DOI 10.1002/mnfr.201200196 *Mol. Nutr. Food Res.* 2012, *56*, 1748–1754

RESEARCH ARTICLE

Validation of questionnaire-based long-term dietary exposure to polychlorinated biphenyls using biomarkers

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Received: April 10, 2012
Revised: July 20, 2012
Accepted: August 1, 2012

Scope: The health consequences of lifelong low-level exposure to polychlorinated biphenyls (PCBs) via food are largely unknown, mainly due to the lack of large population-based prospective studies addressing this issue. We validated long-term food frequency questionnaire (FFQ)-based dietary PCB exposure against concentrations of six PCB congeners in serum.

Methods and results: Dietary PCB exposure was estimated in the Swedish Mammography Cohort by constructing a recipe-based database of CB-153, an indicator for total PCBs in food. The Spearman rank correlation (adjusted for within-person variability) was assessed between concurrent (2004–2006), past (1997), and long-term (mean of 1997 and 2004–2006) FFQ-based dietary PCB exposure, respectively, and the following serum PCB congeners, CB-118, CB-138, CB-153, CB-156, CB-170, and CB-180, in women (56–85 years of age, $n = 201$). The correlation between FFQ-based dietary PCB exposure and serum CB-153 was 0.41 ($p < 0.001$) for the concurrent (median 1.6 ng/kg body weight) and 0.34 ($p < 0.05$) for the past (median 2.6 ng/kg body weight) exposure assessment. Long-term validity of FFQ-based PCB estimates and the six serum PCB congeners ranged from 0.30 to 0.58 ($p < 0.05$).

Conclusion: FFQ-based PCB exposure estimates show acceptable validity in relation to PCB concentrations in serum, justifying their use in large-scale epidemiological studies.

Keywords:
Dietary exposure / Food frequency questionnaire / Polychlorinated biphenyls / Serum / Validation study

1 Introduction

Polychlorinated biphenyls (PCBs), a chemical mixture of 209 congeners, were manufactured in large quantities during the 20th century [1] for a wide variety of applications including electronic equipment, heat transfer and hydraulic fluids, sealants, and plastics [2]. Once released into the environment, PCBs can resist biodegradation, travel long distances, and accumulate and magnify up the food chain [3]. More than 95% of the total PCB exposure in the general population is through the diet, predominantly through consumption of fatty foods of animal origin [4, 5]. Despite the fact that the production and use of PCBs were restricted or banned in many countries in the 1970s, followed by a global act in 2001 [6], they are still prevalent in the environment and in food.

Exposure to PCBs has been associated with a wide range of health effects, including cardiovascular disease, diabetes, and cancer [7–9]. However, there is very limited knowledge about the possible public health consequences of lifelong low-level PCB exposure via food. Previous studies linking PCB exposure to human health effects are based on chemical analysis of PCBs in serum. Due to high analytical costs, sample sizes are restricted and can therefore lead to discrepancies in results because of low statistical power and chance [10]. To increase sample size in prospective cohort studies, the exposure could be assessed via food frequency questionnaires (FFQs) [11] if reasonable validity is obtained.

PCBs in serum are biomarkers of exposure that reflect differences in dietary intakes [12–15], allowing them to be used as a reference to validate FFQs. The aim of the present

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Abbreviations: CI, confidence interval; **FFQ**, food frequency questionnaire; **PCBs**, polychlorinated biphenyls

study was to validate FFQ-based estimates of concurrent, past, and long-term dietary PCB exposure against concentrations of individual PCB congeners in serum of women from the population-based Swedish Mammography Cohort (SMC). For this purpose, we created two extensive recipe-based databases of PCBs in foods taking into account the time-trend of PCB concentrations in food [16] and the effect of processing on the PCB concentrations in cooked food [17, 18].

2 Materials and methods

2.1 Study population

The study population is part of the SMC, a population-based prospective cohort established during a mammography screening program between 1987 and 1990 [19]. The source population consisted of 90 303 women who were born between 1914 and 1948 and living in Uppsala and Västmanland counties in central Sweden (response rate 74%). In 1997, a second self-administered questionnaire on diet and lifestyle factors was sent out to 56 030 eligible cohort members (70% response rate) still living in the study area.

Starting from November 2003, 8311 women from the cohort living in the town of Uppsala received a third questionnaire (65% response rate) together with an invitation to participate in a health examination. For the purpose of this study, we included the first 201 consecutive women with complete data on serum lipids and total body fat mass and who were also nonusers of fish oil supplements to avoid the influence of additional sources of PCB exposure [20, 21].

All women ($n = 201$) donated blood samples and completed a FFQ between 2004 and 2006 (herein referred to as the concurrent exposure assessment). Of these women, 165 completed the same FFQ 1 year later (herein referred to as the reproducibility study), and 142 had complete data from the 1997 FFQ (herein referred to as the past exposure assessment). The study was approved by the Regional Ethical Review Board, Stockholm, Sweden (Dnr 2006/1490). Informed consent was obtained from the participants before the study started.

2.2 Data collection

The FFQs from 1997 (96-item) and 2004–2006 (expanded to 123-item) primarily reflected the women's average consumption of different foods and beverages during the previous year. The FFQs were based on open-ended questions with prespecified serving sizes for frequently consumed foods (e.g. dairy products) and eight predefined frequency categories (never, 1–3 times/month, 1–2, 3–4, and 5–6 times/week, and one, two, and three or more times/day) for other foods. Age-specific portion-sizes were estimated from 5922 weighted food records kept by 213 randomly selected women from the study area.

Questions on fish consumption were categorized into five groups; high fat fish (Atlantic herring, Baltic herring, and mackerel), medium fat fish (Arctic char, salmon, and whitefish), low fat fish (cod, saithe, and pollock), caviar, and shellfish. The FFQ from 2004 to 2006 also included one question on consumption of tuna and one question on "other fish." The FFQ has previously been validated in 129 women reporting a Pearson correlation coefficient (r) between the FFQ and mean of four 1-week weighted diet records of 0.6 for fatty fish (salmon, herring, and mackerel), 0.5 for other fish, 0.5–0.7 for dairy products, and 0.4 for cheese.

Body weight was self-reported in the 1997 questionnaire and measured at the health examination in 2004–2006. During the health examination, a blood sample was taken and total fat mass was measured using DXA (dual-energy X-ray absorptiometry; Lunar Prodigy, Lunar Corp., Madison, WI, USA; precision error 1.5% for triple measurements in 15 subjects).

2.3 Assessment of dietary PCB

In order to account for time-related changes in PCB concentrations in food, two large recipe-based databases were developed to estimate the dietary exposure to CB-153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) for the 1997 and the 2004–2006 exposure assessment. Concentration data on PCBs in foods sold on the Swedish market was obtained from regular control and monitoring programs run by the Swedish National Food Agency. When concentration data for the specific time periods was not available, concentrations retrieved for the time period between 1992 and 2009 (over 1200 samples) were used with extrapolation backwards or forward to reflect the concentrations of PCBs in food during the years 1997 and 2004. The extrapolation was based on an average PCB concentration decline of 8% per year, which mirrors the temporal trend of CB-153 in Swedish breast milk samples between 1996 and 2006 [22] as well as an average decline of CB-153 concentrations in food sold on the Swedish market [23]. Before extrapolating the data, values below the LOQ were set to half of this limit (in total 12% of all samples). To account for changes in PCB concentrations of fried and boiled fish or of cooked meat products, the PCB concentrations (expressed on a lipid weight basis of raw food) were multiplied by the lipid weight of the cooked/processed food [17, 24]. For stews and similar dishes and cooking methods, no loss of fat was assumed and, instead, a factor for the loss of water during cooking was applied to the cooked/processed food. Data on the lipid content and the percentage of water loss in cooked food was obtained from the Swedish National Food Agency.

Dietary exposure to PCB (ng/day) was estimated by multiplying the average PCB (CB-153) concentration in various foods with the respective consumption frequency and portion size, and then adjusting for total energy intake (mean in the study cohort) using the residual-regression method [25].

Long-term FFQ-based PCB estimates were based on mean values from the 1997 and the 2004–2006 exposure assessments ($n = 142$).

2.4 Chemical analysis of PCBs

Venous blood samples were collected at the health examination after a 12-h overnight fast. Samples were immediately centrifuged, separated, and stored at −80°C. Six PCB congeners (CB-118, CB-138, CB-153, CB-156, CB-170, and CB-180) were measured at the National Institute for Health and Welfare in Finland according to Rantakokko et al. [26]. The analytical method has been modified to include a large number of PCBs. Briefly, 200 μL of serum, ethanol, ^{13}C-labeled internal standards of each compound, dichloromethane-hexane (1:4), and activated silica were mixed and eluted through a solid-phase extraction cartridge and analyzed by GC high-resolution MS (GC-HRMS). The quantitation of PCBs was performed by selective ion recording using a HP 6890 GC/Waters Autospec Ultima HRMS with DB-5MS column (J&W Scientific, 30 m, id 0.25 mm, 0.25 μm). The average concentrations of the reference materials for PCBs in human serum (National Institute of Standards and Technology, NIST Standard Reference Material 1589a) varied from 94 to 107% and the relative standard deviation varied from 1.5 to 4.4% depending on the congener. The LOQ varied from 2 to 5 pg/mL and none of the concentrations were below the LOQ. This analytical method is accredited. Serum sumPCB was calculated by summing six congeners (CB-118, CB-138, CB-153, CB-156, CB-170, and CB-180).

2.5 Adjustment of serum PCB for serum lipids

Total serum lipids, i.e. triglycerides, cholesterol (measured at Uppsala University Hospital), and phospholipids (measured at Karolinska University Hospital, Stockholm) were used for adjustment of PCBs in serum [27, 28]. Total lipid concentrations in serum were calculated according to Grimvall [29] and based on the assumed molecular weights for triglycerides, phospholipids, and cholesterol of 807, 571, and 714, respectively, and a proportion of free and esterified cholesterol in plasma of 1:2.

2.6 Statistical analysis

Since PCB concentrations were not normally distributed, we used Spearman rank correlation coefficients (r_s) and 95% confidence intervals (CI) to test the relationship between FFQ-based estimates of dietary PCB exposure and PCB concentrations in serum. The amount of accumulated PCBs in the body is dependent on age and body weight [13, 15, 30, 31]; therefore, we adjusted the dietary PCB exposure for body weight and serum PCBs for age in the correlation analyses. Age- and lipid-adjusted serum PCB concentrations (ng/g lipid) were estimated by dividing the levels of PCB in serum with total serum lipid concentrations and the women's age (years). The within-person variability of the FFQ-based PCB exposure was assessed by calculating the intraclass correlation coefficient from the reproducibility study based on the two identical FFQs completed 1 year apart. To take into account the within-person variability in the validity analysis, the Spearman correlation was adjusted for the intraclass correlation coefficient resulting in a deattenuated Spearman correlation coefficient [32]. Cross-classification of tertiles of FFQ-based PCB exposure and serum levels of CB-153 and sumPCB was used to test the agreement of classification. All statistical analyses were performed in STATA (Intercooled STATA software version 11; StataCorp, LP, College Station, Texas, USA). Reported p values were from two-sided statistical tests and a p value of ≤ 0.05 was considered statistically significant.

3 Results

The median FFQ-based estimate of dietary PCB exposure in the study group was 105 ng/day (1.6 ng/kg bw) in the concurrent ($n = 201$), and 171 ng/day (2.6 ng/kg bw) in the past exposure assessment ($n = 142$), reflecting 7–9 years earlier exposure, which was similar to the estimated median exposure of the whole cohort in 1997 (166 ng/day; $n = 33, 638$; Table 1). In the reproducibility study, the median FFQ-based dietary PCB exposure was 105 ng/day in 2004–2006 and 112 ng/day 1 year later, with an intraclass correlation coefficient of 0.48. The major dietary source of PCB exposure was fish, accounting for 72% of the total exposure in the concurrent exposure assessment. Dairy products, meat and poultry products, egg, and other fat containing products including butter and oil accounted for 13, 8, 2, and 5% of the PCB exposure, respectively.

The most abundant PCB congener in serum was CB-153 followed by CB-180, CB-138, CB-170, CB-118, and CB-156 (Table 2). Serum CB-153 was statistically significantly correlated with the other serum PCB congeners (r_s 0.76–0.88, p value < 0.001) and with serum sumPCB (r_s 0.99, p value < 0.001). The sum of six serum PCB congeners was positively correlated with age at blood sampling (r_s 0.41, p value < 0.001) and inversely correlated with weight (r_s –0.23, p value < 0.001) and total fat mass (r_s −0.17, p value < 0.05).

The Spearman correlation coefficients between FFQ-based dietary PCB exposure and individual PCB congeners in serum are presented in Table 3. In general, the correlations became stronger after adjusting the serum concentrations for age and the dietary estimates for body weight (Table 3). The estimated dietary PCB exposure based on both the concurrent exposure assessment and the past exposure assessment, were correlated with serum CB-153; r_s 0.41 and r_s 0.34 adjusted for within-person variability ($p < 0.05$ for both), respectively, and with serum sumPCBs; r_s 0.45 (Fig. 1) and r_s 0.38 ($p < 0.05$ for both), respectively.

Table 1. Major characteristics of Swedish women included in the validation study; median (5th–95th percentile)

Study groups				
	Current 2004–2006 ($n = 201$)	Past 1997 ($n = 142$)	Long term 1997/2004–2006 ($n = 142$)	The Swedish Mammography Cohort 1997 ($n = 33\,638$)[a)]
Age (years)	66 (58–78)	57 (50–69)	61 (54–73)	59 (50–77)
Weight (kg)	70 (55–92)	67 (54–86)	69 (56–90)	66 (52–88)
Body mass index (kg/m^2)	26 (17–39)	25 (21–30)	25 (21–32)	24 (20–32)
Energy intakes (kcal)	1680 (1106–2610)	1701 (1171– 2502)	1735 (1255–2506)	1683 (575–4664)
Dietary PCB (ng/day)[b)]	105 (48–200)	171 (78–348)	140 (82–249)	166 (71–372)
Dietary PCB (ng/kg body weight per day)[b)]	1.6 (0.7–3.0)	2.6 (1.1–5.0)	2.1 (1.1–3.4)	2.5 (1.1–5.8)

a) The 1997 baseline cohort, excluding women with prevalent cardiovascular diseases, malignant neoplasms, and diabetes (identified through National Patient Registry, Cancer and Diabetes registries) as well as those reporting implausible energy intakes (±3 SD from the log mean of the energy intakes).
b) Food frequency questionnaire-based PCB (polychlorinated biphenyls—CB-153) exposure energy adjusted to the mean energy intake in the whole cohort.

Table 2. Serum concentrations of lipids and individual PCB congeners of 201 Swedish women in 2004–2006

	Median (5th and 95th percentile)	Mean
Lipids (g/L)		
Triglycerides	0.9 (0.4–2.0)	1.0
Total cholesterol	3.4 (2.5–4.3)	3.4
Phospholipids	2.6 (2.0–3.2)	2.6
Total lipids	7.6 (6.2–9.1)	7.6
Adjusted PCBs (ng/g lipid weight)		
118	15 (5.8–35)	17
138	56 (26–111)	62
153	114 (63–195)	124
156	11 (5.8–18)	11
170	38 (21–62)	40
180	79 (46–130)	83
sumPCB	312 (184–528)	337

PCBs, polychlorinated biphenyls.

Significant correlations were also observed between dietary exposure and individual serum PCB congeners ranging from 0.30 to 0.52 (95% CI from 0.11 to 0.68) in the concurrent and 0.24 to 0.44 (95% CI; from 0.04 to 0.64) in the past exposure assessments. The correlations between the long-term exposure assessment (mean 1997 and 2004–2006 FFQs) and some of the individual serum PCB congeners (CB-153–CB-180) as well as sumPCB were slightly higher compared to the concurrent and past exposure assessments, ranging from 0.30 to 0.58 (95% CI from 0.07 to 0.76). When substituting body weight for the amount of total fat mass (from DXA measurements), the Spearman correlations for concurrent exposure assessment became stronger for some of the higher chlorinated PCB congeners; CB-156 $r_s = 0.57$, CB-170 $r_s = 0.59$, CB-180 $r_s = 0.66$, and sumPCB $r_s = 0.47$.

The degree of cross-classification of tertiles of serum CB-153 concentrations was evaluated against tertiles of the FFQ-based PCB exposure estimates of the concurrent exposure assessment. In the lowest tertile of serum CB-153, 32 of 67 women (48%) had low levels of both serum CB-153 and dietary PCB exposure (corresponding result was 51% for serum sumPCB). In the highest tertile of serum CB-153, 30 of 67 women (45%) were categorized into the same dietary category (the same result was obtained for serum sumPCB). Very similar results were obtained for the FFQ-based PCB estimates of the past exposure assessment in 1997. The percentage of women classified into the same or adjacent tertile was 89% for the concurrent and 85% for the past exposure assessment.

4 Discussion

This is the first study to validate FFQ-based estimates of long-term PCB exposure against concentrations in serum. In middle-aged and elderly women, we obtained acceptable validity of concurrent and past (7–9 years prior to blood sampling) exposure assessment of PCBs in comparison to concentrations of individual PCB congeners (CB-118, CB-138, CB-153, CB-156, CB-170, and CB-180) and the sum of serum PCBs. The correlation was stronger for the higher chlorinated PCB congeners (CB-153–CB-180) and sumPCB than for the lower chlorinated congeners (CB-118 and CB-138).

The serum PCB concentrations of this population of middle-aged and elderly women are comparable to levels observed in women from other countries [15, 33, 34]. In addition, the dietary CB-153 exposure is consistent with the estimated exposure in middle-aged and elderly women of the general Swedish population [35]. Since the 1970s when PCBs were banned, the levels of PCBs have decreased in the environment and subsequently they have also decreased in the food supply. Dietary exposure to PCBs was reported to have decreased in the general Swedish population from 139 ng/day in 1999 to 85 ng/day in 2004 [16].

Table 3. Spearman correlation coefficients (95% confidence interval) between FFQ-based PCB exposure assessments (concurrent, past, and long term) and individual PCB congeners measured in serum in Swedish women, 2004–2006

	IUPAC no.						
Serum	118	138	153	156	170	180	sumPCB
Concurrent (2004–2006) FFQ-based PCB estimates ($n = 201$)							
PCB[a)]	0.29**	0.21*	0.24**	0.26**	0.21*	0.22*	0.26**
	(0.16–0.41)	(0.07–0.34)	(0.11–0.37)	(0.13–0.39)	(0.08–0.34)	(0.09–0.35)	(0.12–0.38)
PCB[b)]	0.26**	0.21*	0.29**	0.36**	0.33**	0.35**	0.31**
	(0.12–0.38)	(0.07–0.34)	(0.15–0.41)	(0.23–0.47)	(0.20–0.45)	(0.23–0.47)	(0.18–0.43)
PCB[c)]	0.37**	0.30*	0.41**	0.52**	0.47**	0.51**	0.45**
	(0.18–0.55)	(0.11–0.49)	(0.22–0.59)	(0.33–0.68)	(0.29–0.64)	(0.33–0.68)	(0.27–0.63)
Past (1997) FFQ-based PCB estimates ($n = 142$)							
PCB[a)]	0.21*	0.20*	0.21*	0.20*	0.18*	0.19*	0.22*
	(0.05–0.37)	(0.04–0.35)	(0.05–0.36)	(0.03–0.35)	(0.02–0.34)	(0.03–0.35)	(0.05–0.37)
PCB[b)]	0.19*	0.17*	0.23*	0.30**	0.28**	0.31**	0.26*
	(0.03–0.34)	(0.003–0.32)	(0.07–0.38)	(0.14–0.44)	(0.13–0.43)	(0.15–0.45)	(0.10–0.41)
PCB[c)]	0.27*	0.24*	0.34*	0.43*	0.41*	0.44*	0.38*
	(0.04–0.50)	(0.004–0.47)	(0.10–0.55)	(0.20–0.63)	(0.18–0.62)	(0.21–0.64)	(0.15–0.59)
Long-term FFQ-based PCB estimates ($n = 142$)							
PCB[a)]	0.27*	0.23*	0.23*	0.23*	0.20*	0.21*	0.25*
	(0.11–0.42)	(0.07–0.38)	(0.07–0.38)	(0.07–0.38)	(0.04–0.35)	(0.05–0.37)	(0.08–0.39)
PCB[b)]	0.23*	0.21*	0.29**	0.38**	0.37**	0.40**	0.33**
	(0.07–0.38)	(0.05–0.36)	(0.14–0.44)	(0.23–0.51)	(0.22–0.50)	(0.25–0.53)	(0.18–0.47)
PCB[c)]	0.34*	0.30*	0.42**	0.54**	0.53**	0.58**	0.48**
	(0.10–0.55)	(0.07–0.52)	(0.20–0.63)	(0.33–0.74)	(0.31–0.73)	(0.36–0.76)	(0.25–0.68)

a) Food frequency questionnaire (FFQ) based CB-153 (ng/day) and serum polychlorinated biphenyls (PCBs, ng/g lipid).
b) FFQ-based CB-153 adjusted for body weight (ng/kg body weight/day) and serum PCB adjusted for age (ng/g lipid/age).
c) Additional adjustments for within-person variability intraclass correlation of FFQ-based PCB (CB-153) estimates.
*p value < 0.05; **p value < 0.001.

In the present study, we developed two extensive recipe-based databases to reflect the time trend of PCB concentrations in food for the two FFQs (1997 and 2004–2006) based on dietary concentrations of CB-153 in food. CB-153 is the most abundant PCB congener in human biological media and has been proposed as an indicator biomarker for total PCB body burden [36–38]. We observed serum CB-153 to be highly correlated with the other PCB congeners, including the dioxin-like (CB-118 and CB-156) and nondioxinlike (CB-138, CB-170, CB-180) congeners, as well as with sumPCB. The dioxinlike PCBs are of special toxicological concern because they have similar properties to the most potent and toxic compound, 2,3,7,8-tetrachlordibenzo-p-dioxin, which has been classified as a human carcinogen by the International Agency for Research on Cancer [39].

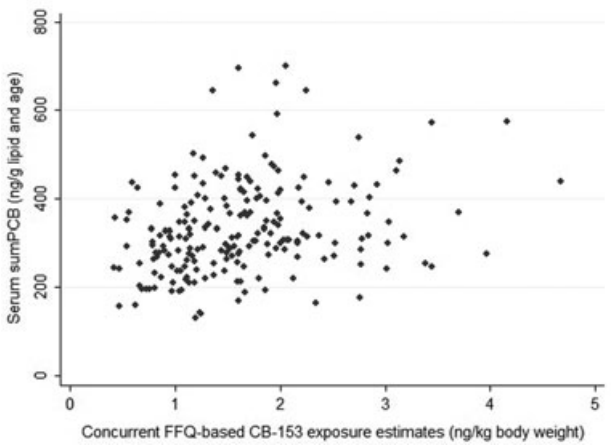

Figure 1. Scatter plot between concurrent FFQ-based CB153 exposure estimates (ng/kg body weight) and sum of six PCB congeners in serum (ng/g lipid and age) adjusted to the mean age of the study group (66 years).

Apart from diet [12, 13, 15], age is a strong predictor of PCB concentrations in the body [13, 30, 31], as also observed in the present study, because PCBs accumulate in adipose tissue over time [29, 40]. The mean CB-153 concentration in blood has been shown to increase by 1.8% per year in Swedish women of similar age [13]. By adjusting for age, we are able to compensate for age-related cumulative exposure to PCBs. The long half-lives of PCBs in the human body range from a couple of years to decades depending on the congener (half-life for CB-153 is 14.4 years) [41, 42], allowing us to validate FFQ-based PCB exposure estimates 7–9 years prior to blood sampling.

The present validation study has several strengths. The women in both the validation and reproducibility studies were representative of the whole SMC cohort with respect to dietary CB-153 exposure, body weight, and age. We were able to exclude women using fish oil supplements since fish oils have previously been shown to be contaminated with PCBs [20, 21].

The blood samples were collected after a 12-h overnight fast that avoided the influence of recent food intake on serum PCB concentrations and the chemical analysis were performed with high analytical accuracy. In addition, the construction of the extensive CB-153 food database accounted for the effect of food processing on the PCB concentrations. The CB-153 food database was also time specific, taking into consideration the decline in exposure that took place between our two dietary exposure assessments (1997 and 2004–2006). By using data from FFQs completed by the same women 7–9 years apart, we were able to assure long-term validity.

One limitation is the use of a simplified average 8% change per year extrapolation of PCB concentrations in food from the date of the chemical analysis, as this may not fully capture the change in exposure over time. Furthermore, the FFQ from 2004/2006 contained more items (123) than the FFQ from 1997 (96-item). Nevertheless, we observed no major differences in the validity of the long-term exposure assessment compared to the short-term exposure assessment.

In conclusion, the present study showed acceptable validity of FFQ-based PCB exposure estimates in relation to PCB concentrations in serum, thereby justifying the use of FFQs in large-scale epidemiological studies of the association of PCBs with health outcomes.

We would like to thank Associate Professor Paolo Parini at the Division of Clinical Chemistry, Department of Laboratory Medicine, Karolinska University Hospital Huddinge for the analysis of phospholipids in serum, and Professor Bengt Vessby at the Department of Public Health and Caring Sciences, Clinical Nutrition and Metabolism, Uppsala University for the analysis of triglycerides and cholesterol in serum. The study was financed by the Swedish Research Council Formas and the Swedish Research Council/Research Infrastructure.

The authors have declared no conflict of interest.

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