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PCDD/F and PCB in human serum of differently exposed population groups of an Italian city

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ABSTRACT

A chemical plant located in Brescia, an industrial city in North-Western Italy, produced polychlorobiphenyls (PCBs) during a 30–50 year period, causing widespread pollution of the surrounding agricultural area. This area contains several small farms, which principally produce veal meat for private consumption of the farmers' families.

The pollution went undiscovered for many years, during which period contaminated food was regularly consumed. This paper reports the polychlorodibenzodioxin (PCDD), polychlorodibenzofuran (PCDF) and PCB levels of a serum sample pooled from the consumers of contaminated food, compared to six population groups of the city of Brescia. Four of these groups were selected in order to represent, respectively, the local general population and the residents of three zones of the polluted area, while the last two groups represented, respectively, the present and the former workers of the plant. One human milk sample from one of the consumers of contaminated food was also analyzed.

Results show that the consumers of the contaminated food and the former workers of the plant display considerably higher levels than all other groups. The levels of general population and of all other groups were generally similar both to each other and to the range of literature values for unexposed populations. The respective contribution of PCDDs, PCDFs, mono-*ortho* and non-*ortho* PCBs (dioxin-like PCBs) to (Toxicity Equivalents) TEQ of the population groups of this study were also compared to literature data: the two groups with a high contamination level, together with the human milk sample, displayed a higher incidence of mono-*ortho* PCBs and a lower contribution of PCDD, possibly correlated with the source of contamination.

1. Introduction

This is the third part of a study dealing with PCB, PCDD and PCDF contamination in Brescia, a city in the North-West of Italy. The first part dealt with soil and forage contamination (Turrio-Baldassarri et al., 2007) whereas the second part dealt with contamination of meat and milk produced in the polluted area. The results of the latter study were presented as a summary (La Rocca et al., 2004) not yet in complete form (Turrio-Baldassarri et al., in preparation). The present paper represents the third part of this study and deals with serum levels of various population groups of the area. This study therefore documents the overall progression of the contamination from the environment to man.

The source of pollution was the only Italian PCB-producing plant. It has been reported in the literature that about 31000 tons of PCB were produced in Italy during the period 1958–1983 (Brievik et al., 2002) even though, according to local sources, the plant began production of PCBs in the late thirties. PCB and PCDD/F soil levels higher than the legal limits were found in a wide area where approx. 11,000 inhabitants live, and as a result precautionary measures were taken by town authorities in order to minimize contact with contaminated materials such as soil and grass. The most polluted soils were however, found in an agricultural area about 1 km^2 wide adjacent to the southern limit of the plant. In this area, the contamination values ranged from 15 to 1034 pg WHO-TE/g of soil (WHO Toxicity Equivalency Factors, Van den Berg et al., 1998); considering only PCDDs + PCDFs, the range was from 8 to 592 pg WHO-TE/g of soil (Turrio-Baldassarri et al., 2007). By comparison the background PCDD + PCDF soil contamination in Italy falls in the range from 0.1 to 4.3 pg I-TE/g of soil (International Toxicity Equivalency Factors, NATO/CCMS, 1988), as reported by a 1993 study (di Domenico et al., 1993).

A particular feature of this polluted area is the presence of several small farms, a fact quite uncommon inside an industrial and residential urban area. The farmers produced meat and milk mostly for their own needs and to provide their families with home-produced (supposedly healthy) food. In fact, three out of

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the four farms kept only few animals (from one to five cows). However, the fourth farm kept 18 cows for the production of veal. The size of this farm far exceeds the consumption of one family, thus suggesting that most of the contaminated products were sold directly to consumers.

As soon as the contamination was discovered, all animals fed with contaminated feed were slaughtered and the consumption of their products was forbidden. All pooled samples from cattle had contamination levels in the range 75–103 pgWHO-TE/g fat (La Rocca et al., 2004).

Although there is no reliable information on the time when contamination began to diffuse into the environment, it is known that the PCB production lasted for decades and ended in 1983. It is therefore probable that contamination begun during production and lasted up to the present day, even after the end of production. As a result it is likely that the owners of the farms and their families had been consuming the contaminated food for a long time, some of them throughout most of their lives and in a few cases even for generations, thus representing cases of chronic exposure. High PCB levels were indeed found in the blood of the farmers (Apostoli et al., 2005). PCB analyses on the blood of the Brescian general population were performed in order to get information on the background levels (Apostoli et al., 2005). However, as PCB industrial mixtures contain various levels of the 12 congeners with dioxin-like activity and ppm levels of PCDF and PCDD, it was thought fit to measure blood levels not only of the most abundant congeners of PCB, but also of all the PCDD, PCDF and PCB with dioxin-like toxic properties in different groups of inhabitants of the city of Brescia.

It is known from the literature that significant human exposure to these products may occur mainly through consumption of contaminated food and from dermal contact; however, in a contaminated area, contact with soil, vegetation and other surfaces or consumption of garden vegetables may contribute to exposure. The main aim of the present study is to compare the levels of PCCD, PCDF and PCB found in a pooled sample of consumers of the contaminated food to the levels of other groups of the Brescian population. The groups were chosen in order to represent, respectively, the three population groups living in the three parts of the polluted area (South-East, South and South-West of the plant), the present workers of the firm and the former workers of the plant and, finally, the general population of Brescia.

2. Experimental

Seven serum samples were formed by pooling sera from a minimum of 41 to a maximum of 169 individual donors, which were grouped according to different exposure; each donor contributed a 1 mL sample to the pool. The pools were collected in two different campaigns: the first six pools (PWF; R-S, R-SE; R-SSW; GPB, CCF) were collected in 2004 and analyzed in 2005, the seventh pool (FWP), consisting of sera from 46 former workers of the plant, was collected from March to June 2005 and analyzed in 2006. The mean age of the donors of this group at the time of blood sampling was 67 and about 20 of them were contaminated by PCBs during an industrial accident in 1982.

The pool of the consumers of contaminated food consisted of sera from the farmers and their relatives.

Details of pooled samples (number of male and female donors, mean age, lipid concentration and exposure group characteristics) are reported in Table 1. To each pooled sample 1/10 (v/v) of acid solution (pH 1) was added to sterilize the sample.

Pools GPB, R-SE, R-S, R-SSW, PWF and FWP were spiked with labeled $^{13}C_{12}$ recovery standards (nine 2,3,7,8 substituted congeners of PCDD/PCDF, PCB 77, 81, 126, 169 and ten labeled PCB and labeled pesticides, hexachlorobenzene (HCB), and 1,1-bis(*p*-chlorophenyl)-2,2-dichloroethylene (DDE)).

Table 1
Description of the main characteristics of the sera pools

Pooled sample	Females	Males	Mean age	Lipid amount (%)	Exposure group
GPB	42	52	51	0.52	General population of Brescia
R-SE	88	81	52	0.55	Residents of the area SE of the plant
R-S	69	78	51	0.55	Residents of the area S of the plant
R-SSW	73	54	53	0.57	Residents of the area SSW of the plant
PWF	9	133	46	0.61	Present workers of the firm
FWP	0	46	67	0.62	Former workers of the plant
CCF	24	17	56	0.62	Consumers of contaminated food

Only in the case of the sample CCF two different aliquots were taken and separately spiked and analyzed. The first aliquot was used for the analysis of PCDDs, PCDFs and non-*ortho* PCBs; the second one for the other PCB congeners and for the pesticides. Microcontaminant extraction was carried out with a separatory funnel by adding formic acid, sodium oxalate, diethyl ether and *n*-hexane to the serum samples. The *n*-hexane extracts were eluted with *n*-hexane on a column packed with concentrated sulfuric acid coated on an inert support (Extrelut, Merck). The concentrated eluate was further purified with the automated multi-column Power Prep system. A procedural blank was associated with each batch of three-samples and processed in the same manner.

The Power Prep fraction for the analysis of PCDDs, PCDFs, non-*ortho* PCBs was concentrated and injected into an Autospec HRGC–HRMS system operating at resolution of 10,000 for PCDD/F and non-*ortho* PCB determination.

The fraction containing mono-*ortho*, non dioxin-like PCBs and organochlorine pesticides was analyzed by high resolution gas chromatography coupled with low resolution mass spectrometry (HRGC/LRMS).

Relative response factors were determined daily with an external standard mixture containing all of the compounds to be determined.

In each sample the following compounds were individually determined:

PCDDs: 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD.

PCDFs: 2,3,7,8-TCDF, 1,2,3,7,8-PeCDF, 2,3,4,7,8-PeCDF, 1,2, 3,4,7,8-HxCDF, 1,2,3,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF, 2,3,4,6,7, 8-HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF, OCDF.

Mono-*ortho* congeners: 2,3,3′,4,4′-PeCB (105); 2,3,4,4′,5-PeCB (114); 2,3′,4,4′,5-PeCB (118); 2′,3,4,4′,5-HxCB (123); 2,3,3′,4,4′,5-HxCB (156); 2,3,3′,4,4′,5′-HxCB (157); 2,3′,4,4′,5,5′-HxCB (167); 2,3,3′,4,4′,5,5′-HpCB (189).

Non-*ortho* congeners: 3,3′,4,4′-TeCB (77); 3,4,4′,5-TeCB (81); 3,3′,4,4′,5-PeCB (126); 3,3′,4,4′,5,5′-HxCB (169).

Non dioxin-like PCB congeners: 2,2′,4-TrCB (17); 2,2′,5-TrCB (18); 2,4,4′-TrCB (28); 2,4,6-TrCB (30); 2,2′,3,4-TeCB (41); 2,2′,3,5′-TeCB (44); 2,2′,4,4′ + 2,2′,4,5-TeCB (47 + 48); 2,2′,4,5′-TeCB (49); 2,2′,5,5′-TeCB (52); 2,3,4,4′-TeCB (60); 2,3,4′,6-TeCB (64); 2,3′,4,4′ + 3,3′,5,5′-TeCB (66 + 80); 2,3′,4′,5-TeCB (70); 2,4,4′,5-TeCB (74); 2,2′,3,4,4′-PeCB (85); 2,2′,3,4,5′-PeCB (87); 2,2′,3,4′,6-PeCB (91); 2,2′,3,5′,6-PeCB (95); 2,2′,3′,4,5-PeCB (97); 2,2′,4,4′,5-PeCB (99); 2,2′,4,4′,6-PeCB (100); 2,2′,4,5,5′-PeCB (101); 2,2′,3,3′,4,4′-HxCB (128); 2,2′,3,3′,5,6′-HxCB (135); 2,2′,3,3′,6,6′-HxCB (136); 2,2′,3,4,4′,5-HxCB (137); 2,2′,3,4,4′,5′ + 2,3,3′,4′,5,6-HxCB (138 + 163); 2,2′,3,4,5,5′-HxCB (141); 2,2′,3,4′,5,5′-HxCB (146); 2,2′, 3,4′,5′,6-HxCB (149); 2,2′,3,5,5′,6-HxCB (151); 2,2′,4,4′,5,5′-HxCB (153); 2,2′,4,4′,6,6′-HxCB (155); 2,2′,3,3′,4,4′,5-HpCB (170); 2,2′,3,3′,4,4′,6-HpCB (171); 2,2′,3,3′,4,5,5′-HpCB (172); 2,2′,3,3′,4,5,6′-HpCB (174); 2,2′,3,3′,4,6,6′-HpCB (176); 2,2′,3,3′,4′,5,6-HpCB (177); 2,2′,3,4,4′,5,5′-HpCB (180); 2,2′,3,4,4′,5′,6-HpCB (183); 2,2′,3,4′,5,5′,6-HpCB (187); 2,2′,3,3′,4,4′,5,5′-OCB (194); 2,2′,3,3′,4,4′,5,6-OCB (195); 2,2′,3,3′,4,5′,6,6′-OCB (200); 2,2′,3,3′,4′,5,5′,6-OCB (201); 2,2′,3,3′,5,5′,6,6′-OCB (202); 2,2′,3,4,4′,5,5′,6 + 2,2′,3,3′,4,4′,5′,6-OCB (203 + 196).

Chlorinated pesticides and metabolites: HCB, DDE.

Quality control: The laboratory took part successfully, since its first edition in 2000, in the annual "Dioxin in food" exercises organized by the Folkehelsa, Norwegian Institute of Public Health, for the determination of PCDDs, PCDFs and dioxin-like PCBs in food matrices. As the main parts of the analytical procedure (clean-up and instrumental determination) used for food analysis are identical to those used in the present work, the results obtained in the exercises can be used to illustrate the quality of the data produced in the present work. The exercise does not rank the overall laboratory performance but gives a consensus value for each analyte in each matrix (three different matrices each year). Although the quality of the data produced by our laboratory throughout the six annual exercises (18 matrices) is fairly homogeneous, the 2005 exercise, carried out in the same period as most of the serum analyses, is here considered. All results determined for the 17 PCDD/Fs and 12 dioxin-like PCBs (including only those with a concentration higher than the lower limit of determination) had acceptable deviations from the consensus value, with a maximum of +62% (OCDD) and a minimum of 3% (1,2,3,4,7,8-HxCDD) for PCDD/Fs, while for the dioxin-like PCBs a maximum of +88% (105) and a minimum of −1% (123) were observed.

In 2006 the laboratory participated to the "Interlaboratory study of PCDDs, PCDFs, dioxin-like PCBs, marker PCBs and PBDEs in human blood plasma", organized by the University of Lieges, Centre d'Analyse des Résidues en Traces. The final report ranks "good" the performance of our laboratory as 82% of its results had a z-score <|2|. The samples of the plasma interlaboratory study were analyzed in the same batch of samples as the FWP pool of the present study.

3. Results and discussion

For ease of interpretation and comparison with literature data, the results on PCDD/Fs and on PCBs are presented and discussed separately.

Results for PCDDs and PCDFs of the various pools are reported in Table 2. Data are expressed as pg/g of lipid. The percentage lipid was calculated from cholesterol and triglycerides data using the formula proposed by Phillips et al. (1989): total serum lipid (mg/dl) = 2.27 serum cholesterol (mg/dl) + triglycerides (mg/dl) + 0.623. In the same Table the results of one mother's milk sample, from one of the consumers of contaminated food, are also reported. Päpke (1998) proposed data illustrating a correspondence of lipid based TEQ levels between blood and human milk, so that a direct comparison between the two different matrices can be made; differences in contamination profiles in the two matrices were relevant for some congeners, such as the highly chlorinated PCDDs, as confirmed by Schecter et al. (1998a) in a partitioning study. Human milk is, however, the only sample in Table 2 coming from a single person rather than from a group, and it is shown for a visual comparison of the contamination level, not for correlation purposes.

The upper bound approach was adopted in Table 2 in order to take into account the TEQ contribution of the congeners below the limit of determination (LOD), i.e. the analytical concentration of the analyte is considered equal to the LOD. As a consequence, total TEQ values may be overestimated, particularly when congeners with high toxic equivalency factors (TEFs) (such as 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD) are not determinable.

It is evident that the both the serum pool of the consumers of contaminated food (CCF) and the serum pool of the former workers of the plant (FWP) show a level of contamination far exceeding all the others; their TEQs are, between 2.9 and 4.8 times higher than the remaining five pools. The other five pools display levels similar to each other: differences are within a factor 1.6, the higher values being exhibited by the pool of the inhabitants of the area R-SSW of the plant. Differences among contamination levels of these five samples are minor and may not be related to the soil contamination; however, for the three groups of inhabitants of the contaminated area, contamination levels are slightly higher than that of the general population, but generally within the range of literature data. The lowest values are shown by both the present workers of the firm (PWF) and the general population (GPB). The low levels of the PWF pool may appear unexpected; however, it should be noted that some of the donors were hired after production of PCB was terminated (1983), and that the pool included many sera from clerks of the firm working in buildings far from production plants; it may also be relevant that the components of this group had a mean age lower than all the others.

Table 2
PCDD and PCDF concentration (pg/g lipid) and TEQ (WHO, upper bound, pg TE/g lipid)

	GPB	R-SE	R-S	R-SSW	PWF	FWP	CCF	Breast milk
2,3,7,8-TCDD	<2.3	2.2	3.0	4.1	1.8	<3.1	5.0	<1.6
1,2,3,7,8-PeCDD	<6.5	7.2	5.9	10.8	7.0	28.3	21	5.6
1,2,3,4,7,8-HxCDD	<3.9	2.7	2.3	4.9	1.4	7.8	13	<2.5
1,2,3,6,7,8-HxCDD	14.9	22	23	19	10	37.2	30	7.3
1,2,3,7,8,9-HxCDD	<3.3	2.4	3.8	2.7	1.2	<5.3	<6.9	<2.3
1,2,3,4,6,7,8-HpCDD	20.3	26	31	20	15	23.1	18	11
OCDD	176	224	273	190	137	186	221	60
2,3,7,8-TCDF	<2.2	<0.9	4.7	<2.6	1.4	<5.3	<4.7	<1.6
1,2,3,7,8-PeCDF	<5.5	<1.6	<2.6	<3.0	<2.3	<6.8	<7.6	2.7
2,3,4,7,8-PeCDF	18.2	27	24	32	20	120	136	36
1,2,3,4,7,8-HxCDF	4.3	6.8	6.0	8.9	6.6	35.3	41	23
1,2,3,6,7,8-HxCDF	4.3	6.6	7.2	6.8	5.2	14.9	21	6.8
1,2,3,7,8,9-HxCDF	<1.5	<0.9	<2.1	<1.6	<1.3	<6.1	<3.4	<2.4
2,3,4,6,7,8-HxCDF	<1.3	1.6	2.3	<1.4	1.2	<5.7	5.5	2.6
1,2,3,4,6,7,8-HpCDF	4.0	5.2	5.9	5.8	5.9	6.2	5.9	3.3
1,2,3,4,7,8,9-HpCDF	<3.4	<0.6	<1.0	<1.2	<1.2	<4.6	<5.2	<3.4
OCDF	<8.4	<2.1	<3.6	<9.8	<4.0	<7.1	<12	<3.1
PCDD pgTE/g up	11	12	12	18	10	37	31	8
PCDF pgTE/g up	11	15	14	18	12	67	76	22
PCDD + PCDF pgTE/g up	22	28	27	36	22	104	107	30

Casual differences between the donors such as dietary habits, age, sex may influence contamination levels of the pools. It should also be considered that unaware consumers of contaminated meat of local production may be present anywhere in the city and consequently also in any of the population groups analyzed here. In fact, a single consumer with high contamination levels may increase the mean concentration and modify the profile of the whole pool.

PCDD + PCDF contamination levels of the five pools are on the upper side of the range reported for pools in the recent literature worldwide (Fürst and Päkpe, 2002; Schwenk et al., 2002; Maruyama et al., 2003; Tsuchiya et al., 2003; De Felip et al., 2004; Leondiadis et al., 2004; Schuhmacher et al., 2004; Kim et al., 2005; Masuda et al., 2005; Turyk et al., 2006), which all lie in the range of 7.5–28.5 pg WHO-TE/g lipid.

The human milk sample, taken from one of the consumers of contaminated food, displays contamination levels on fat basis lower than the sera of both pool CCF and FWP, and rather close to the other groups. This may be due to the decrease of contamination levels during the lactation period (Schecter et al., 1998b): this sample was taken at about the end of the third month of lactation, so possibly the contamination level at the beginning of lactation was higher. However, both the low ratio PCDD/PCDF, as will be discussed later, and the comparatively high level of PCBs of this sample are consistent with its provenience from a consumer of contaminated food. When compared to recent literature data on PCDD + PCDF in breast milk (range 3.5–15.2 pg WHO-TE/g lipid), this sample reveals a higher contamination level (Norén and Meironyté, 2000; Fürst and Päkpe, 2002; Kunisue et al., 2004; Wang et al., 2004).

In Table 3 the results obtained on PCBs are reported. The individual concentrations of 18 congeners are reported (the 12 dioxin-like and the six indicator congeners) together with the total PCB concentration, evaluated both as the sum of the 63 congeners determined and the sum of the six indicators. The TEQ contribution of non-*ortho* and mono-*ortho* congeners are also listed together with the overall TEQ of PCDDs + PCDFs + dioxin-like PCBs.

The levels of total PCBs and dioxin-like PCBs of both CCF and FWP are considerably higher than all the other groups.

For total PCBs, differences between the sera pools of both CCF and FWP with respect to the other five pools are even higher than for PCDD/F, reaching a value of 18.5 for the ratio maximum/minimum (CCF/PWF).

It is relevant to point out that levels of all groups, except for the CCF and the FWP pools, are in good agreement with the reference values reported by Apostoli (2005) in a study, targeted at the general population of Brescia, which focussed on assessing reference values for PCB in blood. In that study it was found that the mean value (calculated on both sexes and all ages) for serum content of PCB for the unexposed general population of Brescia was 897 ng/g lipid evaluated as the sum of 24 congeners. When the results of the present study are reported in terms of the same congeners determined by Apostoli (2005), our results turn out to be very similar to the mean value of Apostoli: general population of Brescia (GPB), 866 ng/g lipid; residents South South-West (R-SSW), 875 ng/g lipid; residents South-East (R-SE), 1104 ng/g lipid; residents South (R-S), 1347 ng/g lipid; present workers of the firm (PWF), 586 ng/g lipid; these values are, respectively 3% (GPB) lower, 2%lower (R-SSW), 23% (R-SE) and 50% (R-S) higher and 35% (PWF) lower than the mean value (897 ng/g fat) of the general population reported by Apostoli (2005), while for both the consumers of contaminated food and the former workers of the plant our results (CCF 10869 ng/g lipid; FWP 10482 ng/g lipid) are about four times higher than the 95° percentile (2643 ng/g fat) of the general population of the Apostoli study (2005). This supports in a statistically significant way the self evident observation that the contam-

Table 3
PCB concentrations (ng/g lipid) and TEQ (WHO, upper bound, pg TE/g lipid)

	GPB	R-SE	R-S	R-SSW	PWF	FWP	CCF	Breast milk
PCB indicator								
28	<0.3[a]	3.7[b]	4.9[b]	3.7[c]	3.6[c]	5.7	<2.3	9.9
52	<0.4[a]	0.9[b]	1.7[b]	0.8[b]	0.6[b]	<7.2	0.2	0.5[b]
101	<0.4[a]	1.3[b]	3.4[c]	0.6[b]	1.3[c]	<9.2	0.2	1.5
138 + 163	108	125	151	80	66	935	904	214
153	242	293	344	205	141	2459	2622	660
180	303	391	472	321	190	3905	4221	832
Non-ortho PCB								
77	<0.008[a]	0.057[b]	0.037[b]	0.066[b]	0.012[b]	0.051	<0.010[a]	0.006[b]
81	<0.015	0.011	0.008	0.009	<0.020	0.007	0.029	0.011
126	0.082	0.133	0.125	0.228	0.136	0.255	0.602	0.427
169	0.182	0.238	0.244	0.352	0.211	0.509	1.983	0.454
Mono-ortho PCB								
105	4.3	6.5	6.0	4.1	3.8	30.0	31.9	28.0[c]
114	2.3	3.1	3.2	1.8	1.4	<10.9	23.1	8.0
118	31	42	42	25	20	120	219	147.4
123	0.3	0.6	0.5	<0.1	<0.1	<10.7	<0.2	<2.3
156	26.4	35.3	39.9	24.5	14.8	342.0	299.6	76.9
157	6.3	9.3	9.3	5.7	3.0	69.2	79.8	14.4
167	8.0	11.01	13.4	6.4	4.1	67.5	68.2	17.1
189	4.9	5.4	6.9	4.6	3.2	55.8	55.2	15.8
Sum of indicator PCB	654	816	977	611	403	7342	7750	1719
Total PCB ng/g	1136	1446	1766	1147	768	12008[d]	14244	3598
Mono-*ortho* pg TE/g up	22	29	32	20	12	233	233	69
Non-*ortho* pg TE/g up	10	16	15	26	16	31	80	47
PCB + PCDD + PCDF pg TE/g up	54	73	73	82	50	368	419	147

The letters a-c indicate an incidence of the blank on the sample signal (Turrio-Baldassarri et al., 2005).
[a] The blank incidence ranges from 75% to 125%, the analyte is considered not determinable at the method detection limit.
[b] The blank incidence ranges from 25% to 75% and is subtracted from sample signal.
[c] The blank incidence ranges from 5% to 25% and is considered negligible.
[d] Sum of only 41 congeners.

ination level of the CCF and FWP pools is higher than the mean level. The PCB concentration of the human milk sample is also slightly higher than the 95° percentile of blood, indicating an exposure higher than the general population.

Among the residents in the polluted area, two of the three groups (R-SE, R-S) have total PCB levels higher than the GPB pool; the third (R-SSW), with the higher PCDD + PCDF level, had total PCB almost coincident to the GPB group. Donato et al. (2006) found that consumers of fruit and vegetables produced in the contaminated area had PCB levels higher than non-consumers.

The data here presented for serum, with the exclusion of the groups CCF and FWP, are generally within the range of the literature data, once the comparison is made on the basis of the same congeners. The more frequently reported is the 2,2′,4,4′,5,5′-hexachlorobiphenyl (153), whose concentration ranges from 91 to 300 ng/g lipid (Pauwels et al., 2000; Glynn et al., 2000; Wingfors et al., 2000; Turci et al., 2006; Marchand et al., 2004). The level of 2,2′,4,4′,5,5′-hexachlorobiphenyl (153) in the human milk of this study is higher than the literature data range (53–152 ng/g) (Schecter et al., 1998a,b; Norén and Meironyté, 2000; Polder et al., 2003).

The data presented clearly indicate that the pools from the CCF and FWP groups display levels of contamination, for either PCDD/F or PCB, higher than both literature data (for the general population) and other local population groups, so confirming that these two groups were subject to additional exposure.

It is worth pointing out that either for PCDD/F or for PCBs, both levels and profiles in the CCF and FWP groups are very similar to each other, although the route of exposure were very different.

In the case of the former workers the contamination derived from occupational exposure, probably with dermal exposure prevailing over inhalatory exposure, which ended at least 22 years before blood sampling, while for the consumers of contaminated food the chronic exposure through diet ended only three years before sampling. Too much information is missing at the moment to understand whether the strict similarity of levels may be casual or not, and analyses of individual serum samples instead of pools are surely a better tool to obtain information on relations between exposure and blood levels. However, the similarity of the profiles is probably due to the source of contamination, i.e. in both cases the PCB mixtures produced in the plant.

The main contamination difference between the CCF and FWP pools lies in the level of non-*ortho* PCBs. It must be pointed out, however, that, for the FWP sample, recoveries of the isotopically labeled standards were unusually high, and this may result in an underestimation of these compounds.

Table 4 reports the pesticide concentration of six of the seven serum pools and of the breast milk: CCF displays the highest concentration for HCB and DDE; however, as the FWP pool was not analyzed for these pesticides, some elements are missing to speculate on the possible trace presence of HCB and DDT in the PCB mixtures that caused the exposure.

Some comments can be made by discussing the data presented in Table 5a and 5b, where the percentage contributions to total TEQ of, respectively, mono-*ortho* PCBs, non-*ortho* PCBs, PCDDs and PCDFs from our results and from literature data on both serum

Table 4
Pesticide concentration in six pools and in breast milk, ng/g lipid

	GPB	R-SE	R-S	R-SSW	PWF	CCF	Breast milk
HCB	74	83	72	80	60	149	20
DDE	796	826	767	876	573	2317	303
DDD	0.4	1.2	3.1	<0.3	<0.2	<0.4	<0.02

Table 5a
Percentage contribution to the total TEQ (WHO) of the various components and the PCDD/PCDF ratio for blood and similar matrices

Author	Turrio-Baldassarri (in preparation)	Leondiadis (2004)	Tsuchiya (2003)			This study							De Felip (2004)		Fürst (2002)
Nation	Italy	Greece	Japan			Italy							Italy	Belgium	Germany
Matrix	Adipose tissue	Blood	Blood			Serum							Blood		Blood
Population	Obese population	Athenian general population	Fishermen	Farmers	General population	GPB	R-SE	R-S	R-SSW	PWF	FWP	CCF	Women		Lactating women
% mono-*ortho*TEQ	26.1%	25.3%	18.8%	17.1%	16.5%	40.3%	40.4%	43.5%	23.8%	24.7%	63.5%	55.5%	25.2%	23.0%	33.7%
% non-*ortho*TEQ	30.1%	22.4%	34.8%	25.9%	37.2%	18.7%	21.6%	20.4%	32.1%	31.4%	8.3%	19.1%	21.1%	20.7%	18.9%
% PCDD TEQ	25.3%	25.3%	23.9%	34.0%	26.9%	20.9%	17.0%	16.7%	21.7%	20.6%	10.0%	7.3%	34.9%	35.2%	28.9%
% PCDF TEQ	18.4%	27.0%	22.5%	23.0%	19.4%	20.1%	21.1%	19.5%	22.4%	23.2%	18.3%	18.1%	18.9%	21.0%	18.5%
PCDD/PCDF	1.37	0.94	1.06	1.48	1.38	1.04	0.80	0.85	1.97	0.89	0.55	0.40	1.85	1.68	1.56

Table 5b
Percentage contribution to the total TEQ (WHO) of the various components and the PCDD/PCDF ratio for human milk samples

Author	This Study	Norén (2000)	Wang (2004)	Fürst (2002)	Kunisue (2004)	
Nation	Italy	Sweden	Taiwan	Germany	China (Dalian)	China (Shenyang)
Matrix				Breast milk		
Population	CCF	General population	General population	General population	General population	
% mono-*ortho*TEQ	47.1%	19.7%	18.4%	20.9%	9.7%	13.1%
% non-*ortho*TEQ	32.2%	27.7%	22.2%	30.4%	25.7%	31.0%
% PCDD TEQ	5.8%	30.9%	37.3%	26.8%	25.8%	21.9%
% PCDF TEQ	15.0%	21.7%	22.2%	21.9%	38.8%	34.0%
PCDD/PCDF	0.38	1.42	1.68	1.22	0.66	0.64

and breast milk are reported. Unfortunately, the studies where results on dioxin-like PCB are reported together with PCDD and PCDF are not numerous, so that the following observations are based on a limited number of data.

Two features deserve to be discussed: the percentage contributions of PCDDs to TEQ and its relation to PCDF.

PCDF percentage contributions to TEQ have relatively low variations throughout the data presented, while PCDDs display higher variations. Consequently, the PCCD/PCDF ratio, is either higher than 1 or only slightly lower, except for the CCF and FWP groups, for which minimum values of 0.4 and 0.54 are reached as shown in Table 5a and 0.38 in Table 5b.

It is also worth commenting on the PCB contribution. While non-*ortho* PCBs have a limited variation range when FWP is excluded, the mono-*ortho* PCBs continuously vary in the range from 9.7% (Kunisue et al., 2004) to 63.5% (serum, pool FWP). The three highest contributions of mono-*ortho* and the three lowest contributions of PCDD are associated with the samples of exposed people of this study, i.e. CCF and FWP for serum and CCF for breast milk.

The reason for the prevalence of PCDFs on PCDDs in the three cases of relevant contamination in this study may lay in the source of contamination, as it is known that PCB mixtures are richer in PCDFs than in PCDDs.

The reason for the high contribution of mono-*ortho* PCBs, although possibly related to the source, is less evident: a contribution higher than 40% is present also in three of the population groups examined (GPB, R-SE, R-S).

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