than the data provided by the Italian Residue Control Plan (RCP) 2001[5]: in fact RCP data of milk and bovine meat from the same region show that, in about 80 samples randomly chosen, most of the samples have levels lower than the limits of determination (LOD), with only a few of them presenting the prevailing congeners (153 and 138) higher than the LOD (2 ng congener/g of lipid)[6]. Comparison with data obtained during the Belgian crisis of 1999[7], expressed as sum of the 7 indicator PCB congeners, show that our data are in the same range of bovines from farms that received contaminated feed (246-1060 ng/g of lipid), but significantly higher than the mean of these samples (487 ng/g of lipid). Comparison with milk levels show that results obtained in this study are significantly higher than what was then measured in Belgium (6-160 ng/g of lipid for milk).

Contamination levels of both the pooled and individual samples are generally similar, only sporadically displaying values differing for a factor higher than 2. The exam of the contamination levels of soils and forages can possibly help explaining this.

Table 2. Total PCBs (ng/g of lipid).

Farms	Perirenal fat	Liver	Milk
A	1106	2669	493
A1	1323	1351	845
B	418	1371	-
C	1164	1543	344
D	1118	1606	554

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