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Polychlorinated biphenyl serum levels, thyroid hormones and endocrine and metabolic diseases in people living in a highly polluted area in North Italy: A population-based study

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ABSTRACT

Polychlorinated biphenyls (PCBs) are persistent organic pollutants produced until the 1980s, which they are still present worldwide. They have been associated with metabolic and endocrine diseases and hypertension in humans, but definite evidence is lacking. A chemical factory producing PCBs caused a heavy pollution in an urban area in Northern Italy. We aimed to evaluate present PCBs serum levels according to demographic and lifestyle variables and their associations with endocrine and metabolic diseases and hypertension in the resident general population. A random sample of 816 adults aged 20–79 years (mean ± SD: 49.1 ± 16.5 years) was enrolled in a cross-sectional population-based study. The participants provided a fasting blood sample for laboratory analysis and were face-to-face interviewed about the presence of chronic diseases. The serum level of total PCBs was computed as the sum of 33 PCB congeners. The median serum level of lipid-adjusted total PCBs was 435.2 and 95th centile was 2154.9 ng/g lipid. Medium and high chlorinated PCBs with immunotoxic and endocrine disrupting activity contributed most to total PCB serum levels, particularly PCBs 138, 153, 170, 180 and 194. The serum levels of total PCBs and of PCB functional groups were positively associated with age and negatively with female gender, education, smoking habit and BMI, and not associated with serum levels of thyroid hormones and TSH and glycaemia and with presence of endocrine diseases, diabetes and hypertension by multivariable analysis. Subgroup analyses according to gender, age and BMI provided similar results. In conclusion, this study shows a long-term persistence of past exposure to PCBs and does not support the hypothesis of an association between PCB exposure and prevalence of endocrine and metabolic diseases and hypertension.

1. Introduction

Polychlorinated biphenyls (PCBs) are organochlorine compounds produced in the past century up to the 1970s-1980s, when they were banned in most countries due to their toxicity and environmental persistence (USEPA, 1979 ATSDR, 2000). For these characteristics, PCBs tend to accumulate in soil, plants and animals and in the food chain (ATSDR, 2000). They have been included among the Persistent Organic Pollutants (POPs) and been found to determine deleterious effects to environment and animals. In fact, various experimental studies on animals and epidemiological studies on humans have demonstrated an association between PCBs and metabolic and endocrine diseases, damage to nervous systems and development and immunological and reproductive disorders (IARC , 2015). Moreover, PCBs have been recently updated to carcinogens to humans (group I) by the International Agency for Research on Cancer (IARC , 2015).

PCBs have been classified as endocrine disruptors because they can interact with several functions of endocrine system, particularly the thyroid and reproductive organs (El Majidi et al., 2014). Various studies have also found that PCBs are associated with diabetes, insulin resistance and unhealthy metabolic phonotype (Kuo et al., 2013; Taylor et al., 2013; Gasull et al., 2017), and cardiovascular diseases,

particularly hypertension (Everett et al., 2008, 2011). However, current evidence on the role of PCBs as determinants of endocrine, metabolic or cardiovascular disease is still inconclusive (Warner et al., 2013; Lee et al., 2014; Henríquez-Hernández et al., 2017; Benson et al., 2018), Brescia is a highly industrialized town in North Italy where a chemical factory produced PCBs from 1938 to 1984 (CTS , 2003). In the 2000s, a high concentration of PCBs and other organochlorines, particularly polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/PCDFs), was found in the soil and in locally produced animal food. A survey on a random sample of the general population living in the town was performed in 2003, showing high PCB levels in subjects living in the area, especially those who had consumed locally produced food (Apostoli et al., 2005; Donato et al., 2006; Turrio-Baldassarri et al, 2007, 2008, 2009). In 2013–14 the survey was repeated and included also two villages adjacent to the PCB polluted area. The data regarding the only residents in Brescia town have been already shown for comparing PCB serum levels between the two surveys (Magoni et al., 2016).

This study reports the data collected in the 2013-14 survey in the whole sample to evaluate: i) PCBs serum levels in a population living in a PCB polluted area according to age, gender, education, smoking habits and BMI; ii) the association between serum levels of PCBs and of thyroid hormones and TSH and glycaemia; and iii) the association between PCBs serum levels and the presence of self-reported endocrine and metabolic diseases and hypertension.

2. Material and methods

2.1. Study population

The study design was the same as that of the 2003 survey (Apostoli et al., 2005; Donato et al., 2006; Magoni et al., 2016). Briefly, a random sample of people aged 20–79 years was selected. The sampling design included stratification by gender, age (6 age groups) and residence in one out of four areas at different soil concentration of PCBs of Brescia, a town of about 200,000 inhabitants in North Italy (Magoni et al., 2016), and in two adjacent villages (range of soil concentration of total PCB: 0.06–2.5 mg/kg) (ARPA, 2014). The sample size was determined using the results of a previous study (Donato et al., 2006; Apostoli et al., 2005), with the aim of showing significant differences among people living in the most and least polluted areas as previously reported (Magoni et al., 2016). For each of the 6 areas (4 in the town and 2 in the adjacent villages) a minimum of 120 subjects was needed. Initially, 300 individuals were sampled for each area, but oversampling was performed in some areas subsequently, due to the low response rate, to achieve the minimum sample size established.

An invitation letter was sent to each eligible subject, and non-responders were re-contacted by mail and phone. Participants were face-to-face interviewed by a trained nurse using the same structured questionnaire used in the 2003 survey. The interview lasted 30–45 min and collected information on the subject's demographic variables, weight and height, residential and occupational history, smoking habit and alcohol drinking. Body max index (BMI, kg/m^2) is defined as the weight in kilograms divided by the square of the height in meters (kg/m^2) and it was categorized as normal (<25), overweight (25–30) and obese ($\geq$30) (WHO, 2000). Subjects were also asked whether they were affected by, or were taking medicines for, chronic diseases. According to their self-reported history of disease and/or use of medicines, participants were classified as affected or not affected by thyroid and other endocrine diseases, diabetes and hypertension. These diseases were chosen because of some evidence that PCBs may act as endocrine disruptors (IARC , 2015).

The Local Ethics Committee approved the project and each participant provided informed consent.

2.2. Blood sampling and storage

A 20 ml blood sample was collected by each participant under fasting conditions. The serum was separated by centrifugation at 3500 rpm (5 min) and stored at -20 °C until determination.

Fasting plasma glucose was determined using the glucose-oxidase method. Serum lipid profile, including total cholesterol and triglycerides, was evaluated using enzymatic methods.

Hormone analysis was performed at the Laboratory of the Local Health Authority of Brescia and serum FT3, FT4 and TSH concentrations were measured with immunocyte DTC (Medical Systems S.p.A.; Genoa, Italy), an automated solid-phase immunoassay analyzer that has a chemiluminescent detection system.

2.3. PCB analysis

We determined the serum concentration of the following 33 congeners: 28, 31, 52, 74, 77, 81, 99, 101, 105, 114, 118, 123, 126, 128, 138, 146, 153, 156, 157, 167, 169, 170, 172, 177, 180, 183, 187, 189, 194, 196/203, 201, 206 and 209. PCBs were measured using a Hewlett-Packard 6890N gas chromatograph coupled with an MSD HP 5973. Analytical procedure, limit of quantification and accuracy assessment of the method are reported elsewhere (Apostoli et al., 2005). PCB analysis was performed at the Laboratory of Occupational Hygiene and Toxicology, Brescia University, Italy. The limit of quantification (10 times the signal-to-noise ratio peaks) varied among PCBs but was generally <0.1 ng/ml for each congener.

Total PCB concentration was computed by summing values of PCB congeners in subjects with non-null values for one or more congeners, as reported in Apostoli et al. (2005). All subjects had detectable values for at least some of the PCBs investigated. Since PCB concentration is influenced by the amount of serum lipids, the ratio of PCB concentration to total lipid level was computed (lipid-adjusted PCB concentration), using the Phillips et al. (1989) formula to determine total lipid (TL) content in serum when only triglycerides (TG) and total cholesterol (TC) are available: TL (g/L) = 2.27*TC + TG + 0.623, where both TC and TG are expressed as g/L. All the analyses were performed using lipid-adjusted total PCB values, expressed as ng/g lipid.

2.4. Statistical analysis

Due to asymmetric, non-normal distribution of PCB values, the median, range and 95th centile of the distribution are reported together with geometric mean. The differences of PCBs serum levels according to age, gender, education, smoking habits and BMI and presence of chronic diseases were evaluated using the non-parametric Kruskal-Wallis test at a first step. At a second step, multiple regression models were fitted with log-transformed PCB serum level as the dependent variable and age, gender, education, smoking habits and BMI and presence of any chronic disease as independent variables (age was included as a covariate).

The association of each chronic disease with PCB serum values was also evaluated by multivariable analysis, using multiway ANOVA for comparison of log-transformed PCB serum values according to presence of each investigated disease, including also age, gender, education, smoking habits and BMI as independent factors. Furthermore, these association were assessed fitting logistic regression models with presence of chronic disease as the dependent variable and the PCB serum concentration categorized in tertiles as an independent ordinal variable, controlling for age, gender, education, smoking habits and BMI as possible confounders. The odds ratios (ORs) were computed by logistic regression analysis as measures of associations.

The relationships between serum levels of PCBs and of thyroid hormones, TSH and glycemia were evaluated by Spearman's correlation coefficients and by fitting multiple regression models with log-transformed PCB serum level as the dependent variable, and serum levels of hormones and glycemia and age, gender, education, smoking

habits and BMI as independent variables.

Each analysis was performed for total PCB concentration and PCB congener functional groups according to the classification proposed by Cocco et al. (2008) as: a) congeners with immunotoxic activities (PCB 138, 153 and 180); b) low-chlorinated PCBs with pseudo-oestrogen activity (PCB 28, 52 and 153); c) high-chlorinated PCBs with anti-oestrogenic activities (PCB 170, 180 and 194); d) PCBs that can induce phenobarbital (PCB 101, 153, 180 and 194). We also examined PCB 153 alone, which is an endocrine disruptor non-dioxin congener associated with thyroid and metabolic diseases in a few studies.

The analyses of the associations of PCB serum levels with prevalence of chronic diseases and with serum levels of thyroid hormones, TSH and glycemia were performed in all subjects. Stratified analysis by gender, age (dichotomized at the median), and BMI (dichotomized as normal vs overweight and obese) were also performed, because of the possible role of these variables as effect modifiers.

The agreement between subjects' self-reported diagnoses and those recorded in the LHA database was assessed using the Cohen's kappa statistic.

All the statistical tests were two sided with a threshold of 0.05 for refusing the Null hypothesis. The 95% confidence intervals (95% CIs) were also reported for each estimate. Statistical analyses were performed using the STATA software (Stata Statistical Software release 12.1, 2013; Stata Corporation, College Station, Texas).

3. Results

An invitation letter was sent to 1523 adult residents in Brescia town and 489 adults residents in two adjacent villages, for a total of 2012 subjects. A total of 816 subjects (40.5% of the invited) agreed to participate and underwent an interview and a blood sample, including 614 subjects living in the urban areas and 202 ones living in the villages.

Their mean $\pm$ SD age was 49.1 $\pm$ 16.5 years and 454 of them were females (55.7%).

The lipid-adjusted serum levels of total PCBs and PCB congeners detected in at least 10% of subjects according to chlorination level (low, medium, high) are shown in Table 1. The geometric mean and the median of the serum levels of total PCBs were 364.3 and 435.2 ng/g lipid, respectively, with a range of 0–22882.9 ng/g lipid. The medium chlorinated PCB 138 and 153 and high chlorinated PCB 170, 180 and 187 showed the highest serum concentration. Various congeners had a median of 0 because they were not detectable in the majority of subjects.

The distribution of serum total PCBs according to age, gender, education, smoking habits and BMI are reported in Table 2. PCB serum levels were strongly associated with age (p < 0.001), with more than 10-fold higher median value in the oldest (60–79 years: 1163.4 ng/g lipid) compared to the youngest (20–39 years: 110.8 ng/g lipid) subjects. No differences were found between males and females. PCB serum levels were inversely associated with smoking habits and education, with lower concentration in former and current smokers compared to never smokers and in subjects with 5 years of less compared to those with more than 8 years of school, and positively associated with BMI, with higher values in subjects with BMI >30 compared to those with BMI <25.

Multiple regression with log-transformed PCB serum value as the dependent variable and all the variable in Table 2 as independent variables confirmed the that PCB serum levels were associated positively with age (p < 0.001), and negatively with female gender (p = 0.02), BMI (p = 0.001), tobacco smoking (p = 0.004) and education (p = 0.04) (data not shown in Table).

The median serum values of the total PCB, PCB 153, and four PCB functional groups according to presence of thyroid and other endocrine gland diseases, diabetes and hypertension are shown in Table 3. Higher total PCB serum levels were found in subjects with thyroid diseases, diabetes, hypertension. However, these differences were mainly due to older age of subjects with, compared to those without, chronic diseases. Indeed, these associations were not confirmed by multiway ANOVA with

log-transformed PCB serum value as the dependent variable and each chronic disease, age, gender, education, smoking habits and BMI as independent variables (data not shown in Table). Accordingly, no association was found between any chronic disease and serum levels of PCB 153 and PCB functional groups (data not shown in Table).

An analysis of the associations between tertiles of the distribution of total PCB serum levels and prevalence of self-reported chronic diseases

Table 1

Serum levels of some PCB congeners according to chlorination degree and of total PCBs: number and percentage of subjects with values above the detection limit, geometric mean, median, range and 95th centile of distribution (in ng/g lipid). Only PCB congeners detected in at least 10% of subjects are reported.

PCB congeners	Serum levels (ng/g lipid)				
	No. (%)	Geometric mean	Median	Range	95th centile
Total PCBs	816 (100)	364.3	435.2	<dl-22822.9	2154.9
Low chlorinated PCBs					
28	105 (12.8)	21.05	<dl	<dl-71.4	22.4
52	172 (21.0)	11.76	<dl	<dl-46.3	14.5
74	446 (54.6)	14.55	8.42	<dl -189.3	28.7
99	395 (48.4)	14.06	<dl	<dl -340.4	26.3
101	132 (16.1)	12.54	<dl	<dl -41.62	14.43
118	516 (63.2)	18.86	11.0	<dl -598.1	55.8
Medium chlorinated PCBs					
138	738 (90.4)	63.86	53.48	<dl -2578.9	246.4
146	382 (46.8)	16.0	<dl	<dl -253.4	32.8
153	789 (96.7)	99.9	93.5	<dl -3701.1	478.9
156	517 (63.3)	20.07	12.09	<dl -368.9	49.3
167	162 (19.8)	12.66	<dl	<dl -80.94	15.8
High chlorinated PCBs					
170	586 (71.8)	55.71	35.82	<dl -1314.0	161.6
172	233 (28.5)	16.51	<dl	<dl -178.9	26.3
180	765 (93.7)	124.1	117.7	<dl -4797.1	613.8
183	340 (41.6)	16.14	<dl	<dl -315.2	33.3
187	556 (68.1)	29.19	16.16	<dl -1627.6	99.45
194	484 (59.3)	60.65	27.15	<dl -1534.2	179.5
201	466 (57.1)	30.04	12.51	<dl -1033.3	88.6
209	405 (49.6)	22.9	<dl	<dl -3671.4	87.7

<dl: less than the detection limit, 0.1 ng/ml.

Table 2

Lipid-adjusted PCB serum levels (ng/g lipid) in adults of the general population living in a highly PCB polluted area in North Italy, according to age, gender, education, smoking habits and body mass index (BMI).

	PCB serum levels (ng/g lipid)				
	No. (%)	Geometric mean	Median	Range	95th centile
Age (years)**					
20–39	238 (29.2)	97.2	110.8	<dl -671.4	471.5
40–59	310 (38.0)	375.8	395.1	5.8–7035.1	1452.9
60–79	268 (32.8)	1136.4	1065.0	198.1–22882.9	3899.1
Gender					
Female	454 (55.7)	341.8	424.6	<dl -13100.3	2564.0
Male	362 (44.3)	394.7	437.0	<dl -22882.9	2045.9
Smoking habits*					
Never smoker	435 (53.3)	389.7	460.9	7.8–22882.9	2763.5
Former smoker	215 (26.3)	407.7	527.5	5.8–7035.1	1968.0
Current smoker	166 (20.4)	263.9	276.4	7.9–2622.5	1431.9
Education (years)**					
≤5	129 (15.8)	1177.9	1121.2	137.9–14768.5	4177.7
6–8	242 (29.8)	467.2	567.0	<dl -13100.4	2465.3
>8	444 (54.4)	227.2	224.0	<dl -22882.9	1484.1
BMI (kg/m²)					
<25	491 (60.2)	302.6	334.7	<dl -22882.9	1971.5
25–30	235 (28.8)	468.0	550.2	<dl -14768.5	2465.3
≥30	90 (11.0)	528.6	665.5	137.9–14768.5	2468.9

*p < 0.01; **p < 0.001 by Kruskal-Wallis test.
<dl: less than the detection limit, 0.1 ng/ml.

Table 3
Median PCB serum levels according to history of chronic diseases.

Disease		No. Subjects (n = 816)	% with disease	PCBs serum levels (ng/g lipid)					
				Total PCBs	PCB 153	Immunotoxic PCBs (PCB138 + PCB153 + PCB180)	Pseudo-oestrogen PCBs (PCB28 + PCB52 + PCB153)	Highly chlorinated anti oestrogenic PCBs (PCB170 + PCB180 + PCB194)	Phenobarbital inducer PCBs (PCB101 + PCB153 + PCB180 + PCB194)
Thyroid diseases	Yes	82	10.0	518.5	119.6	324.3	135.1	189.8	282.2
	No	734	-	422.7	90.1	266.7	97.3	180.2	236.3
Diabetes	Yes	38	4.7	918.3	202.8	565.0	203.8	417.5	542.1
	No	778	-	406.9	89.3	257.3	95.6	173.4	229.6
Hypertension	Yes	151	18.5	861.2	174.3	514.6	178.5	389.4	496.7
	No	665	-	315.5	73.6	212.2	81.0	126.0	182.7
Endocrine diseases[a]	Yes	17	2.1	611.5	163.8	417.3	163.8	266.9	384.3
	No	799	-	426.2	92.6	268.1	100.2	179.5	237.9

[a] thyroid diseases excluded. Percentages computed on the total females or males.

stratified by BMI, dichotomized as normal/overweight and obese, is set out in Table 4. No association was observed for any disease and PCB serum values in both normal and overweight/obese subjects. We performed also subgroup analyses of the associations between total PCB serum levels in tertiles and presence of self reported chronic diseases stratifying subjects by the dichotomic variables: age (<49 vs ≥ 49 years), gender, education (<8 vs ≥ 8 years of school) and smoking habits (current smokers vs non-smokers and former smokers). No statistically significant associations were found for any disease.

A good correlation was found between serum levels of lipid-adjusted PCBs and age as reported in Fig. 1: the oldest subjects had the highest values of total PCBs (r = 0.6).

A weak inverse correlation was found between serum levels of total PCBs and those of free T3, free T4 and TSH using non-parametric methods (Table 5). However, no association was found when adjusting for age, gender, education, smoking habits and BMI by multiple regression models with log-transformed PCB serum value as the dependent variable. The joint distribution of the serum concentration of free thyroid hormones, TSH and glycaemia and log-transformed total PCBs is shown in Fig. 2. No clear pattern is evident.

The record linkage between our data set and the LHA database was successful for 752 of the 816 subjects (92%). The percentage of agreement for diabetes was 98.6 % (kappa = 0.85, p < 0.0001) and for hypertension was 91 % (kappa = 0.71, p < 0.0001).

4. Discussion

This cross-sectional study on the general population living in an area polluted by a factory producing PCBs until the 1980s showed that

subjects' PCB serum levels are still high and strongly associated with age, with the highest values in the oldest subjects, suggesting high body storage of these compounds by cumulative past exposure. The serum levels of total PCBs, PCB 153 and four PCB functional groups were also negatively associated with female gender, education level and smoking habit, and positively associated with BMI by multivariable analysis. However, we found no association of PCB serum levels, PCB 153 and four PCB functional groups with either prevalence of endocrine and metabolic diseases and hypertension or serum levels of thyroid hormones and TSH and glycaemia when controlling for age and other covariates.

PCBs are still a matter of concern, in spite of their dramatic decline in the world in last decades, after the ban of their production and use in most countries in the 1970–1980s. In fact, they are still present world-wide, due to long environmental persistence, and are suspected to play a role in the occurrence of metabolic, endocrine and cardiovascular diseases and to have an impact on fetal and newborn development, also for relatively low concentration (Solecki et al., 2017; Kabir et al., 2015; Haddow et al., 1999). Furthermore, emissions of unintentionally produced PCBs by some factories, from e-waste contaminated sites so in e-waste dismantling workers increased in various countries, particularly in China, in the 2000s (Cui et al., 2015), determining relatively high PCB serum levels in e-waste workers and in the general population living inside e-waste recycling areas compared to people living outside polluted areas (Ma et al., 2018; Lv et al., 2015). It is noteworthy that predominant PCB congeners found in e-waste workers and in people living in e-waste recycling areas were low-chlorinated PCBs, especially tri- and tetrachloro-PCBs, probably due to exposure by inhalation of contaminated air or dermal contact, contrary to the finding that ingestion of contaminate food is the most relevant source of PCB exposure in Western

Table 4
Prevalence of self-reported chronic diseases according to tertiles of total PCB serum levels stratified by BMI (normal weight vs overweight and obese).

Disease	PCB Tertiles	Normal weight			Overweight and obese			Total subjects		
		N. subjects with disease/total subjects (%)	OR (95% CI)[a]	P value	N. subjects with disease/total subjects (%)	OR (95% CI)[a]	P value	N. subjects with disease/total subjects (%)	OR (95% CI)[a]	P value
Thyroid diseases	1st	13/184 (7.0)	1		7/65 (10.7)	1		20/249 (7.4)	1	
	2nd	19/122 (15.6)	1.97 (0.8–4.7)	0.1	8/85 (9.4)	0.7 (0.2–2.7)	0.6	27/207 (11.5)	1.46 (0.7–3.0)	0.3
	3rd	19/144 (13.2)	1.29 (0.4–3.9)	0.6	16/134 (11.9)	0.7 (0.2–3.2)	0.7	35/278 (11.2)	1.12 (0.4–2.7)	0.2
Diabetes	1st	2/195 (1.0)	1		2/70 (2.8)	1		4/265 (1.5)	1	
	2nd	1/140 (0.7)	0.3 (0.02–4.6)	0.4	5/88 (5.7)	0.5 (0.07–3.6)	0.5	6/228 (2.5)	0.5 (0.1–2.2)	03
	3rd	7/156 (4.5)	1.1 (0.09–13.7)	0.9	21/129 (16.2)	0.8 (0.1–5.7)	0.8	28/285 (9.0)	0.8 (0.2–3.8)	0.8
Hypertension	1st	5/192 (2.6)	1		4/68 (5.8)	1		9/260 (3.3)	1	
	2nd	13/128 (10.1)	0.97 (0.3–3.2)	0.9	27/66 (40.9)	2.7 (0.8–9.3)	0.1	40/194 (17.1)	1.7 (0.7–3.8)	0.2
	3rd	37/126 (29.3)	1.0 (0.3–3.6)	0.9	65/85 (76.4)	2.9 (0.8–10.5)	0.09	102/211 (32.6)	1.6 (0.7–4.0)	0.2
Endocrine diseases[b]	1st	3/194 (1.5)	1		1/71 (1.4)	1		4/265 (1.5)	1	
	2nd	1/140 (0.7)	0.1 (0–1.7)	0.1	4/89 (4.5)	1.5 (0.1–21.5)	0.7	5/229 (2.1)	0.5 (0.09–2.4)	0.3
	3rd	4/159 (2.5)	0.2 (0.1–2.3)	0.1	4/146 (2.7)	0.4 (0.01–8.1)	0.5	8/305 (2.6)	0.2 (0.04–1.5)	0.1

[a] OR estimated adjusted for age, educational level, gender and tobacco smoking by logistic regression.
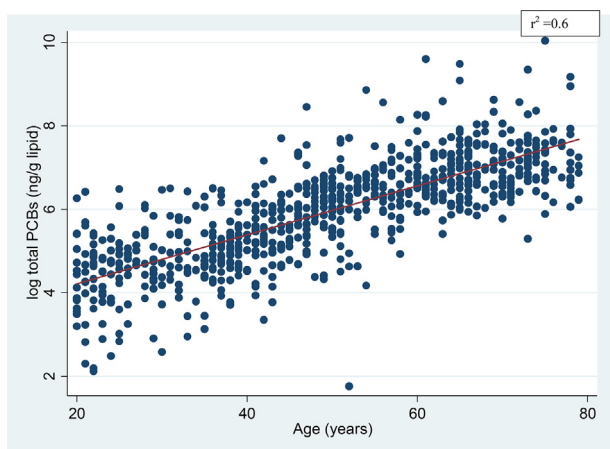[b] Thyroid diseases excluded.

Fig. 1. Serum levels of lipid-adjusted total PCBs (log-transformed) according to age.

Table 5
Non-parametric correlation (Spearman rho) and regression coefficients of free T3 and T4 and TSH serum levels on log-transformed PCB serum levels in subjects without history of thyroid diseases.

	Rho	P-value	Regression coefficient[a]	
			Coeff	P-value
Free T3 (pcg/ml)	− 0.2	<0.0001	−0.03	0.6
Free T4 (ng/dl)	−0.1	0.0009	−0.17	0.3
TSH (μIU/mL)	−0.09	0.008	−0.01	0.5

[a] Adjusted for age, gender, education, smoking habits and BMI.

countries. Furthermore, the PCB serum concentration does not vary by age or is higher, especially for more volatile PCB congeners, in younger than older people, in recent Chinese studies (Wang et al., 2018).

Overall, PCB serum levels in our population are similar to those observed in other areas in Spain, France, Germany and Belgium (Pirard et al., 2018; Fernández-Rodríguez et al., 2015; Mrema et al., 2014), though higher than in the USA (Sjödin et al., 2014), in Chinese cities (Wang et al., 2018) or in other industrial areas or waste contaminated area of Italy (Esposito et al., 2014; De Felip et al., 2008, 2014).

As regards the possible effects of PCBs on human health, several studies have focused endocrine and metabolic diseases in last decades. Particularly, some authors claim that these compounds could cause thyroid diseases acting in several steps in hormone thyroid synthesis, with synergic or antagonist effects, based on the interference with binding of THS to receptors or to transport proteins (Boas et al., 2006). However, epidemiologic research has provided contrasting results, with some but not all studies showing associations of PCB serum levels with thyroid diseases and/or serum levels of thyroid hormones or TSH (reviewed in Donato et al., 2008; Salay and Garabrant, 2009; Boas et al., 2006). In Anniston, Alabama, where a plant producing PCBs lead to a substantial contamination in the area, residents showed elevated PCB blood levels compared to the US population, but no associations between PCBs and thyroid hormones, antibodies, or combined indicators of thyroid function (Benson et al., 2018). A study on pregnant women living in a e-waste recycling area in China who had with PCB serum levels 3–4 time higher than those living in other areas found a weak inverse association with the serum levels of TSH but not of total or free T3 and T4 (Lv et al., 2015).

Recently, PCBs, especially PCB 153, have shown a positive association with type 2 diabetes when also taking account of the major risk factors for this disease, though some inconsistencies were. The Anniston community survey showed a positive association between serum PCB levels and self-referred diabetes prevalence, but the association was found in females only and was imprecise in older people, who however had the highest PCB serum levels (Silverstone et al., 2012). PCB serum concentration was positively associated with diabetes, prediabetes and unhealthy metabolic pattern also in a cross-sectional study in a Spanish general population, with a stronger association in normal-weight than overweight-obese subjects (Gasull et al., 2012, 2017). In another cross-sectional study in a Native American population, however, an association was found between diabetes and only low-chlorinated PCB congeners, which are the most volatile congeners, suggesting that the
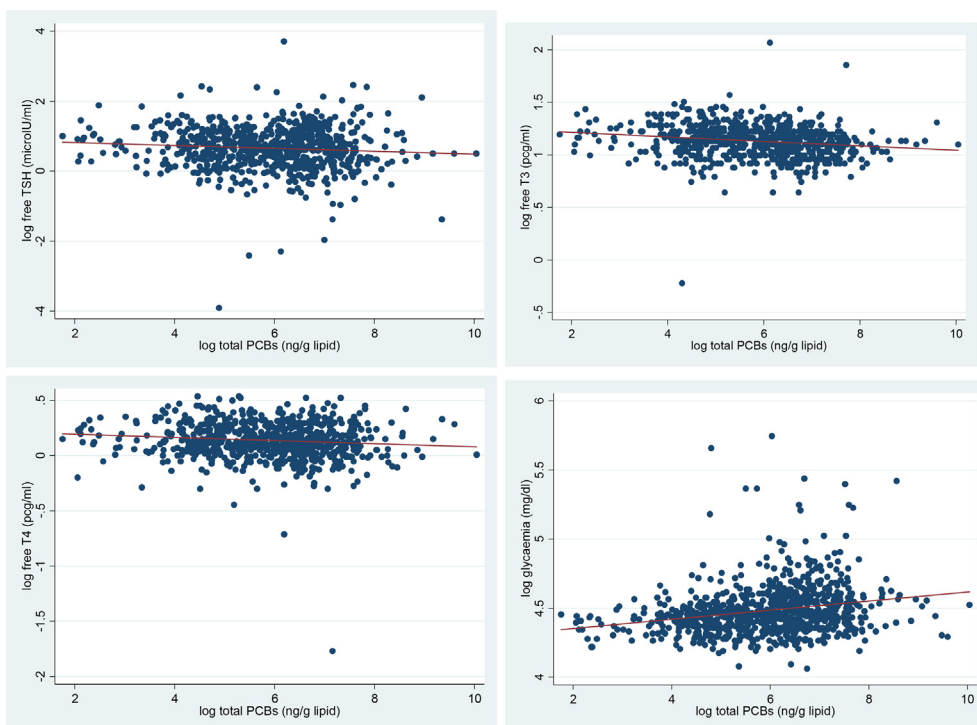

Fig. 2. Serum levels of free T3, free T4, TSH and glycaemia and total PCBs (A, B, C, D, respectively) (log-transformed data).

main route of exposure was inhalation (Aminov et al., 2016). Other studies showed a weak, not significant, association (Grice et al., 2017) or no association at all (Henríquez-Hernández et al., 2017) between PCBs serum levels and type 2 diabetes. Some systematic reviews showed that overall evidence of epidemiologic studies suggests an association between PCB serum levels and diabetes, although inconsistency was found among the studies, possibly due to differences among populations, sources of exposure, PCB congeners detected and adjustment for relevant risk factors for diabetes, which did not allow to draw firm conclusions on the cause-effect relationship (Kuo et al., 2013; Tang et al., 2014; Taylor et al., 2013).

An association of POPs serum levels, including PCBs, with hypertension has been reported in some studies. In the Anniston community survey, serum PCB levels were significantly associated with prevalence of hypertension and with systolic and diastolic blood pressure (Goncharov et al., 2011). However, the 1999–2002 NHANES found that most PCB congeners were associated with hypertension, especially dioxin-like PCBs, but only among men (Everett et al., 2008; Ha et al., 2009), and other studies showed no association (Henríquez-Hernández et al., 2014), or a positive association of hypertension with some PCB congeners but a negative association with others (Van Larebeke et al., 2015). A recent meta-analysis observed an association between the sum of dioxin-like PCBs, though not the sum of non-dioxin-like PCBs, and hypertension (Park et al., 2016). It is noteworthy, however, that dioxin-like PCBs are less commonly found than non-dioxin-like PCBs in the general population worldwide, and that some non-dioxin-like PCBs, particularly PCB 153, are classified as endocrine disruptors.

In our study, we found no association between total PCB serum levels, PCB 153 and PCB functional groups and diabetes, hypertension and thyroid diseases when also performing stratified analyses according to gender, age, and BMI. Overall, these findings are in agreement with those from our 2003 cross-sectional survey (Donato et al, 2006, 2008; Zani et al., 2013). In our population, however, medium- and high-chlorinated PCBs contributed most to the total PCBs, whereas dioxin-like PCBs and low chlorinated PCBs, which were the only PCB congeners associated with diabetes and hypertension in some studies, contributed less than 5% to the sum of total PCBs, in agreement with the pattern of PCB congeners assayed in most studies carried out in Western countries.

Our study has various strengths. First, it was a population-based study aimed to investigate PCB serum levels and their possible impact on the prevalence of some chronic disease in a random sample of the general adult population living in the area. Second, the subjects enrolled in the study had lived in a PCB polluted area for many years, and their present serum values probably reflect a cumulative past exposure. Third, the relatively high number of adult people enrolled (n = 816), the large age range (20–79 years), and the high number of PCB congeners examined (n = 33) provided enough power for showing associations between PCB serum levels and presence of chronic diseases and blood hormones. For instance, the subjects affected by various chronic diseases in our study were more than the minimum number of 75 "cases" for having 80% power to detect an odds ratio of 2.0 with a higher than 1:4 case/control ratio and alpha = 0.05.

This study has some limitations, too. First, participation rate was moderate (40.5%), although this is in agreement with the decline of people's participation in epidemiological surveys worldwide, especially when a blood sample is requested (Galea and Tracy, 2007). Second, the cross-sectional study design and the collection of subjects' self-reported data on the presence of chronic diseases may be of concern. However, residents were unaware of their PCB serum levels when interviewed, and the proportion of those referring history of chronic diseases is on line with the national prevalence (http://www.cuore.iss.it), therefore selection bias seems unlikely to have occurred. The use of self-reported data on presence of chronic diseases is in agreement with various recent population-based studies investigating the relationship between POPs exposure and chronic diseases (Aminov et al., 2016; Everett et al., 2011; Henríquez-Hernández et al., 2017), which does not seem to have

introduced substantial information bias. Anyway, to assess validity of self-reported diagnoses, we made a record linkage of our data set with the Brescia Local Health Authority (LHA) health-care database, based on routinely collected administrative data, which have been shown to have a satisfactory completeness and accuracy according to a previous study performed by some of us (Lonati et al., 2008). We found an overall good agreement between subject's self-reported data and the LHA database as regards the presence of diabetes and hypertension. No evaluation could be done for thyroid diseases because some subjects with past diagnosis of these diseases no longer require health services for their diseases and therefore are not included in the LHA database (Lonati et al., 2008).

In conclusion, the serum levels of total PCBs, PCB 153 and PCB functional groups were not associated with thyroid diseases, diabetes and hypertension or with hormone serum levels and glycemia when taking account of age, gender, education, smoking habits and BMI in the general population living in a highly PCB polluted area.

Declarations

References

Aminov, Z., Haase, R., Rej, R., Schymura, M.J., Santiago-Rivera, A., Morse, G., De Caprio, A., Carpenter, D.O., Akwesasne Task Force on the Environment, 2016. Diabetes prevalence in relation to serum concentrations of polychlorinated biphenyl (PCB) congener groups and three chlorinated pesticides in a Native American population. Environ. Health Perspect. 124, 1376–1383.

Apostoli, P., Magoni, M., Bergonzi, R., Carasi, S., Indelicato, A., Scarcella, C., Donato, F., 2005. Assessment of reference values for polychlorinated biphenyl concentration in human blood. Chemosphere 61, 413–421.

ARPA, 2014. Lombardia Agenzia Regionale per la Protezione dell'ambiente. http://www.arpalombardia.it/Pages/Bonifica/Brescia.aspx.

ATSDR Agency for Toxic Substances and Disease Registry, 2000. Toxicological Profile for Selected Polychlorinated Biphenyls. U.S. Department of Health and Human Services, Public Health Service, Atlanta, GA.

Benson, K., Yang, E., Dutton, N., Sjodin, A., Rosenbaum, P.F., Pavuk, M., 2018. Polychlorinated biphenyls, indicators of thyroid function and thyroid autoantibodies in the Anniston Community Health Survey I (ACHS-I). Chemosphere 195, 156–165.

Boas, M., Feldt-Rasmussen, U., Skakkebaek, N.E., Main, K.M., 2006. Environmental chemicals and thyroid function. Eur. J. Endocrinol. 154, 599–611.

Cocco, P., Brennan, P., Ibba, A., de Sanjose` Llongueras, S., Maynadie`, M., Nieters, A., et al., 2008. Plasma polychlorobiphenyl and organochlorine pesticide level and risk of major lymphoma subtypes. Occup. Environ. Med. 65, 132–140.

CTS (Comitato Tecnico Scientifico), 2003. Relazione Finale: 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 (in Italian). http://www.aslb rescia.it/webbs/Web.

Cui, S., Fu, Q., Ma, W.L., Song, W.W., Liu, L.Y., Li, Y.F., 2015 Nov 15. A preliminary compilation and evaluation of a comprehensive emission inventory for polychlorinated biphenyls in China. Sci. Total Environ. 533, 247–255. Epub 2015 Jul 11. PubMed PMID: 26172591.

De Felip, E., Bianchi, F., Bove, C., Cori, L., D'Argenzio, A., D'Orsi, G., Fusco, M., Miniero, R., et al., 2014. Priority persistent contaminants in people dwelling in critical areas of Campania Region, Italy (SEBIOREC biomonitoring study). Sci. Total Environ. 487, 420–435.

De Felip, E., Abballe, A., Casalino, F., di Domenico, A., Domenici, P., Iacovella, N., Ingelido, A.M., Pretolani, E., Spagnesi, M., 2008. Serum levels of PCDDs, PCDFs and PCBs in non-occupationally exposed population groups living near two incineration plants in Tuscany. Italy. Chemosphere. 72, 25–33.

Donato, F., Zani, C., Magoni, M., Gelatti, U., Covolo, L., Orizio, G., Speziani, F., Indelicato, A., Scarcella, C., Bergonzi, R., Apostoli, P., 2008. Polychlorinated biphenyls and thyroid hormone serum concentrations among people living in a highly polluted area: a cross-sectional population-based study. Environ. Res. 108, 380–386.

Donato, F., Magoni, M., Bergonzi, R., Scarcella, C., Indelicato, A., Carasi, S., Apostoli, P., 2006. Exposure to polychlorinated biphenyls in residents near a chemical factory in Italy: the food chain as main source of contamination. Chemosphere 64, 1562–1572.

El Majidi, N., Bouchard, M., Carrier, G., 2014. Systematic analysis of the relationship between standardized biological levels of polychlorinated biphenyls and thyroid function in pregnant women and newborns. Chemosphere 98, 1–17.

Esposito, M., Serpe, F.P., Diletti, G., Messina, G., Scortichini, G., La Rocca, C., Baldi, L., Amorena, M., Monda, M., 2014. Serum levels of polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and polychlorinated biphenyls in a population living in the Naples area, southern Italy. Chemosphere 94, 62–69.

Everett, C.J., Frithsen, I., Player, M., 2011. Relationship of polychlorinated biphenyls with type 2 diabetes and hypertension. J. Environ. Monit. 13, 241–251.

Everett, C.J., Mainous 3rd, A.G., Frithsen, I.L., Player, M.S., Matheson, E.M., 2008. Association of polychlorinated biphenyls with hypertension in the 1999-2002 national health and nutrition examination survey. Environ. Res. 108, 94–97.

Fernández-Rodríguez, M., Arrebola, J.P., Artacho-Cordón, F., Amaya, E., Aragones, N., Llorca, J., Perez-Gomez, B., Ardanaz, E., Kogevinas, M., Castano-Vinyals, G., Pollan, M., Olea, N., 2015. Levels and predictors of persistent organic pollutants in an adult population from four Spanish regions. Sci. Total Environ. 538, 152–161.

Galea, S., Tracy, M., 2007. Participation rates in epidemiologic studies. Ann. Epidemiol. 17, 643–653.

Gasull, M., Castell, C., Pallarès, N., Miret, C., Pumarega, J., Te Llez-Plaza, M., López, T., Salas-Salvadó, J., Lee, D.H., Goday, A., Porta, M., 2017. Blood concentrations of persistent organic pollutants and unhealthy metabolic phenotypes in normal-weight, overweight, and obese individuals. Am. J. Epidemiol. 187, 494–506.

Gasull, M., Pumarega, J., Téllez-Plaza, M., Castell, C., Tresserras, R., Lee, D.H., Porta, M., 2012. Blood concentrations of persistent organic pollutants and prediabetes and diabetes in the general population of Catalonia. Environ. Sci. Technol. 46, 7799–7810.

Goncharov, A., Pavuk, M., Foushee, H.R., Carpenter, D.O., Anniston Environmental Health Reseach Consortium, 2011. Blood pressure in relation to concentrations of PCB congeners and chlorinated pesticides. Environ. Health Perspect. 119, 319–325.

Grice, B.A., Nelson, R.G., Williams, D.E., Knowler, W.C., Mason, C., Hanson, R.L., Bullard, K.M., Pavkov, M.E., 2017. Associations between persistent organic pollutants, type 2 diabetes, diabetic nephropathy and mortality. Occup. Environ. Med. 74, 521–527.

Ha, M.H., Lee, D.H., Son, H.K., Park, S.K., Jacobs Jr., D.R., 2009. Association between serum concentrations of persistent organic pollutants and prevalence of newly diagnosed hypertension: results from the National Health and Nutrition Examination Survey 1999-2002. J. Hum. Hypertens. 23, 274–286.

Haddow, J.E., Palomaki, G.E., Allan, W.C., Williams, J.R., Knight, G.J., Gagnon, J., O'Heir, C.E., Mitchell, M.L., Hermos, R.J., Waisbren, S.E., Faix, J.D., Klein, R.Z., 1999 Aug 19. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N. Engl. J. Med. 341 (8), 549–555. PubMed PMID: 10451459.

Henríquez-Hernández, L.A., Luzardo, O.P., Valerón, P.F., Zumbado, M., Serra-Majem, L., Camacho, M., González-Antuña, A., Boada, L.D., 2017. Persistent organic pollutants and risk of diabetes and obesity on healthy adults: results from a cross-sectional study in Spain. Sci. Total Environ. 607–608, 1096–1102.

Henríquez-Hernández, L.A., Luzardo, O.P., Zumbado, M., Camacho, M., Serra-Majem, L., Álvarez-León, E.E., Boada, L.D., 2014. Blood pressure in relation to contamination by polychlorobiphenyls and organochlorine pesticides: results from a population-based study in the Canary Islands (Spain). Environ. Res. 135, 48–54.

IARC (International Agency for Research on Cancer), 2015. Polychlorinated Biphenyls, Volume 107. WHO-IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Available: http://monographs.iarc.fr/ENG/Monographs/vol107/.

Kabir, E.R., Rahman, M.S., Rahman, I., 2015. A review on endocrine disruptors and their possible impacts on human health. Environ. Toxicol. Pharmacol. 40, 241–258.

Kuo, C.C., Moon, K., Thayer, K.A., Navas-Acien, A., 2013. Environmental chemicals and type 2 diabetes: an updated systematic review of the epidemiologic evidence. Curr. Diabetes Rep. 13, 831–849.

Lee, D.H., Porta, M., Jacobs, D.R., Vandenberg, L., 2014. Chlorinated persistent organic pollutants, obesity, and type 2 diabetes. Endocr. Rev. 35, 557–601.

Lonati, F., Scarcella, C., Indelicato, A., Brioschi, A., Magoni, M., Medea, G., Saleri, N., Orizio, G., Donato, F., 2008. Brescia Local Health Autority Population Database: a

method based on current data for monitoring chronic diseases and management. Epidemiol. Prev. 32, 137–144 [In Italian].

Lv, Q.X., Wang, W., Li, X.H., Yu, L., Zhang, Y., Tian, Y., 2015 Apr. Polychlorinated biphenyls and polybrominated biphenyl ethers in adipose tissue and matched serum from an e-waste recycling area (Wenling, China). Environ. Pollut. 199, 219–226. Epub 2015 Feb 11. PubMed PMID: 25681817.

Ma, S., Ren, G., Zeng, X., Yu, Z., Sheng, G., Fu, J., 2018 Oct. Polychlorinated biphenyls and their hydroxylated metabolites in the serum of e-waste dismantling workers from eastern China. Environ. Geochem. Health 40 (5), 1931–1940. Epub 2017 May 5. PubMed PMID: 28477162.

Magoni, M., Donato, F., Speziani, F., Leonardi, L., Orizio, G., Scarcella, C., Gaia, A., Apostoli, P., 2016. Substantial decline of polychlorinated biphenyls serum levels 10 years after public health interventions in a population living near a contaminated site in Northern Italy. Environ. Int. 95, 69–78.

Mrema, E.J., Rubino, F.M., Mandic-Rajcevic, S., Sturchio, E., Turci, R., Osculati, A., Brambilla, G., Minoia, C., Colosio, C., 2014. Exposure to priority organochlorine contaminants in the Italian general population. Part 2: fifteen priority polychlorinated biphenyl congeners in blood serum. Hum. Exp. Toxicol. 33, 170–184.

Park, S.H., Lim, J.E., Park, H., Jee, S.H., 2016. Body burden of persistent organic pollutants on hypertension: a meta-analysis. Environ. Sci. Pollut. Res. Int. 23, 14284–14293.

Phillips, D.L., Pirkle, J.L., Burse, V.W., Bernert, J.T., Henderson, L.O., Needham, L.L., 1989. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. Arch. Environ. Contam. Toxicol. 18, 495–500.

Pirard, C., Compere, S., Firquet, K., Charlier, C., 2018. The current environmental levels of endocrine disruptors (mercury, cadmium, organochlorine pesticides and PCBs) in a Belgian adult population and their predictors of exposure. Int. J. Hyg Environ. Health 221, 211–222.

Salay, E., Garabrant, D., 2009. Polychlorinated biphenyls and thyroid hormones in adults: a systematic review appraisal of epidemiological studies. Chemosphere 74, 1413–1419.

Silverstone, A.E., Rosenbaum, P.F., Weinstock, R.S., Bartell, S.M., Foushee, H.R., Shelton, C., Pavuk, M., 2012. Polychlorinated biphenyl (PCB) exposure and diabetes: results from the Anniston community health survey. Environ. Health Perspect. 20, 727–732.

Sjödin, A., Jones, R.S., Caudill, S.P., Wong, L.Y., Turner, W.E., Calafat, A.M., 2014. Polybrominated diphenyl ethers, polychlorinated biphenyls, and persistent pesticides in serum from the national health and nutrition examination survey: 2003-2008. Environ. Sci. Technol. 48, 753–760.

Solecki, R., Kortenkamp, A., Bergman, Å., Chahoud, I., Degen, G.H., Dietrich, D., Greim, H., Håkansson, H., Hass, U., Husoy, T., Jacobs, M., Jobling, S., Mantovani, A., Marx-Stoelting, P., Piersma, A., Ritz, V., Slama, R., Stahlmann, R., van den Berg, M., Zoeller, R.T., Boobis, A.R., 2017. Scientific principles for the identification of endocrine-disrupting chemicals: a consensus statement. Arch. Toxicol. 91, 1001–1006.

Tang, M., Chen, K., Yang, F., Liu, W., 2014. Exposure to organochlorine pollutants and type 2 diabetes: a systematic review and meta-analysis. PLoS One 9 (10), e85556.

Taylor, K.W., Novak, R.F., Anderson, H.A., Birnbaum, L.S., Blystone, C., Devito, M., Jacobs, D., Köhrle, J., Lee, D.H., Rylander, L., Rignell-Hydbom, A., Tornero-Velez, R., Turyk, M.E., Boyles, A.L., Thayer, K.A., Lind, L., 2013. Evaluation of the association between persistent organic pollutants (POPs) and diabetes in epidemiological studies: a national toxicology program workshop review. Environ. Health Perspect. 121, 774–783.

Turrio-Baldassarri, L., Abate, V., Alivernini, S., Battistelli, C.L., Carasi, S., Casella, M., Iacovella, N., et al., 2007. A study on PCB, PCDD/PCDF industrial contamination in a 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., Iacovella, N., Indelicato, A., et al., 2008. PCDD/F and PCB in human serum of differently exposed population groups of an Italian city. Chemosphere 73, S228–S234.

Turrio-Baldassarri, L., Alivernini, S., Carasi, S., Casella, M., Fuselli, S., Iacovella, N., et al., 2009. 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 76, 278–285.

US EPA (US Environmental Protection Agency), 1979. Bans PCB Manufacture; Phases Out Uses. Environmental Protection Agency, Washington, DC.

Van Larebeke, N., Sioen, I., Hond, E.D., Nelen, V., Van de Mieroop, E., Nawrot, T., Bruckers, L., Schoeters, G., Baeyens, W., 2015. Internal exposure to organochlorine pollutants and cadmium and self-reported health status: a prospective study. Int. J. Hyg Environ. Health 218, 232–245.

Wang, Q., Yuan, H., Jin, J., Li, P., Ma, Y., Wang, Y., 2018 May. Polychlorinated biphenyl concentrations in pooled serum from people in different age groups from five Chinese cities. Chemosphere 198, 320–326. Epub 2018 Feb 6. PubMed PMID: 29421746.

Warner, M., Mocarelli, P., Brambilla, P., Wesselink, A., Samuels, S., Signorini, S., Eskenazi, B., 2013. Diabetes, metabolic syndrome, and obesity in relation to serum dioxin concentrations: the Seveso women's health study. Environ. Health Perspect. 121, 906–911.

WHO, 2000. Obesity: preventing and managing the global epidemic. Report of a WHO Consultation. WHO Technical Report Series 894. World Health Organization, Geneva.

Zani, C., Donato, F., Magoni, M., Feretti, D., Covolo, L., Vassallo, F., Speziani, F., Scarcella, C., Bergonzi, R., Apostoli, P., 2013. Polychlorinated biphenyls, glycaemia and diabetes in a population living in a highly polychlorinated biphenyls-polluted area in northern Italy: a cross-sectional and cohort study. J Public Health Res 2, 2–8.

http://www.cuore.iss.en.