Chemosphere xxx (2009) xxx–xxx

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

PCB, PCDD and PCDF contamination of food of animal origin as the effect of soil pollution and the cause of human exposure in Brescia

Luigi Turrio-Baldassarri [a,*], Silvia Alivernini [a], Sergio Carasi [b], Marialuisa Casella [a], Sergio Fuselli [a], Nicola Iacovella [a], Anna Laura Iamiceli [a], Cinzia La Rocca [a], Carmelo Scarcella [b], Chiara Laura Battistelli [a]

[a] Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy
[b] ASL di Brescia, Dip. Prevenzione, via Cantore 20, 25128 Brescia, Italy

ARTICLE INFO

Article history:
Received 21 October 2008
Received in revised form 20 February 2009
Accepted 1 March 2009
Available online xxxx

Keywords:
PCB
PCDD/PCDF
Milk
Liver
Food chain

ABSTRACT

In Brescia a PCB production plant polluted soil and forage of the surrounding fields and caused a significant contamination of meat and milk of the cattle fed with local forage. This in turn induced elevated blood levels of PCDDs, PCDFs and PCBs in the consumers. The contamination levels and profiles measured in the perirenal fat, in the liver and in the milk of the overall 28 contaminated bovines are reported. TEQ levels varied from 30 to 81 pg WHO_{2005}-TEQ g^{-1} (38–103 pg WHO_{1997}-TEQ) for perirenal fat, from 107 to 138 pg WHO_{2005}-TEQ g^{-1} fat (128–168 pg WHO_{1997}-TEQ) for liver and from 45 to 50 pg WHO_{2005}-TEQ g^{-1} fat (56–65 pg WHO_{1997}-TEQ) for milk; all these values are roughly tenfold higher than the European limits. Non-*ortho* dioxin-like (dl)PCBs are by far the largest contributors to TEQ and PCDF contribution also largely prevail over PCDD's; both these features are also present in both the contaminated forages and in the serum of consumers of contaminated food. The indicator PCB levels are in the following ranges: 226–664 ng g^{-1} for perirenal fat; 929–1822 ng g^{-1} fat for liver; 183–477 ng g^{-1} fat for milk; their level is about 100 times higher than the regional background.

The liver samples displayed an overall TEQ several times higher than the perirenal fat from either the same animal or the same pool of animals; the increase in liver concentration was significantly higher for PCDD and PCDF congeners than for dlPCBs, and it was maximum for OCCD.

1. Introduction

This paper is the third part of a study investigating the contamination of an area in the city of Brescia, Italy, where the only Italian plant producing PCBs operated from 1930 to 1984.

In the year 2001 (CTS, 2003) high levels of PCB were measured in soils located South of the plant; further investigations eventually showed also high levels of PCDD and PCDF (Turrio-Baldassarri et al., 2007); the maximum measured contamination level was as high as 1034 pg WHO_{1997}-TEQ g^{-1} of soil as the sum of dlPCBs, PCDDs and PCDFs. The contamination occurred probably decades earlier, as production of PCB was terminated in 1984, and went undetected for many years; the contamination process was not caused by a single major release of products and was instead a continuous leak lasted for a long time. The vehicle of the propagation of the contamination into the environment is the dispersion with irrigation water of contaminated sediments of a water stream passing through the plant (ASL Brescia, 2008).

The most severely contaminated area had some very peculiar features: four small farms were located in it, and they included a sort of self contained food chain. There, in fact, cows and calves were grown and fed, partially or totally, on contaminated forage, while the farmers themselves, together with the families of their sons and daughters, consumed most of the meat and milk produced in their farms. In this way these families experienced a long-lasting exposure to these chemicals, and exhibited a mean blood level of PCB, PCDD and PCDF of 419 pg WHO_{1997}-TEQ g^{-1} lipid, roughly eight times higher than the general population from the same area (Turrio-Baldassarri et al., 2008).

The whole study, presented here and in the previous two papers, has some unique characteristics: it deals with an industrial contamination affecting first soil and consequently forage, and then meat and milk and finally humans. This cycle of contamination lasted for many years and there are people affected by it presumably throughout their whole life or for a relevant part of it. The persons presenting elevated blood levels of PCDD, PCDF and PCB are now in a sanitary program, involving regular analyses and medical check-up.

In this paper we report the results obtained on samples of bovine perirenal fat, liver and milk from cattle fed with contaminated forage from the four small farms. The concentration of 17 dibenzo-*p*-dioxin (PCDDs) and dibenzofurans (PCDFs), of the four non-*ortho*-substituted polichlorinated biphenyls (PCB 77, PCB 126,

* Corresponding author. Tel./fax: +39 (0)6 4990 3073.
E-mail address: luigi.turrio@iss.it (L. Turrio-Baldassarri).

0045-6535/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2009.03.002

PCB 169 and PCB 81), of other 56 PCB congeners (including 8 mono-*ortho* congeners) were determined. Levels, profiles and contribution to toxic equivalent (TEQ) concentration of PCDDs, PCDFs and dlPCBs (non-*ortho* and mono-*ortho*-substituted) are presented and discussed in relation, whenever possible, to the contamination of soil and forage on the one side and of the serum of the consumers on the other side.

The contamination of food of animal origin is indeed the central issue of the whole study, being both the result of soil and forage contamination (part I, Turrio-Baldassarri et al., 2007) and the main cause of human exposure (part II, Turrio-Baldassarri et al., 2008).

The aim of the whole study is to document as accurately as possible the contamination in all the environments interested and to explore the possible relations among them. However, it was impossible to retrieve all the information relative to the different kinds of samples analyzed in this study. For instance, forage samples were the ones found in the barns and it was not always known the exact location or the time when they were collected. This as a consequence of a real life event and not of a laboratory planned study.

The transfer of contaminants from soil and forage through animals to humans across the food chain will be discussed in terms of contamination profiles and TEQ contribution; their accumulation in different tissues of the same animal will also be commented.

2. Experimental

2.1. Sample description and preparation

After contamination was discovered, 28 bovines from four different farms (indicated as A, B, C and D) were butchered and their meat, after analyses, disposed of. One sample of perirenal fat and one of liver was taken from each animal and frozen. Three farm-pool milk samples were also available together with a single milk sample from a specific cow (A1). The samples from different animals of the same farm were pooled to make farm-pools so that one pool of perirenal fat and one pool of liver were made for each farm. 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. Farm B yielded then a set of two samples (fat and liver) from a single animal; a second single animal set of three samples (milk, fat and liver) was analyzed from farm A as it came from the only one cow (A1) whose milk was also singularly available. A blank sample was analyzed with each batch of samples.

In each sample were singularly determined seventeen 2,3,7,8-substituted PCDDs and PCDFs, four mono-*ortho* PCB congeners, eight non-*ortho* PCB congeners, 48 non-dioxin-like PCB congeners, as reported elsewhere (Turrio-Baldassarri et al., 2008).

2.2. Extraction and clean-up

2.2.1. Bovine perirenal fat

The perirenal fat pooled sample was dissolved in *n*-hexane at 40 °C and filtered. Lipid determination was performed gravimetrically after solvent removal. Two aliquots of each sample were used, the first (aliquot I) for PCDD/PCDF and non-*ortho* PCB analysis and the second (aliquot II) for the other PCB congeners. Both of them were spiked with the respective mixture of fully ^{13}C labeled standards containing: nine 2,3,7,8-substituted congeners of PCDD and PCDF and three non-*ortho* PCB congeners for aliquot I, and ten PCB congeners for aliquot II. Both aliquots were dissolved and eluted with *n*-hexane through columns packed with concentrated sulfuric acid coated on an inert support, Extrelut NT20 (Merck, Darmstadt, Germany). The determination of the mono-*ortho* and of the other PCB congeners and pesticides was performed on the concentrated eluate from the aliquot II column, directly analyzed by GC–LRMS. The analysis of PCDD, PCDF and non-*ortho* PCB involved further purification of the concentrated eluate from aliquot I column, submitted to the automated procedure of the multi-column Power-Prep system (Fluid Management Systems inc., Waltham, MA, USA, Turrio-Baldassarri et al., 2004) in the following way: the *n*-hexane extracts were passed on the multi-layer silica gel column and then eluted in sequence through an alumina column and a carbon column with *n*-hexane/dichloromethane (98/2 v/v). The PCDDs, PCDFs and non-*ortho* PCBs were back-flushed from the carbon column with toluene. Determination was performed by GC/HRMS.

2.2.2. 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 Accelerated Solvent Extractor (ASE) from Dionex (Sunnyvale, CA, USA) with a 1/1 *n*-hexane/acetone mixture at a temperature of 100 °C and a pressure of 100 atm. The extracts were concentrated and the lipid determination was performed gravimetrically. From this point on, the analytical procedure was the same as for perirenal fat samples.

2.2.3. Milk

The milk samples were placed in separatory funnels, spiked with a mixture of isotopically labeled standards containing PCDDs + PCDFs and non-*ortho* PCBs, added with sodium oxalate, and extracted adding, in sequence, methanol diethyl ether and *n*-hexane. The extracts were dried, concentrated, and the fat content of milk was calculated. Two aliquots were obtained from the extract. The aliquot I was treated as described for bovine perirenal fat. The aliquot II was adsorbed on alumina, spiked with a mixture of isotopically labeled standards (ten PCB congeners) and purified by means of a supercritical CO_2 fluid extractor (SFE mod. 7680T instrument from Agilent Technologies, Santa Clara, CA, USA) as elsewhere described (La Rocca et al., 2004) and directly analyzed by HRGC/LRMS.

2.3. Instrumental analysis

The quantification of PCDDs, PCDFs and non-*ortho* PCBs was carried out on an Autospec HRGC–HRMS system (Fisons Autospec, Manchester, UK, 10 000 resolution) equipped with a BPX-5 column from SGE (Ringwood, Australia, 50 m, 0.32 mm i.d., 0.25 μm film thickness).

The determination of the other PCB congeners and pesticides was carried out on a Trace MS DSQ from Thermo Instrument (Austin, TX, USA) HRGC–LRMS, equipped with a HT-5 column from SGE (25 m, 0.22 mm i.d., 0.25 μm film thickness), in Electron Ionization mode operating at 70 eV.

QA/QC: our laboratory regularly uses certified reference materials; and routinely employs laboratory reference materials; moreover, it participated since the year 2000 to the international "Dioxin in food" interlaboratory exercises, determining PCDDs, PCDFs, dlPCBs, non-dlPCBs and lately polybromodiphenylethers (PBDEs) in three different food matrices each year. The results were always satisfactory and are described in more detail in the serum paper (Turrio-Baldassarri et al., 2008).

The number of isotopically labeled spikes used in the analyses, although lower than what is now generally being used, does not imply a loss in accuracy, as proven through successful participation to interlaboratory exercises in the course of many years.

3. Results and discussion

In Table 1 analytical levels of PCDDs, PCDFs and PCBs, including dioxin-like PCBs (pg g^{-1} of lipid) measured in the three matrices

Please cite this article in press as: Turrio-Baldassarri, L., et al. PCB, PCDD and PCDF contamination of food of animal origin as the effect of soil pollution and the cause of human exposure in Brescia, Chemosphere (2009), doi:10.1016/j.chemosphere.2009.03.002

obtained from each farm under study are reported: sum of PCDDs and PCDFs, ratio PCDDs/PCDFs, sum of mono-*ortho* and non-*ortho* PCBs are also included; finally, to allow comparison with non-dlPCB data, also the sum of the 60 analyzed PCB congeners, the sum of the 6 indicator congeners and the concentrations of PCB 153 are also reported. The toxicity equivalent (TEQ) levels of PCDDs and PCDFs, mono-*ortho* and non-*ortho* PCBs (pg TEQ g^{-1} of lipid), calculated using both WHO_{2005} TEFs (van den Berg et al., 2006), and WHO_{1997} TEFs (van den Berg et al., 1998) are reported in Table 2 for PCCDs + PCDFs and for PCBs + PCDDs + PCDFs. Higher overall TEQ values are obtained with 1997 TEFs, which are to be used for EC regulation compliance.

The ratio of the analytical values PCDFs/PCDDs is always higher than 1 in fat and milk: it ranges from 2.5 to 4 for fat and from 4 to 5 for milk, whilst for liver the range spans from 0.5 to 1.2. In the liver the levels of the high chlorinated, low TEF PCDD congeners, such as HpCDD and OCDD, are much increased with respect to both perirenal fat and milk from the same animals, and this effects the PCDF/PCDD ratio. When the TEQ is considered (Table 2), the PCDF/PCDD TEQ ratio in liver reaches maximum values. The high TEQ ratio PCDF/PCDD together with the high levels of PCBs with respect to the levels found in animals representing background exposure (Weiss et al., 2005), confirm that the origin of the pollution is a PCB mixture.

In industrial PCB mixtures, PCDFs display concentrations up to three orders of magnitude higher than PCDDs (di Domenico et al., 1994; Kodavanti et al., 2001). The PCDD levels found here, although higher than expected keeping into account the sheer PCDF/PCDD ratio of industrial PCB mixtures, are comparable with levels measured in Belgian cows following a PCB contamination of feed (Bernard et al., 2002). Contribution of background contamination of the industrial area and a higher resistance to metabolic degradation may play a role in the PCDD enrichment, as it is visible for liver: it is indeed interesting to point out that the concentrations of all PCDD and PCDF congeners are considerably higher in each liver sample than in the corresponding perirenal fat sample, while for dlPCBs this is not true (except for PCB 126). The higher increases are exhibited by PCDDs, the maximum being shown by OCDD (59 as ratio liver/perirenal fat concentration) followed by HpCDD (ratio = 21).

As shown in Table 2, PCDD + PCDF levels for all the matrices but one are higher than the EU limits of 3 pg WHO_{1997}-TEQ g^{-1} of lipid

Table 1
PCDDs, PCDFs, dlPCBs levels (pg g^{-1} lipid), non-dlPCBs (ng g^{-1} lipid) in the three matrices from farms A–D. A1 and B samples are from one single animal.

Matrix	Perirenal fat					Liver					Milk			
Farm/animal	A	A1	B	C	D	A	A1	B	C	D	A	A1	C	D
PCDDs and PCDFs, pg g^{-1} lipid														
2,3,7,8-TCDD	<0.6	0.5	0.3	0.6	0.6	0.4	0.4	0.4	0.4	0.6	0.4	0.3	0.6	0.2
1,2,3,7,8-PeCDD	1.4	1.3	0.6	1.7	1.7	3.2	2.7	4.2	3.2	3.0	1.2	1.0	1.3	1.1
1,2,3,4,7,8-HxCDD	<1.8	0.7	<0.2	0.9	0.5	8.4	4.7	5.1	6.4	4.4	0.5	0.3	0.3	0.3
1,2,3,6,7,8-HxCDD	<1.0	1.6	0.5	2.0	1.5	7.2	2.8	4.3	4.0	3.9	1.3	0.6	1.6	0.9
1,2,3,7,8,9-HxCDD	<1.1	0.5	<0.2	0.7	0.5	4.7	2.0	3.1	3.7	2.1	0.6	0.3	0.5	0.4
1,2,3,4,6,7,8-HpCDD	1.5	1.5	0.4	2.1	0.9	33.0	17.2	15.6	32.1	17.6	1.4	0.7	0.8	1.1
OCDD	<2.0	1.4	<0.8	2.8	1.2	139	56.2	49.6	199.7	64.2	1.5	0.5	1.6	N.D.
ΣPCDDs	9.4	7.5	2.9	10.8	7.0	195.8	85.9	82.3	249.5	95.9	7.0	3.8	6.6	3.9
2,3,7,8-TCDF	0.4	1.0	0.3	0.5	0.5	0.5	0.9	0.5	0.5	0.4	0.4	0.8	0.3	0.4
1,2,3,7,8-PeCDF	<0.6	0.5	<0.1	<0.3	<0.3	0.7	0.6	0.3	0.3	0.3	0.8	0.5	0.7	0.3
2,3,4,7,8-PeCDF	11.0	12.5	3.2	11.8	14.2	56.6	34.1	24.9	30.7	39.4	16.0	9.4	11.8	8.2
1,2,3,4,7,8-HxCDF	6.3	8.0	1.8	9.4	8.1	76.0	36.6	17.9	44.3	42.8	11.9	5.0	8.4	4.5
1,2,3,6,7,8-HxCDF	1.4	2.1	0.5	2.3	1.9	12.4	5.5	4.6	7.5	5.3	2.8	0.9	2.6	1.1
1,2,3,7,8,9-HxCDF	<1.0	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.3	<0.04	<0.5	<0.1
2,3,4,6,7,8-HxCDF	<0.8	1.4	0.5	1.8	1.6	11.4	4.8	5.6	6.4	7.5	1.7	0.6	2.0	1.0
1,2,3,4,6,7,8-HpCDF	0.7	1.2	0.2	2.0	1.2	13.7	7.9	5.4	10.4	8.6	1.4	0.4	0.9	0.6
1,2,3,4,7,8,9-HpCDF	<0.7	0.3	<0.1	0.6	0.5	5.4	3.7	1.0	3.9	4.0	<0.4	0.1	0.3	<0.1
OCDF	<1.0	0.4	<0.4	0.9	<0.5	27.1	11.5	3.1	22.5	10.0	0.9	0.2	0.7	<0.3
ΣΣPCDFs	24	27	7	30	29	204	106	63	127	118	36.7	17.9	28.2	16.7
ΣPCDDs, PCDFs	33	35	10	41	36	400	192	146	376	214	5.2	21.7	34.8	20.6
Ratio PCDFs/PCDDs	2.5	3.6	2.5	2.7	4.2	1.0	1.2	0.8	0.5	1.2	5.2	4.7	4.3	4.2
Mono-ortho PCBs, pg g^{-1} lipid														
Dioxin-like PCBs														
PCB 105	14848	20168	8993	25076	22469	17755	16468	17312	19223	16613	7493	17961	5112	14432
PCB 114	1940	2027	864	2149	2238	1374	1018	1459	1285	1200	753	1848	589	1218
PCB 118	122709	119390	53003	139099	128956	125926	81565	84610	98844	96740	58064	106331	41694	66983
PCB 123	1464	1807	780	1763	1865	1149	1021	875	1059	909	479	1911	481	1009
PCB 156	14149	15023	5566	15921	16547	15907	14292	7993	16254	39996	3344	8097	2169	6715
PCB 157	2555	2824	1049	3166	3520	2798	2466	978	4098	3950	902	1537	927	1492
PCB 167	7883	8992	2983	8088	8805	12353	9078	5199	12580	19034	1955	5998	1347	4059
PCB 189	<408	<460	<299	<412	<418	<484	<487	<1109	<340	<540	<1961	<751	<133	<730
ΣMono-*ortho* PCBs	165956	170690	73538	195674	184818	177744	126395	119535	153682	178982	74952	144434	54451	96638
Non-ortho PCBs, pg g^{-1} lipid														
PCB 77	35	65	19	48	22	35	59	29	68	10	n.a.	61	n.a.	22
PCB 81	10	27	10	20	15	17	23	16	17	12	n.a.	23	n.a.	11
PCB 126	423	512	244	594	644	986	823	920	836	993	n.a.	392	n.a.	359
PCB 169	66	66	26	66	87	43	40	33	36	59	n.a.	35	n.a.	44
ΣNon-*ortho* PCBs	534	669	300	729	768	1082	945	998	957	1074	n.a.	511	n.a.	436
Non-dlPCBs, ng g^{-1} lipid														
PCB 153	341	375	111	336	337	845	402	392	469	476	155	258	99	162
Σ6 Indicator PCBs	655	752	226	657	664	1822	851	929	1019	1027	285	477	183	321
Σ60 PCBs	1106	1323	418	1164	1118	2669	1351	1371	1543	1606	522	845	374	554

n.a. = Not analyzed.

Please cite this article in press as: Turrio-Baldassarri, L., et al. PCB, PCDD and PCDF contamination of food of animal origin as the effect of soil pollution and the cause of human exposure in Brescia, Chemosphere (2009), doi:10.1016/j.chemosphere.2009.03.002

Table 2
Contribution of the different classes of compounds (PCDDs, PCDFs, non-*ortho* and mono-*ortho* PCBs) to TEQ with both 1998 and 2005 WHO TEFs. The TEQ ratio PCCFs/PCDDs is also reported.

Matrix	Perirenal fat					Liver					Milk			
Farm	A	A1	B	C	D	A	A1	B	C	D	A	A1	C	D
TEQ (pg g^{-1} lipid) of the different classes of compounds using 2005 WHO TEFs														
Mono-*ortho* PCBs	5.0	5.1	2.2	5.9	5.5	5.3	3.8	3.6	4.6	5.4	2.2	4.3	1.6	2.9
Non-*ortho* PCBs	44.3	53.2	25.2	61.4	67.0	99.9	83.5	93.0	84.7	101.1		40.3		37.2
TOT dioxin-like PCBs	49.3	58.3	27.4	67.3	72.6	105.3	87.3	96.6	89.3	106.5		44.6		40.1
PCDDs	2.4	2.1	0.9	2.7	2.6	6.0	4.2	6.1	5.4	4.8	1.9	1.5	2.1	1.5
PCDFs	4.3	5.0	1.3	5.0	5.5	27.2	15.2	10.4	15.3	17.6	6.6	3.6	5.0	3.2
PCDDs + PCDFs	6.8	7.1	2.2	7.7	8.1	33.2	19.3	16.5	20.7	22.4	8.5	5.0	7.0	4.7
Overall PCBs + PCDDs + PCDFs	56.0	65.4	29.6	75.0	80.7	138.5	106.6	113.1	110.0	128.9		49.6		44.8
TEQ ratio PCDFs/PCDDs	1.8	2.4	1.4	1.8	2.1	4.6	3.6	1.7	2.8	3.6	3.4	2.4	2.4	2.2
TEQ (pg g^{-1} lipid) of the different classes of compounds using 1998 WHO TEFs														
Mono-*ortho* PCBs	23.3	24.2	10.1	27.3	26.6	24.7	18.9	15.7	22.9	34.2	9.3	18.5	6.8	13.1
Non-*ortho* PCBs	43.0	51.9	24.7	60.1	65.3	99.1	82.7	92.4	84.0	99.9		39.6		36.3
TOT dioxin-like PCBs	66.3	76.1	34.7	87.4	91.9	123.8	101.6	108.0	106.9	134.2		58.1		49.4
PCDDs	2.4	2.1	0.9	2.7	2.6	5.9	4.2	6.0	5.4	4.8	1.9	1.5	2.1	1.5
PCDFs	6.5	7.5	1.9	7.4	8.3	38.6	22.0	15.4	21.4	25.4	9.8	5.4	7.3	4.9
PCDDs + PCDFs	9.0	9.6	2.9	10.1	10.9	44.5	26.2	21.4	26.8	30.2	11.7	6.9	9.4	6.3
Overall PCBs + PCDDs + PCDFs	75.3	85.7	37.6	97.5	102.8	168.3	127.8	129.5	133.7	164.4		65.0		55.7
TEQ ratio PCDFs/PCDDs	2.7	3.6	2.1	2.7	3.2	6.5	5.3	2.5	4.0	5.3	5.1	3.7	3.5	3.3

for meat and milk from ruminants, 6 pg WHO_{1997}-TEQ g^{-1} of lipid for liver (EC, 2375/2001). Only B farm displays levels slightly lower than the EU regulation limits for fat. However, when also dlPCBs are considered, according to the new European Regulation (EC, 199/2006) not yet enforced when animals were slaughtered, all the limits are exceeded, often more than tenfold: in fact the overall limits (PCDDs + PCDFs + dlPCBs) established by the new regulation are 4.5 pg WHO_{1997}-TEQ for meat products, 6 for milk and 12 for liver. For EC regulation compliance, WHO_{1997} TEFs are to be used, yielding, in the present case, overall TEQ values 25% higher (30% for fat and milk, 21% for liver) than using WHO_{2005} TEFs. The dioxin-like PCB contribution to toxic equivalent concentration is greatly prevailing over the PCDD + PCDF one and it is mostly due to non-*ortho* PCBs, and in particular to PCB 126. The 126 congener is by far the major single contributor. These facts confirm that PCB mixtures are the source of the contamination. In fact, samples exhibiting background contamination, beside showing much lower overall contamination levels, generally display a PCCD/F contribution to TEQ comparable (from equal to one half) to that of dlPCB (SCF, 2000; Fürst, 2001; Santillo et al., 2003; Weiss et al., 2005; Malisch and Dilara, 2007); according to Malisch and Dilara (2007) a dlPCB contribution higher than 85% in butter indicates a PCB contamination source. Congener 126, notwithstanding its low concentration in industrial mixtures (Schulz et al., 1989; Frame et al., 1996), due to its high TEF is almost invariably the single greater contributor to TEQ in background samples worldwide and accounts about 52% of the dlPCB contribution to TEQ (Weiss et al., 2005); in the present case it accounts for about 70%.

The non-*ortho* contribution is made even more evident using WHO_{2005} TEFs, due to the decreased contributions of mono-*ortho* PCBs and of Penta CDFs. This is illustrated in the Figs. 1 and 2, where the percentage contribution to total TEQ are reported in the three matrices using, respectively, WHO_{2005} and WHO_{1997} TEFs.

The fact that the levels found exceed by one order of magnitude the legal limit may well represent the intensity of the contamination. Another way to look at it is to compare the levels found with the background levels in the same area. The non-dioxin like PCB data can be used in this case, as reliable background PCB data are available from the Italian Residue Control Plan (RCP) 2001. PCB 153 is the single congener exhibiting the highest concentration in milk samples; so it is the one that has the highest number of determinable values even in background concentration level samples. It is then the most fit for the present comparison: for this purpose its concentration was reported in Table 1.

The mean value for PCB 153 over the perirenal fat and milk samples analyzed in the present study is 242 ng g^{-1}; the data from RCP, (available in Turrio-Baldassarri et al. (2004) for Italy, and in Report of ISS to Ministry of Health (2003), for detail of Lombardy) indicate values for PCB 153, on 83 milk and fat samples randomly chosen from the same region (Lombardy) and on the same year (2001), ranging from below the 2 ng g^{-1} LOD in 62 samples and a mean value of 4.3 ng g^{-1} in the 21 samples that displayed quantifiable levels of PCB 153. Using the upper bound approach (that is assuming equal to LOD all values reported as <LOD[1]), this gives a mean value of 2.58 ng g^{-1} fat in the 83 milk and meat samples from Lombardy in 2001. So it is visible that a contamination roughly one order of magnitude higher than the legal limits (for overall TEQ of PCDDs + PCDFs + dlPCBs) and two orders of magnitude higher than the local background levels (for PCB 153) was present in the animals from the four farms.

It is interesting also to discuss the contamination profiles, as the origin of the contamination is now being disputed by a report (Porta, 2005), based exclusively on the matching of the PCDD/F literature profiles of airborne particulate matter emitted from various thermal processes with the profiles found in the soil. The report suggests that the PCDD and PCDF contamination of the soil was not caused by the PCB production plant but by thermal processes, especially of the metallurgic industries, widely present in the city (but not close to the polluted area). The absolute contamination levels and the simultaneous presence in the soil samples of relevant amounts of PCBs were not considered. Moreover, the report considers, for the profile matching, PCDD/F reference profiles of airborne particulate matter while it was found (ASL Brescia, 2008) that the PCB pollution was diffused through the polluted sediments of a water channel passing through the plant, whose waters were used for irrigation of agricultural fields. The contamination, in fact, is found only in areas close to the channel, downstream of the plant (figures can be found in ASL Brescia, 2008). In the paper on soil and forage (Turrio-Baldassarri et al., 2007)

[1] This assumption implies an overestimation of the real value.

Please cite this article in press as: Turrio-Baldassarri, L., et al. PCB, PCDD and PCDF contamination of food of animal origin as the effect of soil pollution and the cause of human exposure in Brescia, Chemosphere (2009), doi:10.1016/j.chemosphere.2009.03.002

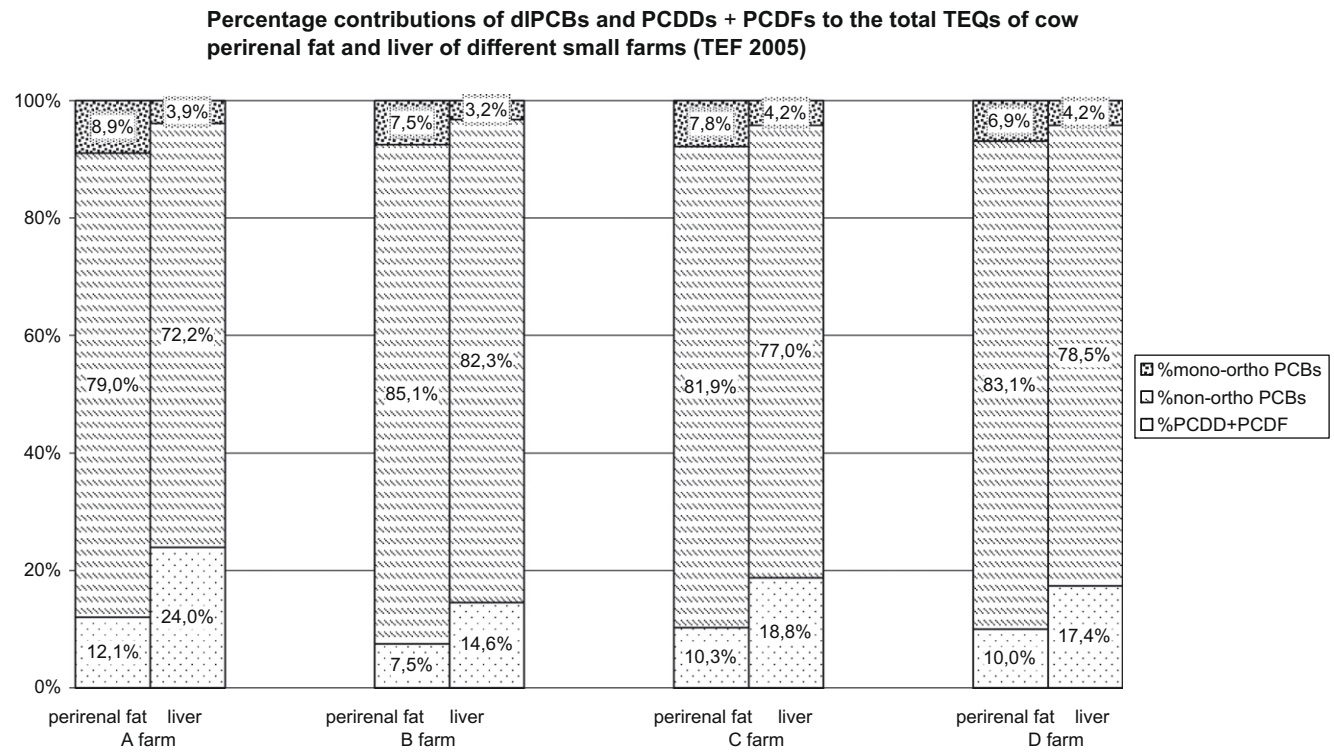

Fig. 1. Percentage contributions of non-*ortho* dlPCBs, mono-*ortho* dlPCBs and PCDDs + PCDFs to the total TEQs of perirenal fat and liver of animals from different farms (TEF 2005).

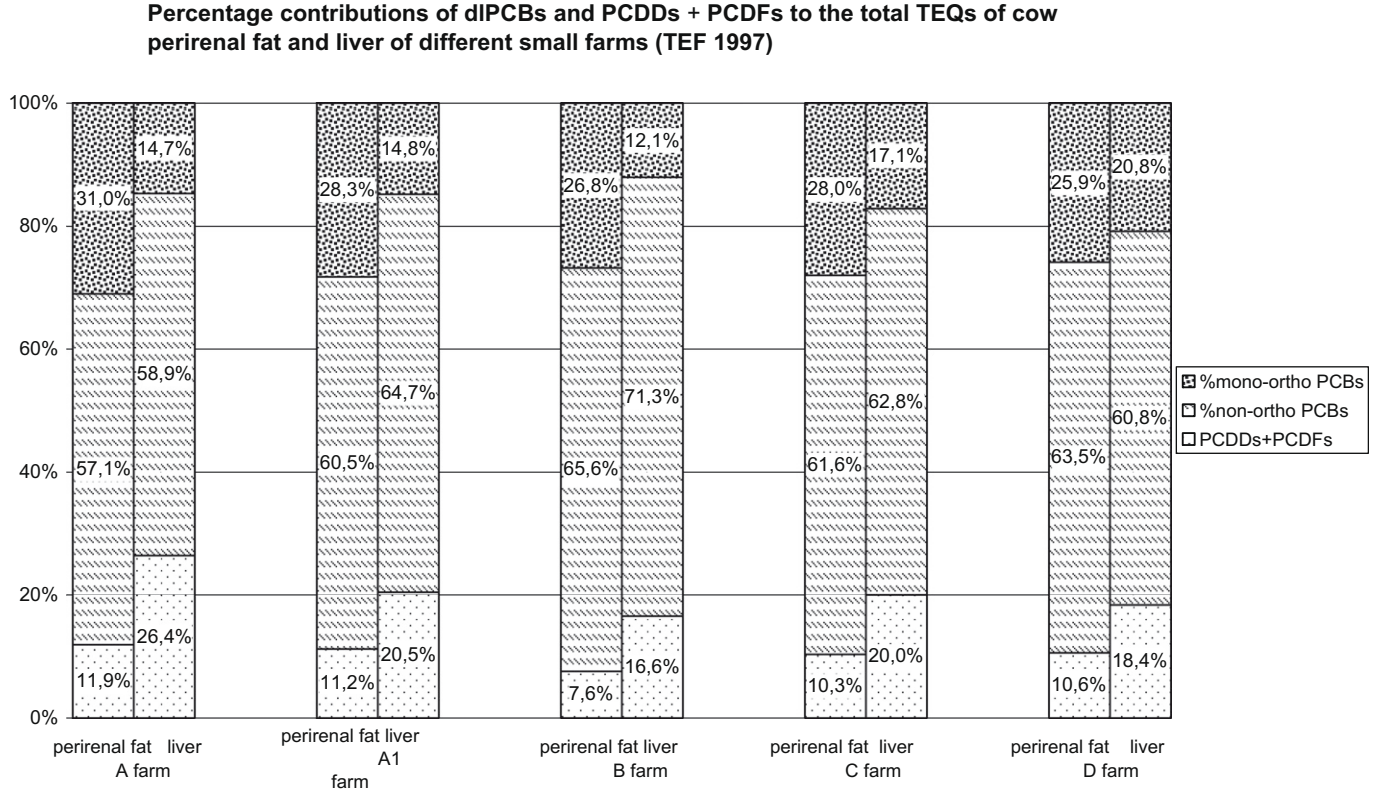

Fig. 2. Percentage contributions of non-*ortho* dlPCBs, mono-*ortho* dlPCBs and PCDDs + PCDFs to the total TEQs of perirenal fat and liver of animals from different small farms (TEF 1997).

we showed that soil samples exhibiting relevant PCB contamination also showed high levels of PCDDs and PCDFs. Moreover, in a previous paper (Turrio-Baldassarri et al., 2005) we showed that the PCB profile of soil contamination was highly correlated

Please cite this article in press as: Turrio-Baldassarri, L., et al. PCB, PCDD and PCDF contamination of food of animal origin as the effect of soil pollution and the cause of human exposure in Brescia, Chemosphere (2009), doi:10.1016/j.chemosphere.2009.03.002

(r = 0.893) with a mixture containing 15% of Aroclor 1248 and 85% of Aroclor 1262. In the present paper we showed that the PCB contamination in animals was at least two orders of magnitude higher than the local background. The results here presented confirm that the contamination concerns simultaneously PCBs, PCDFs and PCDDs, in relative amounts compatible with a PCB contamination. Other characteristics of a PCB contamination present in this case are the high PCDF/PCDD ratio and the relevance of non-*ortho* PCBs contribution to TEQ. All of these features simultaneously present can be regarded as indicators of a PCB contamination.

The overall congener profiles of PCDDs and PCDFs for each matrix were generally constant over animals from different farms. A typical congener profile for the three matrices analyzed in the small farm D is shown in Fig. 3, together with the profile of a pooled sample of forages (Turrio-Baldassarri et al., 2007). In this figure the level of each congener is normalized with respect to the most abundant one. For liver and forage OCDD was the most abundant congener, followed by 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxCDF (from 15% to 65% with respect to the most abundant congener), and 1,2,3,4,6,7,8-HpCDD (15–30%). 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 55% to 80% respect the most abundant congener), and 1,2,3,6,7,8-HxCDF and OCDD (about 15–20%). The profiles of perirenal fat and milk are similar to each other; in all the profiles the prevalence of PCDF over PCDD, OCCD excluded, is evident.

It may be interesting to compare the contamination levels and profiles observed in the present study to the ones reported in the episode occurred in 1999 in Belgium, when a relevant amount of PCB oil was accidentally mixed to vegetable oil and animal fat used to produce animal feed.

The most relevant contamination then was shown by poultry, although some bovine and swine contaminated feed was produced and consumed.

Bernard et al. (2002) reported PCDD, PCDF and non-dioxin-like PCB levels in products from some bovine farms supplied with possibly contaminated feed: levels for PCDD and PCDF in bovine meat were in a range (3.6–13.2 pg WHO_{1997}-TEQ g^{-1} fat) close to the one found (2.9–10.9 pg WHO_{1997}-TEQ g^{-1} fat) in the present study, while the milk level range was somewhat higher in our study (6–12 vs 1.1–6.0). Unfortunately, dlPCBs were not reported in the Bernard study; however comparing the sums of the 7 indicator PCB congeners, it turns out that our data are almost within the range of bovines from farms that received contaminated feed (246–1060 ng g^{-1} of lipid in Belgium, 226–752 in the present study), and comparable as mean value (487 ng g^{-1} of lipid in Belgium, 591 in the present study). Comparison with milk levels show that results obtained in the present study are significantly higher than what was then measured in Belgium (6–160 ng g^{-1} of lipid for milk).

The profile comparison for PCDD and PCDF is shown in Fig. 4, while the one for the profiles of five out of the seven indicator PCB congeners is illustrated in Fig. 5. In both graphs, data from the present study are reported as mean values of all the pools, together with their standard deviations, while the data from the Belgian study no standard deviation was available. The Belgian data illustrated in these two figures were not taken from tables but from graphs: so, some inaccuracy may be present. Similarities between the two couples of graphs are however evident.

A second contamination case that has some similarities to the one described here occurred in Slovakia. There, in the Michalovce area, a PCB production plant operated from 1959 to 1984, causing a widespread contamination of the environment, affecting also freshwater fish and game (Kocan et al., 1999, 2001). In home made butter samples from this area Chovancova et al. (2005) found levels as high as 1.74 pg TEQ g^{-1} fat for PCCDs + PCDFs, 6.11 pg TEQ g^{-1} fat for non-*ortho* PCBs and 3.65 pg TEQ g^{-1} fat for mono-*ortho* PCBs. In the Slovak samples, as in the ones here presented, the non-*ortho* PCB contribution largely prevails over the sum of mono-*ortho* PCBs + PCDDs + PCDFs. It should also be noted, when comparing data, that the mono-*ortho* PCBs in the Chovancova paper are reported using the 1997 TEFs, as in the second part of Table 2.

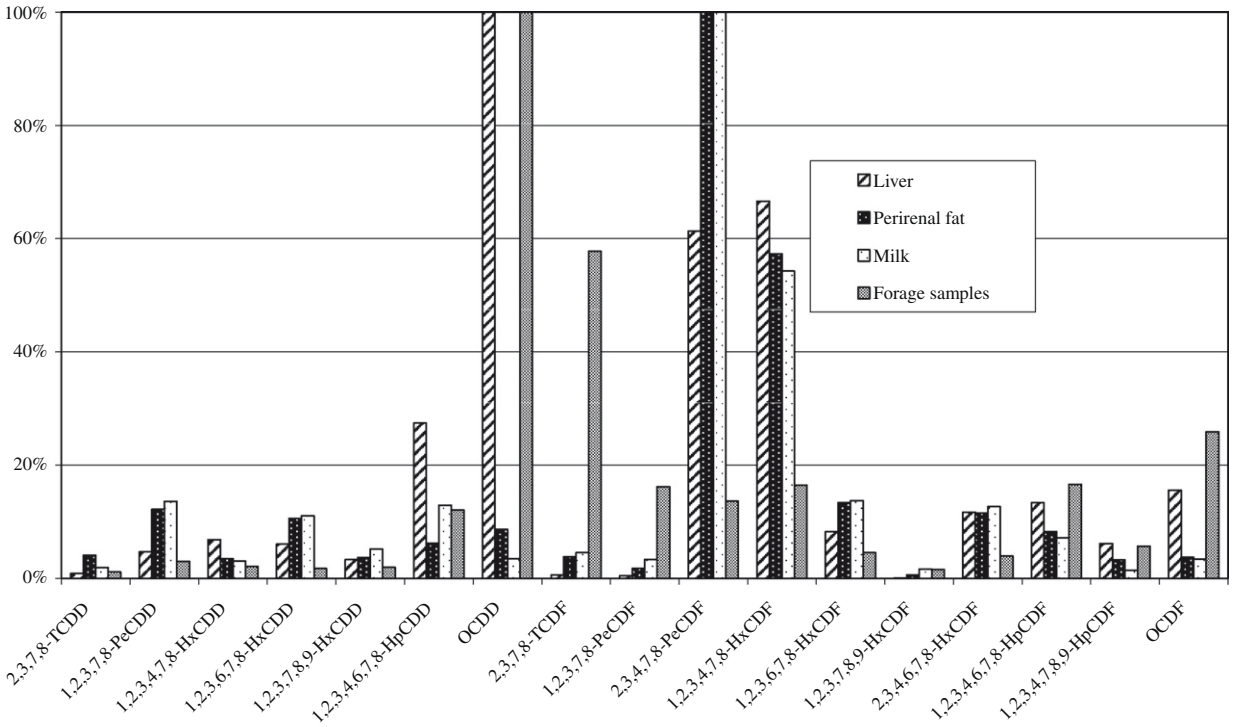

Fig. 3. PCDD and PCDF congener profiles for the three matrices analyzed from farm D, together with a forage sample.

Please cite this article in press as: Turrio-Baldassarri, L., et al. PCB, PCDD and PCDF contamination of food of animal origin as the effect of soil pollution and the cause of human exposure in Brescia, Chemosphere (2009), doi:10.1016/j.chemosphere.2009.03.002

Another similarity between the Slovak and the Italian cases lies in the effect of the contamination on the blood PCB levels of the local population, possibly consuming contaminated food: the general population from the Michalovce area shows blood PCB levels roughly four times higher than the general population from a non-polluted Slovak area. A mean value of 79 pg TEQ g^{-1} lipid was found for dlPCBs in 135 randomly chosen individuals from the Michalovce area, with a mean value of 5836 ng g^{-1} lipid for non-dlPCBs (Jursa et al., 2006). In a serum pooled from 41 habitual consumers of the contaminated food from the Brescia farms, we found 313 pg TEQ g^{-1} lipid for dlPCBs and 14244 ng g^{-1} lipid for non-dlPCBs, whilst in the general population from Brescia we

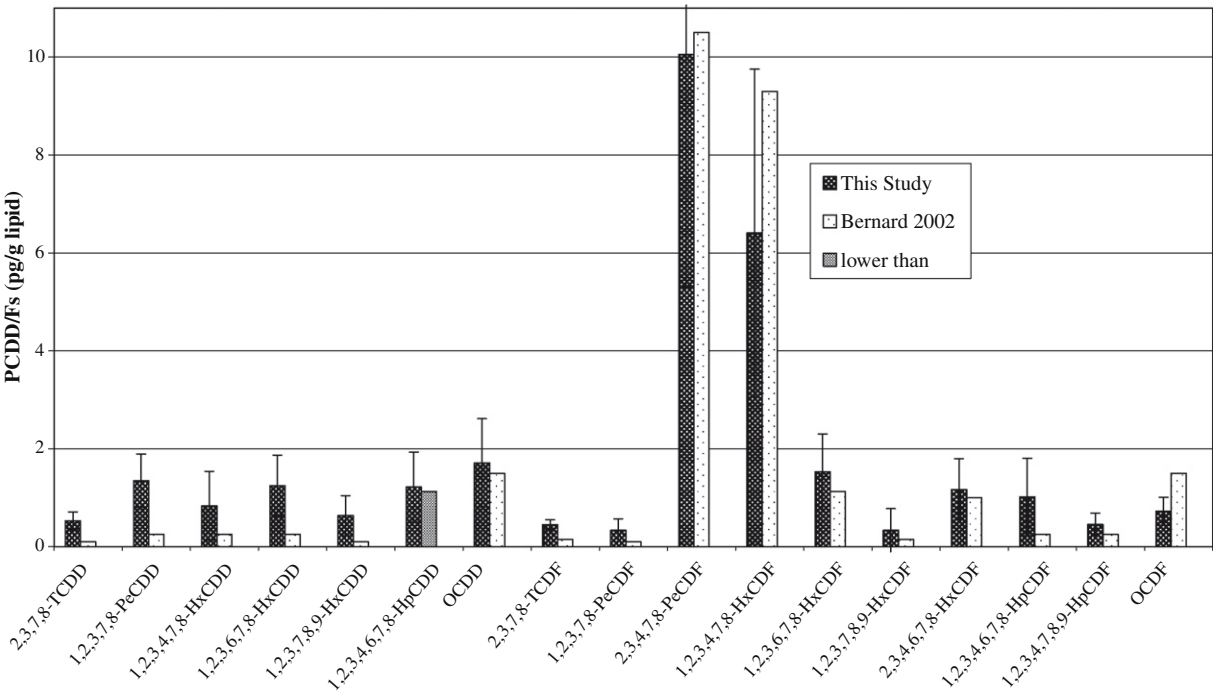

Fig. 4. PCDD and PCDF congener profiles in samples from this study and from the Belgian incident.

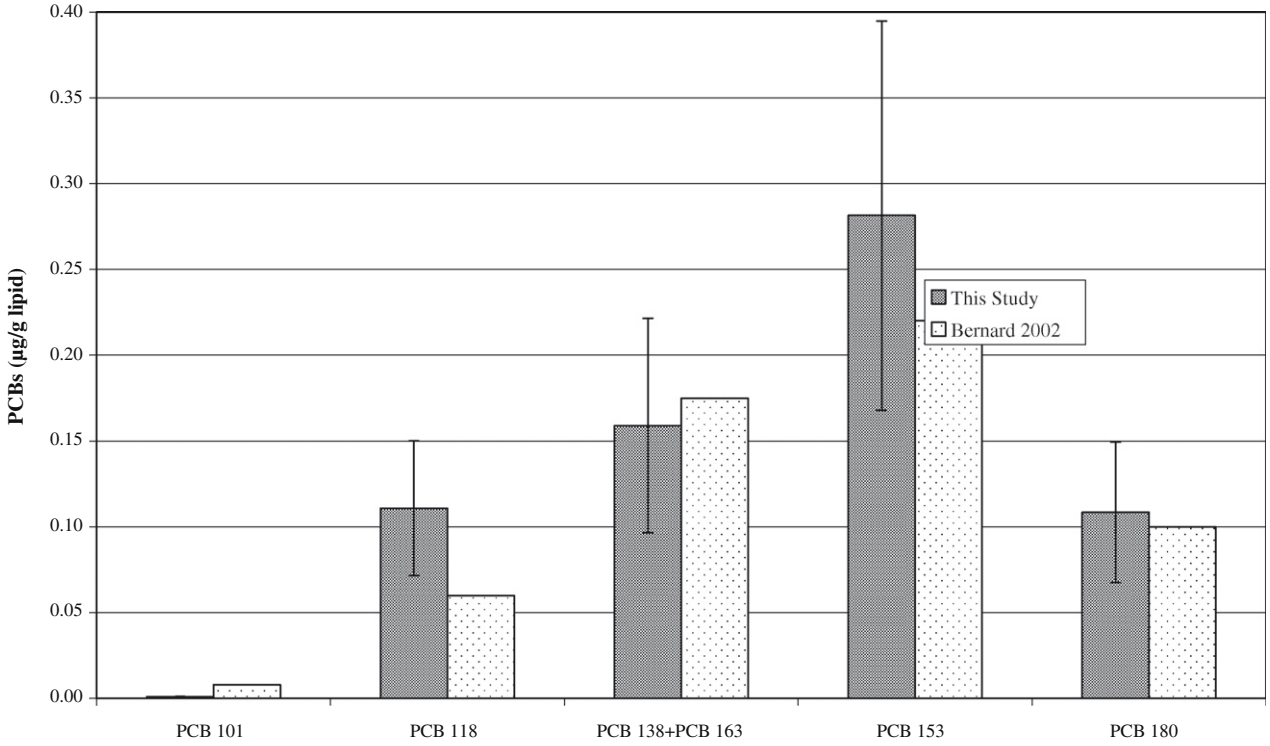

Fig. 5. Congener profiles of indicator PCBs in samples from this study and from the Belgian incident.

Please cite this article in press as: Turrio-Baldassarri, L., et al. PCB, PCDD and PCDF contamination of food of animal origin as the effect of soil pollution and the cause of human exposure in Brescia, Chemosphere (2009), doi:10.1016/j.chemosphere.2009.03.002

found 32 pg TEQ g^{-1} lipid for dlPCBs and 1136 ng g^{-1} lipid for non-dlPCBs (Turrio-Baldassarri et al., 2008). The contamination levels were considerably higher in the consumers of contaminated food from Brescia than in the general population from the Michalovce area, but it must be considered that the data from Brescia refer only to habitual consumers of contaminated food, whilst the data from Michalovce refer to randomly chosen individuals. For the latters, consumption of contaminated food was probably occasional, although some habitual consumer was probably present among them, as Jursa et al. (2006) report a maximum value for dlPCB in serum of 658 pg TEQ g^{-1} lipid.

Acknowledgments

Part of this work was performed in the former Ecotoxicology unit of the Istituto Superiore di Sanità: thanks are due to the personnel of the unit for fruitful cooperation.

References

ASL Brescia, 2008. <http://www.aslbrescia.it/asl/media/documenti/pcb/brescia_aprile_2008/7.7%20-%20Indagini%20ambientali.pdf>.

Bernard, A., Broeckaert, F., De Poorter, G., De Cock, A., Hermans, C., Saegerman, C., Houins, G., 2002. The Belgian PCB/Dioxin incident: analysis of the food chain contamination and health risk evaluation. Environ. Res. Section A 88, 1–18.

Chovancova, J., Kocan, A., Jursa, S., 2005. PCDDs, PCDFs and dioxin like PCBs in food of animal origin (Slovakia). Chemosphere 61, 1305–1311.

CTS, 2003. Relazione finale del Comitato Tecnico Scientifico per la valutazione del rischio per la salute umana, correlato alla presenza nel terreno di sostanze tossiche, PCB e mercurio, nell'area Caffaro del comune di Brescia. <http://www.aslbrescia.it/asl/media/pdf/relfinalects1.pdf>.

di Domenico, A., De Felip, E., Falleni, M., Ferri, F., Iacovella, N., Menale, G., Tafani, P., Tommasino, G., Turrio Baldassarri, L., 1994. Polychlorodibenzodioxin and polychlorodibenzofuran levels in dielectric fluids containing polychlorobiphenyls. Toxicol. Environ. Chem. 46, 239–260.

EC, Commission Regulation 199/2006. Official Journal of the European Communities of 3 February 2006, L32/34, 4.2.2006.

EC, 2375/2001. Official Journal of the European Communities of 29 November 2001, L321/1, 6.12.2001.

Frame, G.M., Wagner, R.E., Carnahan, J.C., Brown, J.F., May, R.J., Smullen, L.A., Bedard, D.L., 1996. Comprehensive, quantitative congener specific analyses of eight aroclors and complete congener PCB assignments on DB-1 capillary GC column. Chemosphere 33, 603–623.

Fürst, P., 2001. Contribution of dioxin-like PCB to total toxic equivalents of dairy products. Organohalogen Compd. 51, 279–282.

Jursa, S., Chovancova, J., Petrik, J., Loks, J., 2006. Dioxin-like and non-dioxin-like PCBs in human serum of Slovak population. Chemosphere 64, 686–691.

Kocan, A., Petrık, J., Chovancova, J., Jursa, S., Drobna, B., 1999. Environmental contamination following PCB manufacture in Eastern Slovakia. Organohalogen Compd. 43, 105–109.

Kocan, A., Petrık, J., Chovancova, J., Jursa, S., Drobna, B., 2001. Environmental contamination with polychlorinated biphenyls in the area of their former manufacture in Slovakia. Chemosphere 43, 595–600.

Kodavanti, P.R.S., Kannan, N., Yamashita, N., Derr-Yellin, E.C., Ward, T.R., Burgin, D.E., Tilson, H.A., Birnbaum, L.S., 2001. Differential effects of two lots of Aroclor 1254: congener specific analysis and neurochemical end points. Environ. Health Persp. 109, 1153–1161.

La Rocca, C., Abate, V., Alivernini, S., Iacovella, N., Mantovani, A., Silvestroni, L., Spera, G., Turrio-Baldassarri, L., 2004. Levels of PCBs, DDT, DDE and DDD in Italian human blood samples. Organohalgen Compd. 66, 2756–2760.

Malisch, R., Dilara, P., 2007. PCDD/Fs and PCBs in butter samples from new European Union member states and a candidate country: analytical quality control, results and certain PCB-specific aspects. Chemosphere 67, S79–S89.

Porta, A., 2005. Identificazione delle sorgenti di emissione di PCDD e PCDF nel comune di Brescia e analisi di fingerprinting sui suoli contaminati nell'area del sito di interesse nazionale Brescia-Caffaro. BATTELLE, Carouge, Switzerland, 199 pages+encl.

Report of the Istituto Superiore di Sanità to the Italian Ministry of Health, 9844/TOC 12, 26 February 2003.

Santillo, D., Fernandes, A., Stringer, R., Alcock, R., Rose, M., White, S., Jones, K., Johnston, P., 2003. Butter as an indicator of regional persistent organic pollutant contamination: further development of the approach using polychlorinated dioxins and furans (PCDD/Fs), and dioxin-like polychlorinated biphenyls (PCBs). Food Addit. Contam. 20, 281–290.

Scientific Committee on Food, 2000. Opinion on the Risk Assessment of Dioxins and Dioxin-like PCB in Food. <http://europa.eu.int/comm/food/fs/sc/scf/out78_en.pdf> (accessed 22.11.2000).

Schulz, D.E., Petrick, G., Duinker, J.C., 1989. Complete characterization of polychlorinated biphenyl congeners in commercial aroclor and clophen mixtures by multidimensional gas chromatography-electron capture detection. Environ. Sci. Technol. 23, 852–859.

Turrio-Baldassarri, L., Iacovella, N., Quattrocchi, W., La Rocca, C., 2004. Control of PCB levels in food of animal origin in Italy: analytical quality control, organization and results. Organohalogen Compd. 66, 1981–1988.

Turrio-Baldassarri, L., Abate, V., Alivernini, S., Battistelli, C.L., Carasi, S., Casella, M., Iacovella, N., Indelicato, A., Scarcella, C., La Rocca, C., 2005. Using a linear correlation as a tool for the identification of a contamination source. Organohalogen Compd. 67, 2073–2075.

Turrio-Baldassarri, L., Alivernini, S., Battistelli, C.L., Carasi, S., Casella, M., Fochi, I., Iacovella, N., Indelicato, A., La Rocca, C., Mariani, A., Scarcella, C., 2007. A study on PCB, PCDF/PCDF industrial contamination in an mixed urban/agricultural area significantly affecting the food chain and the human exposure. Part I: soil and feed. Chemosphere 67, 1822–1830.

Turrio-Baldassarri, L., Abate, V., Battistelli, C.L., Carasi, S., Casella, M., Fochi, I., Iacovella, N., Indelicato, A., La Rocca, C., Scarcella, C., Alivernini, S., 2008. PCDF/PCDF and PCB in human serum of differently exposed population groups of an Italian city. Chemosphere 73, S228–S234.

van den Berg, M., Birnbaum, L.S., Bosveld, A.T.C., Brunström, B., Cook, Ph., Feeley, M., Giesy, J.P., Handberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., van Leeuwen, F.X.R., Liem, A.K.D., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillit, D., Tysklind, M., Younes, M., Wærn, F., Zacharewski, T., 1998. Toxicity equivalence factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Persp. 106, 775–792.

van den Berg, M., Birnbaum, L.S., Denison, M., De Vito, M., Farland, W.F.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, R.E., 2006. The 2005 World Health Organization revaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol. Sci. 93, 223–241.

Weiss, J., Päpke, O., Bergman, Å., 2005. A worldwide survey of polychlorinated dibenzo-*p*-dioxins, dibenzofurans, and related contaminants in butter. Ambio 34, 589–597.