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Polychlorinated biphenyl levels in the blood of Japanese individuals ranging from infants to over 80 years of age

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Abstract Individuals' exposure to various persistent organic pollutants (POPs), including polychlorinated biphenyls (PCBs), and its adverse health effects have been a cause of concern. We measured blood PCB concentrations from samples taken from 507 Japanese individuals ranging from infants to those over 80 years of age. The blood PCB levels increased with age for both male (Spearman's $r=0.69$, $p<0.001$) and female (Spearman's $r=0.70$, $p<0.001$) participants. Adult men and nulliparous women showed similar increases with age. However, the PCB levels of multiparous women were lower than those of nulliparous women in their thirties ($p=0.005$), probably because the PCBs were transferred from the mothers to their children during pregnancy and lactation. Among infants (<2 years of age), some had as high levels of accumulated PCB levels as those in adults >30 years of age. In some cases, the PCB levels were over 0.8 ng/g wet weight, similar to levels observed in adults over 50 years of age. In the future, it will be necessary to do research on the health of the children who are exposed by high concentration level of POPs.

Keywords Polychlorinated biphenyls (PCBs) · Persistent organic pollutants (POPs) · Blood · Infants · Nulliparous · Multiparous · Lactation

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Introduction

Several reports claim that various persistent organic pollutants (POPs) may exist within the human body, such as dioxins, polychlorinated biphenyls (PCBs), *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE), hexachlorocyclohexanes (HCHs), and hexachlorobenzene (HCB; Mori et al. 2002; Fukata et al. 2005; Dirtu et al. 2006; Mori and Todaka 2011; Amodio et al. 2012; Govarts et al. 2012). Although relatively "low" levels of POPs are released into the environment, these contaminants may be bio-accumulated in the human body, mainly through food consumption (Longnecker et al. 2003; Needham et al. 2005; Mori and Todaka 2011). Foetuses and infants are more sensitive to various environmental agents compared to adults (Jacobson and Jacobson 1996; Mori 2004; Mori and Todaka 2011).

Several authors have reported the adverse effects of low-dose PCBs on the immune system (Heilmann et al. 2006); reproductive system (Axmon et al. 2006; Cooper et al. 2005); neurodevelopment, including conditions such as attention deficit-hyperactivity disorder (Jacobson and Jacobson 1996; Stewart et al. 2005); low birth weight (Govarts et al. 2012); and the impact of these contaminants on future generations (Karmaus et al. 2005; Sagiv et al. 2007; Mori and Todaka 2011). Elevated levels of serum organochlorines such as PCBs

have also been positively associated with diabetes (Codru et al. 2007; Everett and Frithsen 2008). In addition, PCBs have been shown to act through P450 enzymes, leading to an increase in the synthesis of cholesterol and triglycerides, which are major risk factors of cardiovascular diseases (Goncharov et al. 2008). Serum concentrations of PCBs, *p,p′*-DDE, and HCB are also associated with elevated circulating thyroid hormone levels in adult men (Meeker et al. 2007).

Human blood PCB concentrations have shown positive correlation with blood concentrations of other POPs such as DDE, HCB, HCHs, and dioxins (Fukata et al. 2005; Mori et al. 2008; Mori and Todaka 2011; Amodio et al. 2012). Maternal blood PCB levels are also indicators of foetal POP contamination (Mori and Todaka 2009, 2011). Previous studies have reported PCB levels in maternal blood, cord blood, and cord tissue (Mori et al. 2002; Fukata et al. 2005; Govarts et al. 2012) as well as the relationship between PCB concentration and subject age (Amodio et al. 2012). However, reports regarding PCB concentrations in the blood of multigenerational individuals have not been reported.

Therefore, this study aimed to assess blood PCB concentrations in Japanese individuals ranging from infants to those over 80 years of age, to determine the differences in blood PCB concentrations between men and women (nulliparous and multiparous), and to estimate the source of high concentrations in children.

Materials and methods

Sample collection

Between 2005 and 2007, blood samples were collected from 507 Japanese individuals, including 460 healthy adult volunteers and 47 patients (<20 years of age) with allergies, who had visited a paediatric clinic. One to 5 ml of blood was pulled from cutaneous vein of the participants. Venoject II® with sodium heparin (Terumo Corporation, Tokyo, Japan) was used to obtain whole blood samples, which were kept at −20 °C until use in analysis (Jotaki et al. 2011). The length of time that the allergy patients had been breastfed was determined through questioning of the patients' mothers by the paediatricians. In addition, the body weight of the 47 patients with allergy was recorded for calculating total body levels of PCBs. The Congress of Medical Bioethics at Chiba University approved this study, and samples were collected after gaining written informed consent.

PCB concentration analysis

Approximately 1 g of whole blood was hydrolysed with 1 mol/L potassium hydroxide (diluted in ethanol) for 18 h, and extracted three times with hexane. The extract was washed three times with distilled water and then dehydrated with anhydrous sodium sulphate. The solution was concentrated by evaporation to approximately 2 mL, eluted through a Florisil column (Florisil PR; GL Sciences, Tokyo, Japan) with 50 mL of hexane, evaporated to a final volume of 1 mL, and analysed by packed-column gas chromatography, with an electron capture detector (GC/ECD; GC-17A, Shimadzu, Kyoto, Japan; Nakagawa et al. 1999; Sakurai et al. 2006; Jotaki et al. 2011). The method requires less than 1 mL of blood from each subject. The PCB concentration was calculated as the sum of the major eight peaks shown after *p,p′*-DDE on the chromatogram (Nakagawa et al. 2003), based on the peaks detected in a Kanechlor mixture standard (Kanechlor 300, 400, 500, and 600=1:1:1:1).

The detection limit was 0.1 ng/g wet weight; samples below the lower level of detection were assigned a value of 0.05 ng/g. For comparison of PCB concentration obtained by packed column GC/ECD and high-resolution gas chromatography and high resolution mass spectroscopy (HRGC/HRMS), 20 adult volunteers' blood samples were measured by both packed column GC/ECD and HRGC/HRMS methods. The detail method of HRGC/HRMS was shown in Jotaki et al. (2011).

Statistical analysis

Median and range were calculated for the blood PCB concentrations. Spearman's rank correlation coefficient and exponential regression analysis were used to assess the relationship between age and blood PCB concentrations. Spearman's rank correlation coefficient and linear regression analyses were used to assess the relationship between whole-body PCB levels and lactation period. Differences between nulliparous and multiparous women were compared using the Mann–Whitney *U* test. The PCB concentration data were not normally distributed, requiring the use of the nonparametric test.

Table 1 Age and gender distribution of the study participants

Age (years)	Males *N* (%)	Females *N* (%)	Total *N* (%)
0–4	13 (2.6)	15 (3.0)	28 (5.5)
5–9	3 (0.6)	4 (0.8)	7 (1.4)
10–14	3 (0.6)	7 (1.4)	10 (2.0)
15–19	0 (0)	2 (0.4)	2 (0.4)
Subtotal	19 (3.7)	28 (5.5)	47 (9.3)
20–29	32 (6.3)	37 (7.3)	69 (13.6)
30–39	39 (7.7)	46 (9.1)	85 (16.8)
40–49	44 (8.7)	54 (10.7)	98 (19.3)
50–59	87 (17.2)	31 (6.1)	118 (23.3)
60–69	37 (7.3)	38 (7.5)	75 (14.8)
70–85	3 (0.6)	12 (2.4)	15 (3.0)
Subtotal	242 (47.7)	218 (47.4)	460 (90.7)
Total	261 (51.5)	246 (48.5)	507 (100)

Table 2 Blood polychlorinated biphenyl concentrations (nanograms per gram wet weight) of the participants

Age (years)	Male participants			Female participants			Total		
	N	Median	Range (min–max)	*N*	Median	Range (min–max)	*N*	Median	Range (min–max)
0–4	13	0.41	(0.05–1.20)	15	0.37	(0.05–0.95)	28	0.41	(0.05–1.20)
5–9	3	0.05	(0.05–0.26)	4	0.24	(0.15–0.27)	7	0.20	(0.04–0.27)
10–14	3	0.20	(0.05–0.22)	7	0.21	(0.05–0.32)	10	0.21	(0.05–0.32)
15–19	NA			2	0.47	(0.20–0.73)	2	0.47	(0.20–0.73)
20–29	32	0.25	(0.09–0.64)	37	0.24	(0.10–0.53)	69	0.25	(0.09–0.64)
30–39	39	0.30	(0.13–1.50)	46	0.30	(0.10–0.87)	85	0.30	(0.10–1.50)
40–49	44	0.54	(0.20–1.30)	54	0.42	(0.10–1.21)	98	0.47	(0.10–1.30)
50–59	87	0.81	(0.26–3.30)	31	0.68	(0.21–2.26)	118	0.76	(0.21–3.30)
60–69	37	0.92	(0.40–5.27)	38	0.89	(0.42–2.24)	75	0.91	(0.40–5.27)
70–85	3	1.20	(0.77–1.90)	12	0.96	(0.42–4.58)	15	0.97	(0.42–4.58)
Total	261	0.54	(0.05–5.27)	246	0.45	(0.05–4.58)	507	0.49	(0.05–5.27)

A two-tailed p value of 0.05 was considered statistically significant. All statistical analyses were conducted using SPSS for Windows (version 13,SPSS, Chicago, IL, USA) and SAS (SAS 9.2, SAS Institute Inc, Cary, NC).

Results

The age and gender distributions of the participants are shown in Table 1. The blood PCB data of 20 volunteers by packed column GC/ECD and HRGC/HRMS were strongly correlated [coefficient of determination (R^2) was 0.996, and the slope of the regression line was 0.625]; the results were in the range of 0.2–5.0 ng/g wet weight. The blood PCB concentrations of five adult participants were measured six times per person and analysed by the GC/ECD method. The average coefficient of variation (Cv) was found to be 0.33±0.14 % [mean±standard deviation (SD)], and the reproducibility was confirmed.

PCB levels in adults

PCBs were detected in the samples obtained from all the 460 healthy adult volunteers (Table 2). The adult participants were classified into three groups: male participants, nulliparous women (never given birth to a baby), and multiparous women (given birth to at least one baby; Table 3). The blood PCB levels increased with age for both male (Spearman's $r=0.69$, $p<0.001$) and female (Spearman's $r=0.70$, $p<0.001$) participants (Tables 2 and 3). Delivery experience affects the PCB concentration level in adults. The male participants and nulliparous women showed similar regression curves between age and PCB levels (Fig. 1a, Table 3). However, for women in their thirties, the PCB levels of multiparous women were lower than those of nulliparous women (Fig. 1b, Table 3). The differences between the nulliparous and the multiparous women were significant, based on the Mann–Whitney U test ($p=0.005$; Table 3).

Table 3 Blood polychlorinated biphenyl concentrations (nanograms per gram wet weight) among male participants and nulliparous and multiparous women

Age (years)	Male participants			Nulliparous women			Multiparous women		
	N	Median	Range (min–max)	*N*	Median	Range (min–max)	*N*	Median	Range (min–max)
20–29	32	0.25	(0.09–0.64)	36	0.25	(0.10–0.53)	1	0.10	(0.10–0.10)
30–39	39	0.30	(0.13–1.50)	35	0.30	(0.14–0.87)*	11	0.20	(0.10–0.32)*
40–49	44	0.54	(0.20–1.30)	15	0.43	(0.20–0.98)	39	0.42	(0.10–1.21)
50–59	87	0.81	(0.26–3.30)	4	0.81	(0.21–0.96)	27	0.64	(0.25–2.26)
60–69	37	0.92	(0.40–5.27)	7	1.13	(0.60–1.32)	31	0.86	(0.42–2.24)
70–85	3	1.20	(0.77–1.90)	2	1.22	(0.94–1.50)	10	0.87	(0.42–4.58)

*$p=0.005$, significant difference between the nulliparous and multiparous women in their thirties (Mann–Whitney U test)

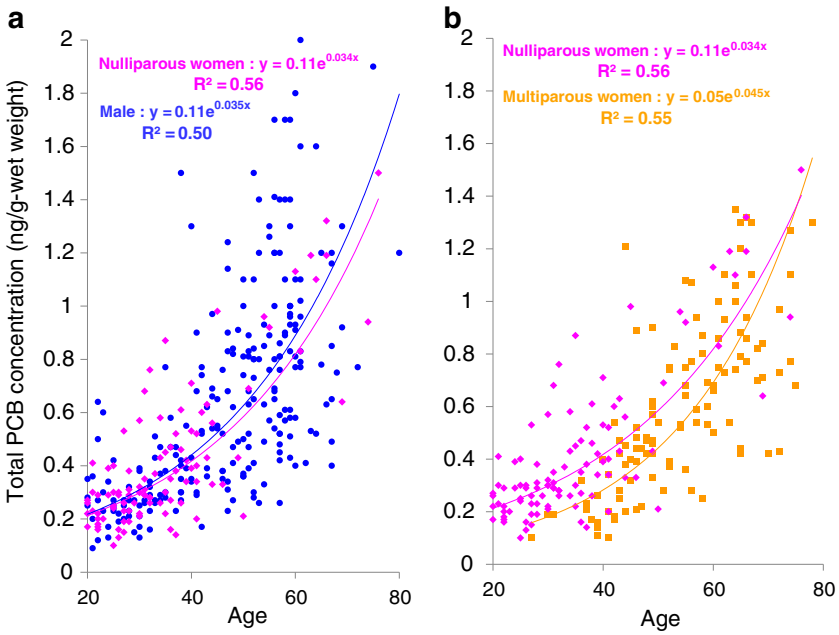

Fig. 1 Association of blood PCB concentration and age, gender, experience of delivery. **a** PCB concentration and age, males (*n*=242, *closed blue circle*), nulliparous women (*n*=99, *closed pink diamond*). **b** PCB concentration and age, nulliparous women (*n*=99, *closed pink diamond*), multiparous women (*n*=119, *closed orange square*)

PCB levels of infants and lactation

Among the 47 subjects <20 years of age, 33 subjects were breastfed more than 3 months (70.2 %). Ten subjects were breastfed shorter than 3 months but more than 1 month (21.3 %). Four subjects were breastfed shorter than 1 month (8.5 %).

The concentration of PCBs in the blood of 8 out of the 47 subjects <20 years of age was below 0.1 ng/g wet weight (Fig. 2a). Some young infants showed high levels of blood PCBs (Table 2 and Fig. 1a). Among infants (<2 years of age), some showed as high levels of accumulated PCB levels as those in adults >30 years of age. Further, among this very young part of the study population, the estimated whole-body PCB levels were strongly correlated with their lactation period (Fig. 2b, Spearman's r=0.85, p<0.001). The whole-body PCB level was calculated as blood PCB concentration (nanograms per gram wet weight)×body weight (grams).

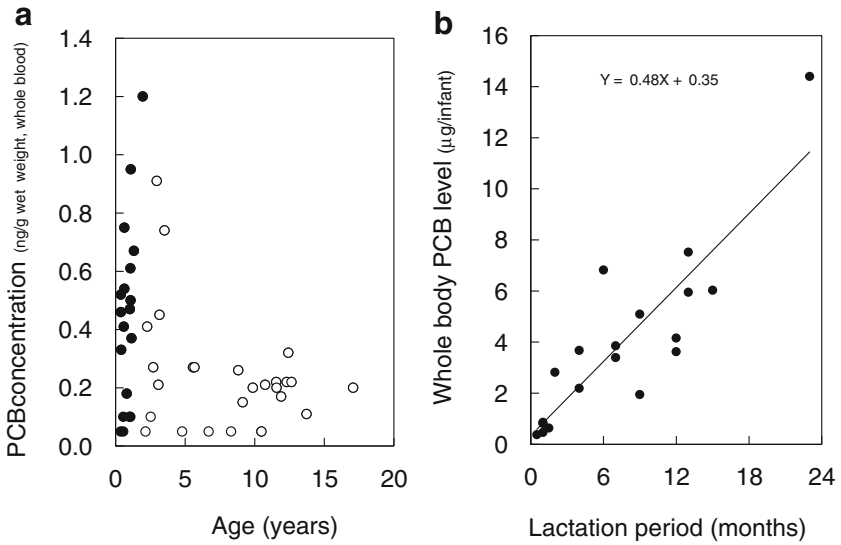

Fig. 2 Association between blood polychlorinated biphenyl (PCB) concentration and age of infants and children (**a**) and between the whole-body PCB level and the duration of the infants' lactation period (**b**). **a** Association between blood PCB concentration and child age, including infants <2 years of age (*closed circles*) and children <20 years of age (*open circles*). **b** Correlation between lactation period and whole-body PCB levels in infants <2 years of age. *Closed circles* show the whole-body levels of PCB. The whole-body PCB level was calculated as the blood PCB concentration (nanograms per gram wet weight)×-body weight (grams). Correlation was found between the lactation period and whole-body PCB level (Y=0.48×+0.35)

Discussion

In adults (≥20 years of age), PCBs were detected in 100 % of the samples, and the levels increased with age, which was similar with previous studies (Mori and Todaka 2011; Amodio et al. 2012). This relationship is commonly assumed to result from continued exposure or intake and absence of excretion of these pollutants (Wolff et al. 2007; Amodio et al. 2012). More than 80 % of the PCBs in Japan are derived from seafood (Mori and Todaka 2011). POPs, including PCBs, are oil- and fat-soluble, and they could be accumulated in the fatty tissues of fish and meat (Longnecker et al. 2003; Needham et al. 2005; Mori and Todaka 2011). It means that Japanese individuals gradually consume and accumulate POPs in their bodies.

Some previous reports indicated that women had lower PCB concentrations, and the authors hypothesised that women excreted the pollutants through lactation and menses (Kang et al. 1997 and Agudo et al. 2009). In the current study, male participants and nulliparous women showed similar associations between ages and PCB levels. On the other hand, multiparous women showed lower PCB levels. In addition, some infants <2 years of age had very high blood PCB levels.

PCB concentrations in breastmilk of ten mothers between 29 and 34 years old have been found to be in excess of 2 ng/g wet weight (unpublished data), and Japanese infants under 5 months of age typically consume 750 mL of breastmilk per day (Dietary Reference Intakes, Ministry of Health, Labour and Welfare, Japan). This means that Japanese babies consume more than 1.5 μg of PCB per day, which may account for the rise in blood PCB levels of breastfed infants that was associated with increased lactation periods (Fig. 2b). PCBs probably transfer from the mother to her child through pregnancy and lactation. This may be the reason for the lower PCB levels in multiparous women. Of course, breastfeeding should not be discouraged despite the contamination of breast milk (Govarts et al. 2012) since it helps to enhance the mother–infant relationship and is perfect nutrition for infants (World Health Organization 2013).

Some studies using cord blood or cord serum have indicated the adverse health effects of foetal exposure to PCBs such as low birth weight (Govarts et al. 2012) and neurodevelopmental disorder including conditions such as attention deficit-hyperactivity disorder (Stewart et al. 2005).

The current study revealed the PCB contamination levels in multiple generations of Japanese individuals, from infants to those over 80 years of age. The PCB levels in some infants <2 years of age were as high as those in individuals older than 30 years (Table 2). In some cases, PCB levels were in excess of 0.8 ng/g (wet weight) (Fig. 2a) which was approximately the same as observed in individuals older than 50-years old. The duration of particularly high PCB concentrations is hypothesised to be short because of their rapid growth; however, since this period represents an extremely important developmental period, the contamination levels should be lowered. For this, decreasing in maternal PCB levels would be necessary. Previously, our group suggested the importance of decreasing the PCB levels in individuals of reproductive age to promote the better health of future generations (Mori and Todaka 2009, 2011). If PCB levels are relatively high among individuals of reproductive age, attempts should be made to decrease contamination levels by changing dietary habits, adopting different cooking methods (Wilson et al. 1998), and consuming specific medicines (Sakurai et al. 2004, 2006).

Two different groups of individuals were involved in the current study. The first group was comprised of healthy, adult (>20 years of age) volunteers who responded to the authors' requests. The second group was comprised of young children group who visited their paediatrician due to allergies. Ideally, data should have been collected from a general population of healthy individuals. However, the collection of blood from healthy children is difficult and presents as a limitation of this study. Further investigation is required to assess the exposure of the overall Japanese population to PCBs and other POPs. To this end, our group is participating in a large-scale birth cohort study, conducted by the Japanese Ministry of the Environment. In this study, 100,000 children and their mothers and fathers will be recruited during pregnancy; as a result, more precise and generalised data are expected.

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