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Plasma levels of polychlorinated biphenyls (PCB) and the risk of soft tissue sarcoma

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Parole chiave: Sarcoma dei tessuti molli; policlorobifenili; epidemiologia; fattori di rischio ambientali; contaminanti chimici; studio caso-controllo

Summary

Background: *Soft tissue sarcoma (STS) is a heterogeneous group of rare neoplasms whose aetiology is largely unknown. Dioxin and dioxin-like compounds, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and polychlorinated biphenyls (PCBs), are potential risk factors for STS.* **Objectives:** *To investigate the relation of 17 PCBs congeners, assessed in human plasma, with STS risk.* **Methods:** *We conducted a case-control study in Italy, including 52 STS cases and 99 hospital-based controls. Selected PCB were extracted by high-performance liquid chromatography (HPLC) and measured with gas chromatography-mass spectrometry (GC-MS). Odds ratios (OR), and the corresponding 95% confidence intervals (CI), were estimated through multivariate logistic regression models.* **Results:** *The most frequently detected PCB congeners were 138, 170, 180 and 149 (detected in 40-77% of controls). The OR for the sum of all 17 PCB congeners was 1.20 (95% CI 0.50-2.92). In categorical analysis no consistent association was found for individual congeners and for groups based on Wolff's classification or the degree of chlorination. For continuous estimates, borderline positive associations emerged for Wolff's groups 2A (OR 1.23, 95% CI 0.97-1.55), 2B (OR 1.34, 95% CI 1.00-1.77, and 3 (OR 1.19, 95% CI 0.96-1.49), for moderately (OR 1.20, 95% CI 0.96-1.51) and highly (OR 1.18, 95% CI 0.99-1.41) chlorinated PCBs, and for congeners 170 (OR 1.26, 95% CI 0.98-1.63), 180 (OR 1.26, 95% CI 0.97-1.64) and 138 (OR 1.45, 95% CI 1.02-2.04).* **Discussion:** *Most associations between PCBs and STS risk were not significant, but, given the limited sample size, we cannot exclude moderate associations.*

Riassunto

«Livelli plasmatici di policlorobifenili (PCB) e rischio di sarcoma dei tessuti molli». **Background:** *Il sarcoma dei tessuti molli (STS) è un eterogeneo gruppo di rare neoplasie la cui eziologia è in gran parte sconosciuta. Diossine e*

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composti diossina-simili, tra cui 2,3,7,8-tetraclorodibenzo-p-diossina (2,3,7,8-TCDD) e policlorobifenili (PCB), sono considerati potenziali fattori di rischio per il STS. **Obiettivi:** I*ndagare la relazione tra 17 congeneri di PCB, misurati nel plasma umano, e il rischio di STS.* **Metodi:** *Abbiamo condotto uno studio caso-controllo in Italia, con 52 casi di STS e 99 controlli ospedalieri. I PCB selezionati sono stati estratti mediante cromatografia liquida ad alta prestazione (HPLC) e misurati con gascromatografia con spettrometria di massa (GC-MS). Gli odds ratio (OR) e i corrispondenti intervalli di confidenza al 95% (CI) sono stati calcolati attraverso modelli di regressione logistica multivariata.* **Risultati:** *I congeneri di PCB più frequentemente riscontrati sono stati 138, 170, 180 e 149 (range 40-77% dei controlli). L'OR per la somma dei 17 congeneri di PCB era 1,20 (IC 95% 0,50-2,92). Nell'analisi categorica non è stata trovata alcuna associazione coerente per i singoli congeneri e per i gruppi basati sulla classificazione di Wolff o sul grado di clorazione. Per le stime continue vi era un'associazione positiva per i gruppi 2A, 2B e 3 (OR 1,23, IC 95% 0,97-1,55, OR 1,34, IC 95% 1,00-1,77 e OR 1,19, IC 95% 0,96-1,49 rispettivamente), per PCB moderatamente e altamente clorurati (OR 1,20, IC 95% 0,96-1,51 e OR 1,18, IC 95% 0,99-1,41 rispettivamente) e per i congeneri 170 (OR 1,26, IC 95% 0,98-1,63), 180 (OR 1,26 , IC 95% 0,97-1,64) e 138 (OR 1,45, IC 95% 1,02-2,04).* **Discussione:** *La maggior parte delle associazioni tra PCB e rischio di STS non erano statisticamente significative, ma la limitata dimensione del campione non permette di escludere associazioni moderate.*

Introduction

Soft tissue sarcoma (STS) is a rare and heterogeneous group of neoplasms whose aetiology is largely unknown (24). Selected jobs and industry titles, and exposure to chemicals, such as herbicides and chlorophenols, have been suggested as risk factors for STS (4, 12). Dioxin and dioxin-like compounds, including 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) and polychlorinated biphenyls (PCBs), are possible risk factor for STS (11). Although manufacturing and use of PCBs have been banned or strongly limited in most countries since the 1970s, these compounds, due to their biochemical characteristics (strongly lipophilic and resistant to biotransformation), persist in the environment and are still commonly detected in air, water, soil, food, wildlife and in human adipose tissue, milk and blood (9).

Epidemiological studies considering the relation between subjects occupationally or accidentally exposed to PCBs and STS risk reported inconsistent results (11, 15, 19). Most studies, however, included a small number of exposed cases, and PCB exposure assessment, based on different approaches including job-exposure matrices (JEM), historical measurements, industry classifications, and contaminated food consumption, was subject to misclassification.

The International Agency for Research on Cancer (IARC) classified PCBs as carcinogenic to humans (group 1), based on sufficient evidence in humans of an association with melanoma, and limited evidence for non-Hodgkin lymphoma and breast cancer, while considered data for other neoplasms, including STS too sparse to draw conclusions (10).

To contribute to the understanding of the association between PCB exposure and STS risk, we measured plasma concentrations of 17 PCB congeners, grouped according to their chemical characteristics and biological mechanism of action, in a case-control study conducted in Piedmont, Northern Italy.

Methods

From July 2010 to March 2013, 64 patients with newly incident histologically confirmed diagnosis of STS, and 114 control patients, were enrolled from the Orthopedic Trauma Center Hospital in Turin.

All newly diagnosed cases of primary and histologically confirmed malignant STS attending the Orthopaedic Trauma Centre Hospital in Turin were enrolled consecutively from from July 2010 and March 2013. Patients were at least 18 years old at diagnosis. Patients with previous malignancies and/or distant metastasis were excluded. Controls subjects were randomly selected from patients admitted to the same Orthopaedic Trauma Centre Hospital with no previous malignancies for an acute or surgical disease that did not appreciably change their lifestyle, and did not represent complication of chronic

diseases: of these 52.5% were non-alcohol-related traumas (such as fractures and sprains), 32.3% were non-traumatic orthopaedic disorders, and 15.2% were acute non-surgical conditions (15.2%).

Up to two controls were individually matched to cases by age and sex. All participants were resident in Italy for at least 15 years, had no previous malignancy and provided informed consent. The proportion of refusals among eligible cases and controls was less than 5%. During hospitalization, trained interviewers administered a structured, validated questionnaire (6) to cases and controls between July 2010 and March 2013, including information on socioeconomic factors, residential history, anthropometric variables, selected lifestyle factors (e.g. tobacco and alcohol consumption, dietary habits), personal medical history of selected disorders, family history of neoplasms, lifetime occupational history (with particular reference to any exposure to pesticides, PCBs or dioxins) and, for women, hormonal and reproductive factors.

Subjects were classified as occupational exposed or not exposed to PCB, according to the WHO PCBs uses classification (25). Occupationally exposed subjects were defined as those exposed to completely closed systems (electrical transformers, electrical capacitors, electrical switches, relays and other electrical cables, electric motors and magnets), nominally closed systems (hydraulic systems, heat transfer systems), and open-ended systems (plasticizers in sealants and caulking material, ingredient in paint and other coatings, ingredient in ink and carbonless copy paper, ingredient in adhesives, pesticide extender, plasticizer in polyvinyl chloride, neoprene and other artificial rubbers, fire retardant in fabrics, carpets, polyurethane foam etc, lubricants) (25).

Participants agreed to donate 7 ml of blood at the time of the interview. Blood samples were collected from patients in the morning in heparinized Vacutainer® tubes before chemotherapy.

Blood samples were not available for 9 cases and 9 controls, and the questionnaire was incomplete for 3 cases and 6 controls, resulting in a total of 52 cases (32 men and 20 women) and 99 controls (63 men and 36 women) with complete questionnaire and biomarker data, who were included in the analysis. Study protocol was approved by the ethical committee (A.O.U. San Giovanni Battista di Torino-A.O "CTO Maria Adelaide di Torino", nr. 448/DG/2010/DS -17/11/2010).

Plasma was obtained by centrifugation at 3600 RPM for 6 minutes, and two ml of plasma for each patient were transferred into Eppendorf vials and frozen at −80°C until PCB analysis. A total of 17 PCB congeners (18, 28, 31, 52, 44, 101, 77, 118, 138, 149, 153, 126, 180, 169, 170, 194, 209) were measured. Plasma samples were denaturated with 1 ml of methanol (HPLC grade). Solid-phase extraction (SPE) columns (C18 endcapped) were prepared with methanol and demineralized water. Plasma samples were applied and washed with the same solvents and the SPE columns dried for 1 hour by aspiration in ambient air. The analytes were eluted from the dry SPE column with iso-octane. Clean-up was performed by column chromatography using silica gel. The analytes were eluted with toluene. The cleaned extracts were reduced to a final volume of 300 µL and 1 µL was analyzed by high resolution gas chromatography with ion trap mass spectrometry (GC-MS/MS). We used bovine serum as 'blank' in the spiked calibration curves, used as standard. Recoveries ranged from 72% to 78%. We used Thermo Fisher Polaris Q equipped with a PTV injector, ion trap detector in MS/MS mode and operated by Excalibur software. Chromatographic separation was accomplished with a fused silical capillary Restek RTX-5 column (30 m X 0.25 mm, i.d. 0.25 microm film til kren). The temperature program used was 50°C initial for 2 minutes, increased to 170°C at 10°C/min and then increased to 315°C at 2.5°C/min and finally held at 315°C for 15 minutes. The limit of quantification (LOQ) was determined as the smallest amount of the analyte that gave a signal to noise ratio ≥5 and was set at 0.1 µg/L. Concentrations below the LOQ were assigned a value of LOQ divided by two.

In the univariate analysis, differences between cases and controls were assessed by the χ^2 or Fisher's exact test, as appropriate, for categorical variables and the Wilcoxon's rank-sum test for continuous variables. We calculated mean (standard deviation, SD) and median values either considering all subjects and assigning a value equal to LOQ/2 to

subjects with PCB level below LOQ, or including only subjects with detectable PCB values. PCB congeners were grouped according to 2 classifications, based on their structure-activity relationships (26) and on the degree of chlorination (14). Low chlorinated included congeners 18, 28, 31, 44, 52 and 77; moderate chlorinated included congeners 101, 118, 126, 138, 149, 153, 169, 170 and 180; and high chlorinated included the remaining PCB congeners 194 and 209. Based on Wolff's classification (26), five PCB groups were formed: Group 1A (potentially estrogenic) including congeners 31, 44 and 52; Group 1B (weak phenobarbital inducers, persistent) included 101; Group 2A (potentially antiestrogenic and immune-toxic, dioxin-like, non-ortho and mono-ortho substituted, and moderately persistent) included congeners 77, 118, 126 and 169; Group 2B (di-ortho substituted, limited dioxin-like, and persistent) including congeners 138 and 170; Group 3 (biologically persistent CYP1A and CYP2B inducers) included congeners 153 and 180.

For the sum of the 17 PCBs, each PCB group and selected individual PCBs, subjects were categorized according to tertiles of concentration among controls or detected vs not detected. We estimated the odds ratios (ORs) of STS, and the corresponding 95% confidence intervals (CIs) for each category using unconditional logistic regression (3). We estimated ORs of STS according to PCB groupings, to the sum of the 17 PCBs and to selected individual PCBs, i.e., those detected in at least 33% of controls and/or those reporting a significant difference in mean concentration between cases and controls.

The ORs for a continuous increase equal to 1 SD were also computed, including detected subjects only. Two logistic regression models were defined, including *a priori* decided covariates: the first one included adjustment terms for sex and age (as continuous term); the second one included adjustment terms for sex, age, smoking habit (never/former/current smokers), alcohol consumption (non-/current drinkers) and body mass index (tertiles). All tests were two tailed, and *p*-values of 0.05 or less were considered statistically significant. Sensitivity analyses were also performed for continuous ORs. All the analyses were conducted using SAS version 9.4 (Cary, NC, USA).

Results

Table 1 shows the distribution of cases and controls according to selected demographic, lifestyle and other characteristics. Cases were older than controls (60.2 vs 54.3 years, respectively, p=0.04) and were more frequently classified as occupationally exposed to PCBs (13.5% vs. 6.1%, respectively, p=0.14). Six cases worked as farmers and one in electrical maintenance, while among controls, three worked as farmers, one each as cattle breeder, electric maintenance worker, and TV repair technician.

The OR for occupational exposure to PCBs was 2.06 (95% CI, 0.61-7.02), using the multivariate model (i.e., adjusting for sex, age, smoking habit, alcohol consumption and body mass index). The proportion of subjects who had lived for at least 10 years in Piedmont was 96.1% among cases and 86.9% in controls (p=0.07). Urban residence at age 15-25 years and at the time of enrolment in the study was reported by about two-thirds of both cases and controls. Similarly, no significant difference in the frequency distribution of body mass index emerged between cases and controls.

Table 2 presents the proportion of cases and controls with detectable level of each PCB group and congeners, and the corresponding mean (SD) and median levels. The most frequently detected PCB congeners were 138, 170, 180 and 149 (positivity among control in the 40-77% range), while for other PCBs the proportion of detection ranged from 3.9% to 25%. Wolff's Groups 1A, 2B and the less chlorinated PCBs, as well as PCB congeners 18, 52, 118, 138 and 149 were detected more frequently in controls than cases. The mean concentrations based only on subjects with detected measures were significantly higher in cases than in controls for PCB 138, PCB 209, for groups 2A and 3, and for the highly chlorinated PCBs. The higher concentration found for PCB groupings 3 and highly chlorinated, and for PCB 209 in cases with detectable values compared to controls was related to different age. In fact, the association was no longer significant after adjusting for age.

The sum of all PCBs was 2.23 μg/L in cases vs. 1.70 μg/L in controls, p=0.13.

Table 3 shows the association of PCB groupings or selected PCB congeners with STS risk. Based

Table 1. Frequency distribution of 52 STS cases and 99 controls according to selected characteristics

	Cases, n (%)	Controls, n (%)	p-value
Sex			
Male	32 (61.5)	63 (63.6)	
Female	20 (38.5)	36 (36.4)	0.80
Age			
<40	5 (9.6)	20 (20.2)	
40-49	7 (13.5)	21 (21.2)	
50-59	13 (25.0)	15 (15.2)	
60-69	12 (23.1)	21 (21.2)	
≥70	15 (28.8)	22 (22.2)	0.21
Mean ± SD	60.2±14.9	54.3±15.8	**0.04**
Smoking habit			
Never smoker	24 (46.1)	41 (41.4)	
Former smoker	19 (36.5)	27 (27.3)	
Current smoker	9 (17.3)	31 (31.3)	0.16
Alcohol drinking			
Non-drinker	16 (30.8)	17 (17.2)	
Current drinker	36 (69.2)	82 (82.8)	0.06
Body mass index (kg/m^2) [a]			
1st tertile (<23.9)	13 (25.5)	33 (33.3)	
2nd tertile (23.9-<27.75)	20 (39.2)	33 (33.3)	
3rd tertile (≥27.75)	18 (35.3)	33 (33.3)	0.59
Residence at age 15-25 years			
Urban	32 (61.5)	68 (68.7)	
Rural	20 (38.5)	31 (31.3)	0.38
Current residence			
Urban	35 (67.3)	67 (67.7)	
Rural	17 (32.7)	32 (32.3)	0.96
Has lived for ≥10 years in Piedmont? [a]			
Yes	49 (96.1)	86 (86.9)	
No	2 (3.9)	13 (13.1)	0.07
Occupational exposure to PCBs			
Yes	7 (13.5)	6 (6.1)	
No	45 (86.5)	93 (93.9)	0.14
Body mass index (kg/m^2) [a]			
1st tertile (<23.9)	13 (25.5)	33 (33.3)	
2nd tertile (23.9-<27.75)	20 (39.2)	33 (33.3)	
3rd tertile (≥27.75)	18 (35.3)	33 (33.3)	0.59

[a] The sums may not add up to the total because of missing values.
Statistically significant results (i.e., p<0.05) are reported in bold.

on Wolff's classification, the multivariate ORs for detected vs. not detected PCB concentrations were 0.37 (95% CI, 0.13-1.02) for group 1A and 0.70 (95% CI, 0.31-1.61) for group 1B, and the corresponding ORs for an increment of 1 SD, computed in subjects with detectable level only, was 0.43 (95% CI 0.18-1.04). The ORs for the highest vs the lowest tertile of plasma concentrations were 1.17 (95% CI, 0.54-2.53) for group 2A, 0.65 (95% CI 0.25-1.65) for group 2B and 0.76 (95% CI 0.27-2.13) for group 3. The corresponding ORs for continuous estimates were 1.23 (95% CI 0.97-1.55, 1.33 (95% CI 1.00-1.77) and 1.19 (95% CI 0.96-1.49). Considering the classification based on the degree of chlo-

Table 2. Plasma concentrations of PCB congeners in 52 STS cases and 99 controls

	Cases					Controls				
		All cases [b]		Detected cases only [c]			All controls [b]		Detected controls only [c]	
	% [a] detected	Mean±SD	Median	Mean±SD	Median	% detected [a]	Mean±SD	Median	Mean±SD	Median
Wolff's classification										
Group 1										
Group 1°										
PCB 31	5.8	0.05±0.02	0.05	0.11±0.02	0.10	8.1	0.05±0.02	0.05	0.11±0.02	0.10
PCB 44	3.9	0.05±0.02	0.05	0.14±0.06	0.14	8.1	0.05±0.02	0.05	0.11±0.03	0.10
PCB 52	3.9 **	0.05±0.01 **	0.05	0.11±0.02	0.11	20.2	0.06±0.02	0.05	0.10±0.01	0.10
∑PCB 1A (31,44,52)	11.5 *	0.16±0.03	0.15	0.23±0.04	0.22	25.2	0.17±0.04	0.15	0.23±0.04	0.20
Group 1B										
PCB 101	21.1	0.07±0.04	0.05	0.14±0.05	0.11	28.3	0.08±0.08	0.05	0.16±0.12	0.10
∑PCB Group 1 (31,44,52,101)	23.1	0.23±0.06	0.20	0.32±0.08	0.30	38.4	0.25±0.11	0.20	0.33±0.14	0.28
Group 2										
Group 2A										
PCB 77	23.1	0.09±0.10	0.05	0.24±0.14	0.19	14.1	0.09±0.13	0.05	0.32±0.25	0.20
PCB 118	17.3 *	0.08±0.09	0.05	0.21±0.16	0.15	33.3	0.09±0.09	0.05	0.16±0.12	0.10
PCB 126	1.9	0.05±0.01	0.05	0.10±NC [d]	0.10	3.0	0.05±0.01	0.05	0.10±0.01	0.10
PCB 169	13.5	0.12±0.23	0.05	0.55±0.46	0.69	5.1	0.05±0.01	0.05	0.11±0.02	0.10
∑PCB 2A (77,118,126,169)	44.2	0.34±0.25	0.20	0.52±0.30 *	0.41	45.4	0.28±0.15	0.20	0.37±0.18	0.29
Group 2B										
PCB 138	73.1 *	0.44±0.62	0.24	0.58±0.67 *	0.35	87.9	0.30±0.28	0.22	0.34±0.28	0.26
PCB 170	40.4	0.13±0.19	0.05	0.24±0.26	0.11	40.4	0.08±0.05	0.05	0.13±0.06	0.10
∑PCB 2B (138,170)	76.9 *	0.57±0.78	0.31	0.71±0.84	0.41	89.9	0.39±0.31	0.29	0.42±0.31	0.32
∑PCB Group 2 (77,118, 126,138, 169,170)	90.4	0.91±0.83	0.62	0.97±0.85	0.63	90.9	0.67±0.39	0.57	0.70±0.39	0.61
Group 3										
PCB 153	19.2	0.07±0.06	0.05	0.15±0.11	0.10	20.2	0.08±0.10	0.05	0.18±0.19	0.11
PCB 180	73.1	0.33±0.50	0.17	0.44±0.55	0.21	69.7	0.20±0.21	0.13	0.26±0.22	0.19
∑PCB Group 3 (153, 180)	76.9	0.40±0.51	0.24	0.50±0.55 *	0.27	77.8	0.28±0.24	0.19	0.33±0.25	0.23
Other PCB congeners										
PCB 18	7.7 **	0.07±0.07 **	0.05	0.28±0.16	0.27	27.3	0.10±0.15	0.05	0.24±0.23	0.13
PCB 28	9.6	0.06±0.03	0.05	0.13±0.05	0.10	18.2	0.06±0.03	0.05	0.12±0.04	0.10
PCB 149	61.5 *	0.17±0.19	0.10	0.25±0.21	0.15	80.8	0.17±0.15	0.12	0.20±0.16	0.14
PCB 194	25.0	0.14±0.23	0.05	0.39±0.37	0.20	22.2	0.09±0.11	0.05	0.22±0.17	0.14
PCB 209	7.7	0.16±0.47	0.05	1.42±1.24 *	1.20	6.1	0.06±0.06	0.05	0.21±0.20	0.11
Classification according to degree of chlorination										
Low[e]	30.8 *	0.38±0.16	0.30	0.56±0.18	0.52	48.5	0.42±0.22	0.30	0.56±0.27	0.44
Moderate[f]	90.4	1.46±1.47	0.94	1.56±1.51	0.99	96.0	1.10±0.73	0.93	1.13±0.73	0.95
High[g]	28.8	0.29±0.64	0.10	0.76±1.09 *	0.32	25.2	0.15±0.12	0.10	0.29±0.18	0.20
Sum of all 17 congeners	92.3	2.12±1.67	1.44	2.23±1.70	1.63	97.0	1.68±0.86	1.45	1.70±0.86	1.46

[a] Detected, for values at least 0.1 μg/L. Where the limit of quantification (LOQ, i.e., 0.1 μg/L) was not reached, we assigned values equal to LOQ/2 (i.e., 0.05 μg/L); [b] Mean and median values were calculated by including all subjects; [c] Mean and median values were calculated by including detected subjects only; [d] Not computable since this congener was detected in 1 case only; [e] Sum of congeners: 18, 28, 31, 44, 52, 77; [f] Sum of congeners: 101, 118, 126, 169, 138, 153, 180, 149, 170; [g] Sum of congeners: 194, 209.

* p-value <0.05, as compared to the corresponding control group; ** p-value <0.01, as compared to the corresponding control group

Table 3. Odds ratios (OR) and 95% confidence intervals (CI) of STS according to plasma concentration levels (approximate tertiles) of selected PCB congeners/classifications[a]

	Cases n (%)	Controls n (%)	OR (95% CI) [b]	OR (95% CI) [c]
Wolff's classification				
Group 1A				
Not detected	46 (88.5)	74 (74.8)	1 (reference)	1 (reference)
Detected	6 (11.5)	25 (25.2)	0.38 (0.14-1.03)	0.37 (0.13-1.02)
Group 1B				
Not detected	41 (78.9)	71 (71.7)	1 (reference)	1 (reference)
Detected	11 (21.1)	28 (28.3)	0.70 (0.31-1.57)	0.70 (0.31-1.61)
Continuous OR[d]			0.64 (0.31-1.30)	0.43 (0.18-1.04)
Group 2A				
0.20 µg/L	29 (55.8)	54 (54.5)	1 (reference)	1 (reference)
>0.20-≤0.25 µg/L	4 (7.7)	16 (16.2)	0.45 (0.13-1.47)	0.47 (0.14-1.62)
>0.25 µg/L	19 (36.5)	29 (29.3)	1.15 (0.54-2.43)	1.17 (0.54-2.53)
Continuous OR[d]			1.26 (1.02-1.56)	1.23 (0.97-1.55)
Group 2B				
≤0.225 µg/L	20 (38.5)	32 (32.3)	1 (reference)	1 (reference)
0.226-≤0.42 µg/L	15 (28.8)	35 (35.4)	0.63 (0.27-1.48)	0.59 (0.24-1.44)
>0.42 µg/L	17 (32.7)	32 (32.3)	0.59 (0.24-1.46)	0.65 (0.25-1.65)
Continuous OR[d]			1.27 (0.98-1.65)	1.33 (1.00-1.77)
Group 3				
≤0.149 µg/L	12 (23.1)	22 (22.2)	1 (reference)	1 (reference)
0.15-≤0.27 µg/L	20 (38.5)	46 (46.5)	0.69 (0.28-1.70)	0.64 (0.25-1.63)
>0.27 µg/L	20 (38.5)	31 (31.3)	0.80 (0.30-2.14)	0.76 (0.27-2.13)
Continuous OR[d]			1.19 (0.95-1.47)	1.19 (0.96-1.49)
Degree of chlorination				
Low				
0.30 µg/L	36 (69.2)	51 (51.5)	1 (reference)	1 (reference)
>0.30-≤0.45 µg/L	6 (11.5)	25 (25.3)	0.33 (0.12-0.89)	0.32 (0.11-0.90)
>0.45 µg/L	10 (19.2)	23 (23.2)	0.66 (0.27-1.58)	0.64 (0.26-1.59)
Continuous OR [d]			0.99 (0.72-1.37)	0.93 (0.65-1.34)
Moderate				
≤0.735 µg/L	13 (25.0)	32 (32.3)	1 (reference)	1 (reference)
0.736-≤1.13 µg/L	20 (38.5)	36 (36.4)	1.36 (0.57-3.25)	1.42 (0.58-3.51)
>1.13 µg/L	19 (36.5)	31 (31.3)	1.12 (0.44-2.81)	1.17 (0.45-3.08)
Continuous OR[d]			1.18 (0.95-1.47)	1.20 (0.96-1.51)
High				
Not detected	37 (71.2)	74 (74.8)	1 (reference)	1 (reference)
Detected				
Continuous OR[d]	15 (28.8)	25 (25.2)	1.09 (0.51-2.35)	1.17 (0.53-2.60)
			1.15 (0.99-1.34)	1.18 (0.99-1.41)
Sum of all examined PCBs				
<1.25 µg/L	15 (28.8)	31 (31.3)	1 (reference)	1 (reference)
1.25-<1.71 µg/L	15 (28.8)	35 (35.4)	0.80 (0.33-1.95)	0.82 (0.33-2.07)
≥1.71 µg/L	22 (42.3)	33 (33.3)	1.13 (0.48-2.66)	1.20 (0.50-2.92)
Continuous OR[d]			1.27 (0.98-1.64)	1.26 (0.97-1.65)

(continued)

Table 3 *(continued)***.** Odds ratios (OR) and 95% confidence intervals (CI) of STS according to plasma concentration levels (approximate tertiles) of selected PCB congeners/classifications[a]

	Cases n (%)	Controls n (%)	OR (95% CI) [b]	OR (95% CI) [c]
For single congener				
PCB 18				
Not detected	48 (92.3)	72 (72.7)	1 (reference)	1 (reference)
Detected	4 (7.7)	27 (27.3)	0.23 (0.07-0.70)	0.21 (0.07-0.67)
PCB 52				
Not detected	50 (96.1)	79 (79.8)	1 (reference)	1 (reference)
Detected	2 (3.9)	20 (20.2)	0.15 (0.03-0.70)	0.15 (0.03-0.69)
PCB 118				
Not detected	43 (82.7)	66 (66.7)	1 (reference)	1 (reference)
Detected	9 (17.3)	33 (33.3)	0.39 (0.17-0.91)	0.32 (0.13-0.82)
Continuous OR (b) [d]			1.18 (0.87-1.60)	1.03 (0.67-1.59)
PCB 138				
≤0.14 µg/L	18 (34.6)	33 (33.3)	1 (reference)	1 (reference)
0.14-≤0.34 µg/L	14 (26.9)	34 (34.3)	0.76 (0.31-1.84)	0.62 (0.24-1.57)
>0.34 µg/L	20 (38.5)	32 (32.3)	0.92 (0.39-2.16)	1.00 (0.41-2.42)
Continuous OR[d]			1.38 (1.00-1.89)	1.45 (1.02-2.04)
PCB 149				
≤0.10 µg/L	30 (57.7)	42 (42.4)	1 (reference)	1 (reference)
0.10-≤0.16 µg/L	7 (13.5)	25 (25.3)	0.35 (0.13-0.94)	0.34 (0.12-0.93)
>0.16 µg/L	15 (28.8)	32 (32.3)	0.53 (0.23-1.19)	0.59 (0.25-1.38)
Continuous OR (b) [d]			1.08 (0.84-1.38)	1.10 (0.85-1.42)
PCB 180				
≤0.10 µg/L	20 (38.5)	42 (42.4)	1 (reference)	1 (reference)
0.10-≤0.20 µg/L	13 (25.0)	26 (26.3)	0.91 (0.38-2.21)	0.88 (0.35-2.16)
>0.20 µg/L	19 (36.5)	31 (31.3)	0.88 (0.37-2.08)	0.86 (0.34-2.14)
Continuous OR[d]			1.25 (0.97-1.62)	1.26 (0.97-1.64)
PCB 170				
0.05 µg/L	31 (59.6)	59 (59.6)	1 (reference)	1 (reference)
>0.05-≤0.10 µg/L	10 (19.2)	23 (23.2)	0.76 (0.32-1.83)	0.66 (0.26-1.67)
>0.10 µg/L				
Continuous OR[d]	11 (21.2)	17 (17.2)	0.90 (0.35-2.30)	0.91 (0.35-2.38)
			1.22 (0.95-1.56)	1.26 (0.98-1.63)

[a] Congeners/classifications considered in approximate tertiles were those detected in at least 33% of controls, otherwise they were divided as "detected" vs. "non-detected".

[b] OR adjusted for age and sex.

[c] OR adjusted for age, sex, smoking habit, alcohol consumption and body mass index.

[d] For an increase equal to an inter-quartile range change (among controls). OR was computed including detected subjects only.

rination the ORs for the highest vs the lowest tertile of PCB exposure were 0.64 (95% CI 0.26-1.59) and 1.17 (95% CI 0.45-3.08) for low and moderate chlorinated PCBs respectively. For high degree of chlorination, the OR was 1.17 (95% CI 0.53-2.60) for detection vs non-detection. For the sum of all 17 PCB congeners the OR for the highest vs the lowest tertile was 1.20 (95% CI 0.50-2.92). The corresponding OR for 1 SD increment was 1.26 (95% CI 0.97-1.65). Specific congeners were considered when they were detected in at least 33% of controls and/or when a significant difference in mean concentrations emerged between cases and controls. Thus, the ORs were 0.21 (95% CI 0.07-0.67) for

PCB 18, 0.15 (95% CI 0.03-0.69) for PCB 52, 0.32 (95% CI 0.13-0.82) for PCB 118; 1.00 (95% CI 0.41-2.42) for PCB 138; 0.59 (95% CI 0.25-1.38) for PCB 149; 0.86 (95% CI 0.34-2.14) for PCB 180 and 0.91 (95% CI 0.35-2.38) for PCB 170 for the highest vs the lowest exposure tertile. When sensitivity analyses were performed for continuous estimates excluding outliers, the corresponding ORs became closer to unity (data not shown).

Discussion

The analysis comparing subjects with detected vs. non detected levels revealed a decreased risk of STS for PCB Wolff's groups 1A and 1B, and for congeners 18 and 52. The categorical analysis of total PCBs, PCB groups, and individual congeners detected in at least one third of the controls (PCB 138, 149, 170 and 180) produced null results. In the analysis restricted to subjects with detectable levels, a borderline positive association was shown for PCB Wolff's groups 2A, 2B, and 3, for moderately and highly chlorinated PCBs, and for congeners 170, 180 and 138. We performed a large number of non-independent analyses, and an adjustment for multiple comparisons is not straightforward; however, caution should be applied in the interpretation of our results, and more emphasis should be given to internal consistency of results rather than significance (5).

In this respect, the apparent discrepancy in results for the same congener or group between different analytical approaches (e.g., categorical analysis of tertiles vs. continuous analysis restricted to detected levels), and the observation of some positive or negative association on few subjects with outlying results detract from a causal interpretation of the results.

The relation between PCBs and STS has been evaluated, in a few epidemiological studies, conducted mostly based on occupationally exposed subjects (11, 15, 19) with no measures of PCB levels in biological materials. In these studies, however, PCB exposure assessment (residential history, job exposure matrix, contaminated food intake) was approximate, the number of events was small, and the results were inconsistent. In a cohort study of 7061 PCB capacitor workers, conducted in the USA, 6 STS deaths were observed with a standardized mortality ratio (SMR) of 1.28, 95% CI 0.47-2.79 (11). Another American cohort study, including 24865 workers exposed to PCBs in three electrical capacitor manufacturing plants, did not find increased mortality for STS (SMR 0.94, 95%CI 0.51-1.57) based on 7 STS deaths (19). As the intake of fatty fish from the Baltic Sea is considered an important source of PCBs and dioxins exposure, cohort studies investigated cancer incidence in fishermen and their wives residing in Swedish coastal areas. The low incidence of STS in this group may be due to a healthier lifestyle among fishermen (i.e. a high consumption of fish and a high level of physical activity), which may have a favourable effect on cancer, including STS (13).

To our knowledge, our study is the first evaluation of the relationship between PCB levels and STS measuring exposure in a biological material. In this population, the most frequent PCB congeners were 138, 170, 180 and 149. Congeners 153, 138 and 180 are the most frequently detected in humans, and they are often used as indicators of overall exposure to PCBs (10). However, PCB 153 was rarely detected in our population. In general, in this population, PCB levels were low compared to those reported in other studies. A decline in PCB concentrations over time was observed in human blood and adipose samples in many countries during the last decade (2,8). A decrease of 3.8% per year in serum concentrations of PCBs (congeners 138, 153, 170, 180, 194 and 209) was observed in a cohort, with a follow-up of 12 years, living close to a chemical factory, located in an industrialized town in North Italy producing PCBs from the 1930s to the 1980s (18).

A limitation of our study is the small sample size due to the rarity of STS, leading to wide confidence intervals around risk estimates and limiting our statistical power. Furthermore, our study may be affected by limitations inherent to the case-control design, such as selection bias. However, this is unlikely to be relevant for cases, who were incident consecutive cases. Moreover, controls were admitted for a spectrum of acute diseases likely unrelated to PCB exposure. In addition, participation was almost complete, and the recruitment area of cases

and controls was similar. The mean concentrations of PCB 138, PCB 209, groups 2A and 3, and the highly chlorinated PCBs were higher in cases than in controls. Cases were slightly older than controls, and age is positively associated with most Persistent Organic Pollutants (POPs) in human adipose tissue and blood (1, 17). However, we adjusted for age in all analyses. The percentage of cases occupationally exposed to PCBs was somewhat higher compared to controls. However, plasma concentrations of PCBs were similar in occupationally exposed and non-exposed individuals, both in cases and controls (data not shown).

Strengths of our study include measurements of PCB levels in a biological tissue. Although PCB congeners are lipophilic substances and tend mainly to accumulate into adipose tissue, a significant correlation was observed between concentrations determined in fat tissue and those measured in blood (16). In addition, STS diagnosis was histologically confirmed. Furthermore, blood specimens for cases were collected before treatment, since weight loss due to therapy may lead to increases in blood levels as the consequence of mobilization of bioaccumulated PCBs stored in fat tissue.

Although we collected blood sample after disease development, due to the long half-lives of PCBs in the blood and adipose tissue, blood concentrations reflect their levels in body and are commonly used as an effective way to determine chronic exposure to these compounds (23). However, any previous weight loss or gain may modify PCB blood concentration. We did not collect information about lifetime gain or loss of body weight, but we adjusted the risk estimates for body mass index, directly associated with STS risk in a previous Italian study (20, 21). In addition, we measured the most representative PCB congeners, which are considered indicators of occupational and environmental exposure. The 17 PCB congeners we measured included the six congeners (28, 52, 101, 138, 153, and 180) recommended by the Stockholm Convention on POPs to characterize PCB contamination (22). We also grouped PCBs according to two classifications based on their degree of chlorination and structural-activity relationship. Although highly chlorinated PCBs have longer half-lives in humans, due to their slower metabolism compared to less chlorinated, evidence in experimental models has shown that less chlorinated PCB congeners might have initiating and promoting activities (10). However, no clear difference in STS risk emerged according to degree of chlorination. Considering Wolff's classification, the dioxin-like PCBs (group 2A) were not associated with STS risk, although some epidemiological studies reported a positive relation between dioxin exposure and STS (7, 27).

In conclusion, we found no consistent association between PCB blood levels and STS risk. However, given the limited sample size, we cannot exclude moderate associations.

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