Effects of *in utero* exposure to polychlorinated biphenyls, methylmercury, and polyunsaturated fatty acids on birth size

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Abbreviations: BMI: body-mass index, B: partial regression coefficient, CI: confidence interval, FFQ: food-frequency questionnaire, Hg: mercury, MeHg: methylmercury, ND: not detectable, OR: odds ratio, SGA: small for gestational age, PCB: polychlorinated biphenyl, TEQ: toxic equivalent

Highlights

- The risk of small for gestational age by weight decreased with increasing hair mercury concentration.
- The concentrations of mercury in maternal hair had no association with birth weight.
- The concentrations of polychlorinated biphenyls in maternal blood had no association with birth size.

Abstract

The adverse effects of *in utero* exposure to polychlorinated biphenyls (PCBs) or methylmercury (MeHg), and the beneficial effects of nutrients from maternal fish intake might have opposing influences on fetal growth. In this study, we assessed the effects of *in utero* exposure to PCBs and MeHg on birth size in the Japanese population, which is known to have a high frequency of fish consumption. The concentrations of PCBs and polyunsaturated fatty acids in maternal blood, and the total mercury in hair (as a biomarker of MeHg exposure) were measured during pregnancy and at delivery. Maternal intakes of fish (subtypes: fatty and lean) and shellfishes were calculated from a food frequency questionnaire administered at delivery. Newborn anthropometric measurement data were obtained from birth records. The associations between chemical exposures and birth size were analyzed by using multiple regression analysis with adjustment for confounding factors among 367 mother–newborn pairs. The birth weight was 3073 ± 37 g (mean ± SD). The incidence of babies small for gestational age (SGA) by weight was 4.9%. The median concentrations of total PCBs and hair mercury were 108 ng/g lipid and 1.41 μg/g, respectively. There was no overall association between mercury concentrations and birth weight, birth length, chest circumference, and head circumference. We observed that the risk of SGA by weight decreased with increasing mercury concentration in regression analyses with adjustment for polyunsaturated fatty acids. Our results suggest that the beneficial effect of essential nutrition may mask the adverse effects of MeHg on birth size. The concentrations of PCBs had no association with birth size.

Keywords: polychlorinated biphenyls, methylmercury, birth size, small for gestational age, *in-utero* exposure, polyunsaturated fatty acids

1. Introduction

Newborn anthropometric measurements (weight, length, and head and chest circumference) reflect fetal growth *in utero*, and are reported to predict infant survival, growth, morbidity, and neurobehavioral performance in early life (Kajantie et al., 2005; Barker, 2006). In Japan, public health concerns have been raised about a marked increase in the prevalence of babies with low birth weight, from 4.2% to 8.3% between 1980 and 2000 (Takimoto et al., 2005). Birth cohort studies reported discrepant findings about the association between maternal intake of fish/seafood during pregnancy and birth size: some found a significant positive association (Olsen et al., 1990, 1993; Olsen and Secher, 2002; Thorsdottir et al., 2004; Drouillet-Pinard et al., 2010; Brantsaeter et al., 2012; Leventakou et al., 2014), whereas others found a null or negative association (Rylander et al., 2000; Oken et al., 2004; Guldner et al., 2007; Halldorsson et al., 2007; Mendez et al., 2010; Heppe et al., 2011).

A plausible explanation is that fish/seafood is a nutrient source of polyunsaturated fatty acids for the mother and, at the same time, exposes the fetus to polychlorinated biphenyls (PCBs) (Grandjean et al., 2001; Halldorsson et al., 2008; Papadopoulou et al., 2013) and methylmercury (MeHg) (Drouillet-Pinard et al., 2010; van Wijngaarden et al., 2014; Vejrup et al., 2014). The adverse effects of *in utero* exposure to environmental contaminants and the positive effects of the nutrients from fish might have opposing influences on fetal growth (Grandjean et al., 2001; Halldorsson et al., 2008; Papadopoulou et al., 2013). PCBs are classified as persistent organic pollutants as they are lipophilic, stable, and show widespread contamination in the environment, food web, and human tissues (Sonneborn et al. 2008). Hg in fish muscle is mostly present in the form of MeHg, which is bioconcentrated up through the aquatic food web, eventually resulting in exposure through the human diet (van Wijngaarden et al., 2014). Fetal exposure to PCBs and MeHg *in utero* has the potential for serious health concerns because these pollutants can cross the placental and blood–brain barriers to reach the immature fetal organs and tissues, which are particularly susceptible to the effects of these toxins (Zahir et al. 2005; National Research Council 2000; Wojtyniak et al., 2010; Casas et al., 2015).

The toxic mechanism of action of PCBs has not yet been fully elucidated; however, it is suspected that their estrogenic activity may play a role (Decastro et al., 2006). Experimental studies have demonstrated that PCBs display endocrine-disrupting effects in their ability to stimulate estrogen and can also function as xenoestrogens (Bonefeld-Jorgensen et al., 2001; Cooke et al., 2001). Estrogenic and antiestrogenic PCBs may have opposite associations with infant anthropometrics (Cooke et al., 2001). Other adverse effects induced by PCBs include dioxin-like activities such as activation of aryl hydrocarbon receptors (Van den Berg et al., 2006), and the potential toxic effects induced by dioxin-like PCB congeners may be stronger than those of non-dioxin-like (NDL) congeners (Giesy and Kannan, 1998). On the other hand, in our previous study, we found that fish/seafood consumption was associated with the concentration of NDL congeners (Miyashita et al., 2015). PCB 153 has been the most frequently used indicator of the effects on fetuses of exposure to PCBs in epidemiological studies. In previous studies, specific PCB congeners 153, 156, 118, 74, and 77 had potential estrogenic and antiestrogenic activities (Cooke et al., 2001; Decastro et al., 2006) and significant associations with birth size (Wojtyniak et al., 2010; Casas et al., 2015).

Epidemiological studies have previously reported inconsistent findings about the effect of prenatal exposure to PCBs at background levels on birth weight: some found significant inverse associations (Patandin et al., 1998; Rylander et al., 1998; Karmaus and Zhu, 2004; Sagiv et al., 2007; Halldorsson et al., 2008; Sonneborn et al., 2008; Tan et al., 2009; Brucker-Davis et al., 2010; Papadopoulou et al., 2013), whereas others found a null or positive association (Vartiainen et al., 1998; Grandjean et al., 2001; Gladen et al., 2003; Longnecker et al., 2005; Givens et al., 2007; Khanjani and Sim, 2007; Wolff et al., 2007; Murphy et al., 2010; Lopez-Espinosa et al., 2011; Kezios et al., 2012; Lignell et al., 2013; Hisada et al., 2014). In populations exposed to relatively high MeHg levels because of high consumption of contaminated seafood or accidental poisoning, epidemiologic studies have reported that prenatal MeHg exposure can lead to harmful effects on children's health such as impaired neurobehavioral development, congenital malformations, and restriction of fetal growth (National Research Council 2000). However, limited epidemiological studies reported no conclusive evidence on the effects of low-level MeHg exposure on birth size (Drouillet-Pinard et al., 2010; Gundacker et al., 2010; Ramirez et al., 2000; Ramon et al., 2009; van Wijngaarden et al., 2014; Vejrup et al., 2014; Zahir et al. 2005).

Moreover, a balance of the opposite effects of contaminants and fish/seafood intakes across populations consuming different types of fish/seafood may have resulted in the discrepant finding among the previous birth cohort studies (Mahaffey, 2004; Halldorsson et al., 2008; Ramon et al., 2009). A meta-analysis study including 19 European cohorts described that the most pronounced effect on birth weight was observed for fatty fish, which is known to be a main source of long-chain polyunsaturated fatty acids (LCPUFAs) (Leventakou et al., 2014). Systematic reviews have suggested that maternal intake of omega-3 fatty acid supplements during pregnancy is associated with small but significant increases in infant birth size (Makrides et al., 2006; Szajewska et al., 2006; Salvig and Lamont, 2011). However, in some Asian countries, including Japan, where there is a high frequency of fish consumption (Miyashita et al., 2015), there is insufficient evidence about the effect of *in utero* exposure to PCBs and MeHg on birth size.

Thus, the aim of this study is to assess the effects of prenatal exposure to PCBs and MeHg on newborn anthropometric measurements, as well as the incidence of babies born small for gestational age (SGA), taking into account the biomarker of LCPUFAs among Japanese pregnant women.

2. Materials and Methods

2.1. Study population

The subjects in this study were all currently enrolled in the Hokkaido Study on Environment and Children's Health. A total of 514 pregnant Japanese women were recruited at the Sapporo Toho Hospital in Hokkaido, Japan, from July 2002 to September 2005 (Kishi et al., 2013). An overview of this study is shown in Figure 1. During their last trimester, the subjects completed a self-administered questionnaire on demographic characteristics, socioeconomic status, tobacco smoking and alcohol habits, and frequency of consumption during pregnancy of food items such as shoreline fish (e.g., saury, Pacific herring, or mackerel), pelagic fish (e.g., tuna, bonito, or salmon), beef, pork, chicken, milk, and eggs. The medical records for 504 mother–newborn pairs were used to gather information on delivery characteristics, including maternal height, maternal prepregnancy weight, pregnancy complications, gestational age, infant sex, parity, congenital anomalies, and newborn anthropometric measurements.

Within 5 days after delivery, the mothers completed a food frequency questionnaire (FFQ) to estimate their fish/seafood intake and history of synthetic hair waving (n = 430). The FFQ provided information about the frequency and portion size for maternal fish intake (Supplementary Table 1). The estimated daily fish intake (g/day) was calculated from the FFQ (Yasutake et al., 2003). We divided maternal fish intake to four subtypes: fatty fish, lean fish, shellfishes, and whole. The fatty fish group consisted of tuna, salmon, yellowtail, sardine, mackerel, saury, eel, Atka mackerel, shishamo smelt, pacific herring, and trout. The lean fish group included bonito, sea bream, flatfish, flounder, horse mackerel, carp, sweetfish, crucian carp, and Pacific cod. The shellfishes group included cuttlefish, octopus, crab, shrimp, shellfish, and fish products (Leventakou et al., 2014).

This study was conducted with written informed consent from all subjects and was approved by the institutional ethics board for epidemiological studies at the Hokkaido University Graduate School of Medicine.

2.2. Exposure assessment

A 40-mL blood sample was taken from the maternal peripheral vein during the last trimester. In subjects with pregnancy-related anemia, the samples were taken during hospitalization immediately after delivery. Consequently, 356 samples were taken during pregnancy and 148 samples were taken after delivery. All samples were stored at -80°C until needed for analysis. The extraction, purification, and analysis of PCBs from whole blood specimens were performed by using a previously reported method (Iida and Todaka, 2003; Todaka et al., 2008a,b). The concentrations of PCBs were analyzed at the Fukuoka Institute of Health and Environmental Sciences by using high-resolution gas chromatography/high-resolution mass spectrometry of 5-g blood samples. To evaluate the accuracy and reliability of the PCB analysis, quality control studies were completed and compared against those done at three other laboratories. The average variation among the concentrations of PCBs in human blood samples was considered acceptable if it was within 10% (Kajiwara et al., 2008, 2009). The concentrations of 70 PCBs congeners were measured in 426 blood samples and adjusted for lipids (pg/g lipid). The sample values below the detection limit for the 70 PCBs congeners were assigned a value of one-half the detection limit. The remaining samples were not analyzed because of unavailable or insufficient sample volumes (<5 g) for measurement. PCB congeners were separated into four groups based on their suggested biological activities and the effect of exposure to them due to fish intake: estrogenic, antiestrogenic, dioxin-like, and NDL PCBs (Cooke et al., 2001). The estrogenic group included congeners 4, 10, 5, 8, 15, 17, 18, 31, 44, 47, 48, 52, 70, 99, 101, 136, 153, and 188. The antiestrogenic group included congeners 77, 110, 105, 114, 126, 156, 171, and 169. The dioxin-like PCBs included congeners 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189 (Van den Berg et al., 2006). NDL PCBs had 58 congeners excluding the 12 dioxin-like PCBs from all 70 congeners measured in our study (Supplementary Table 2) (Miyashita et al., 2015). Additionally, we used the specific PCB congeners 153 (main contributor), 156, 118, 74, and 77 as biomarkers of exposure to PCBs.

Maternal hair was collected within 5 days after delivery (n = 430). For the 1 cm of hair closest to the scalp, the concentrations of total Hg were determined by using the oxygen combustion-gold amalgamation method with the MD-1 atomic absorption detector (Nippon Instruments Co., Ltd., Osaka, Japan) at the National Institute for Minamata Disease (Yasutake et al., 2003). The total Hg concentration in hair was used as a convenient biomarker of MeHg exposure (van Wijngaarden et al., 2014) because >90% of the total Hg in hair is MeHg that is covalently bound to the cysteine residue of hair protein (National Research Council, 2000).

2.3. Maternal polyunsaturated fatty acid assessment

The fatty acid levels in maternal whole blood were determined by using gas chromatography–mass spectrometry (GC–MS) as described in detail in our previous study (Nakashima et al., 2013). Briefly, whole blood lipid was extracted from 25 μL blood (Folch et al., 1957), mixed with 1.2 mL methanol, 75 μL acetyl chloride, and 75 μL of 10 μg/100 μL tricosanoic acid ethyl ester/methanol (internal standard). After adding *n*-hexane (500 μL) and centrifugation of the sample, the upper organic layer was collected and transferred into another vial. The *n*-hexane extraction was repeated once, and then the concentration of fatty acid methyl ester in the *n*-hexane layer was measured with GC–MS. Finally, nine fatty acid species were measured including the omega-6 fatty acids, palmitoleic and oleic acids, linoleic acid, and arachidonic acid (AA), and the omega-3 fatty acids, α-linolenic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). The detection rates for eight fatty acids were >99.0% and that for EPA was 97.8% (Kishi et al., 2015). We used EPA + DHA, AA, omega-3 fatty acids, and omega-6 fatty acids as biomarkers of maternal LCPUFAs (van Wijngaarden et al., 2014; Vejrup et al., 2014).

2.4. Statistical analyses

Some subjects were excluded from analyses because of pregnancy-induced hypertension (n = 11), diabetes mellitus (n = 1), fetal heart failure (n = 1), and multiple births (n = 7). The final study population comprised 367 mother–newborn pairs with completed questionnaire data and birth records, whose PCB and hair Hg concentrations were measured (Figure 1). SGA by weight was defined as a birth weight less than the 10th percentile for the gestational age at delivery, based on growth charts specific for newborn sex and maternal parity for birth size standards by gestational age for Japanese neonates. SGA by length was defined as birth length less than the 10th percentile for the gestational age at delivery, based on growth charts for birth size standards by gestational age for Japanese neonates (Itabashi et al., 2014). Associations between subject characteristics and concentrations of PCBs and hair Hg were evaluated by using the Mann–Whitney U-test and Spearman's rank correlation coefficient. Associations between subject characteristics and birth size were evaluated by using Student's t-test, Pearson correlation, Spearman's rank correlation coefficient, and one-way analysis of variance. For linear regression analyses, we used $\log_{10}$-transformed values for concentrations of PCBs and hair Hg, as well as LCPUFAs, because these variables displayed a skewed distribution. Associations between PCBs or hair Hg (expressed as continuous concentrations) and newborn anthropometric measurements were evaluated by using linear regression analyses. For logistic regression analyses, we used concentrations of PCBs and hair Hg, divided into quartiles, to evaluate potential nonlinear relationships. The associations between PCBs or hair Hg and the incidence of babies born SGA by weight and length were evaluated by using logistic regression analyses. All regression analyses were conducted with or without adjustment of factors—chosen for their significant associations with exposure and birth size in this study ($p < 0.05$)—and possible confounding factors as reported in previous studies (Drouillet-Pinard et al., 2010; Halldorsson et al., 2008; Ramon et al., 2009; Papadopoulou et al., 2013; van Wijngaarden et al., 2014; Vejrup et al., 2014). Specifically, the adjusted factors included maternal age (continuous), height (continuous), prepregnancy weight (continuous), smoking during pregnancy (yes/no), alcohol consumption during pregnancy (yes/no), household income (less than or greater than 5 million Yen annually), blood sampling period (during pregnancy or after delivery), birth order (first-born or later children) reported as maternal parity, infant sex, gestational age, maternal LCPUFAs, and total 70 PCBs or hair Hg. The logistic regression analysis for SGA by weight was not adjusted for birth order, infant sex, and gestational age, because SGA by weight was defined based on growth charts for birth size standards by gestational age specific for newborn sex and maternal parity. Furthermore, the logistic regression analysis for SGA by length was not adjusted for gestational age, because SGA by length was defined based on growth charts for birth size standards by gestational age.

A p-value of <0.05 was considered statistically significant. Statistical analyses were performed by using the Statistics Package for Social Sciences (version 19.0J; IBM, Armonk, NY, USA) software for Windows.

3. Results

The subjects' characteristics are described in Table 1. The percentage of babies born SGA by weight was 4.9% and that of babies SGA by length was 11.7%. Table 2 shows the distribution of maternal biomarkers of fatty acid. The median concentration of the total 70 PCBs in the maternal blood was 108 ng/g lipid (Supplementary Table 2). The distributions of PCB concentrations are shown in Table 3. The geometric mean concentrations of estrogenic, antiestrogenic, dioxin-like, and NDL PCBs were 27.9, 3.98, 10.9, and 93.8 ng/g lipid, respectively, and that of hair Hg was 1.34 μg/g. The concentrations of total PCBs significantly increased with maternal age and intake of fish, EPA + DHA, and omega-3 fatty acids during pregnancy. The concentrations of hair Hg significantly increased with fish intake during pregnancy (Table 4). The concentrations of the total 70 PCBs and hair Hg in subjects with no history of parity; high household income; frequent consumption of pelagic fish, beef, or milk (≥once/week); or for non-SGA babies by weight were significantly higher than those in subjects with a history of parity; low income; infrequent consumption of pelagic fish, beef, or milk; or SGA babies by weight, respectively (Table 4). The newborn anthropometric measurements significantly increased with maternal height, prepregnancy weight, male sex, birth by vaginal delivery, and increasing gestational age (Supplementary Table 3). Incidences of SGA babies by weight and length significantly reduced with increased maternal prepregnancy weight and male sex (Supplementary Table 4).

We found no associations between the concentrations of estrogenic PCBs, antiestrogenic PCBs, dioxin-like PCBs, NDL PCBs, or hair Hg and newborn anthropometric measurements of birth weight, length, chest circumference, and head circumference in the multiple linear regression models with or without adjustment for factors (Supplementary Table 5). As shown in Table 5, we found no significant associations of SGA by weight with any quartile of estrogenic, antiestrogenic, dioxin-like, or NDL PCB levels, for all models. We also found no significant associations between the incidence of SGA by length and levels of estrogenic PCBs, antiestrogenic PCBs, dioxin-like PCBs, NDL PCBs, and hair Hg in all models. The adjusted odds ratios (ORs) for SGA by weight among the third (OR: 0.12, 95% confidence interval [95% CI]: 0.02–0.68), and fourth quartiles (OR: 0.17, 95% CI: 0.04–0.79) for hair Hg significantly reduced as compared with those in the first quartile (reference) with a significant trend (Table 5). The overall results analyzed by using regression analyses remained statistically significant after adjusting for omega-3 fatty acids (Table 5, Supplementary Table 5), and EPA + DHA, AA, omega-6 fatty acids, fish intake, fatty fish intake, and frequent consumption of pelagic fish, beef, and milk (data not shown). Additionally, we found no interaction effect of PCBs or Hg and omega-3 fatty acids on SGA risk (Table 5), as well as EPA + DHA, AA, and omega-6 fatty acids on birth weight, birth length, chest circumference, head circumference, and SGA risk (data not shown).

PCB 153, 156, 118, and 74 were detected in all subjects, and PCB 77 was detected in 64% of the subjects. The median concentrations of PCB 153, 156, 118, 74, and 77 were 21.4, 1.95, 5.78, 3.12, and 0.011 ng/g lipid, respectively. The contribution rates of PCB 153, 156, 118, 74, and 77 according to total PCBs were 20.3%, 1.8%, 5.4%, 3.0%, and 0.01%, respectively. PCB 153 was the main contributor to PCB exposure in this study (Supplementary Table 2). In congener-specific analyses, after sample values below the detection limit were assigned a value of one-half the detection limit, associations between PCB 153, 156, 118, 74, or 77 and birth size were evaluated by regression analyses with adjustment for confounding factors. There were no associations between concentrations of specific PCB congeners and newborn anthropometric measurements or the incidence of babies born SGA in any of the regression analyses (data not shown).

4. Discussion

Prenatal exposure to PCBs and birth size

We found that prenatal exposure to PCBs, including antiestrogenic PCBs as well as specific PCB congeners, has no association with newborn anthropometric measurements at birth, or the incidence of babies born SGA after adjusting for confounding factors, including hair Hg, demographic characteristics, socioeconomic status, and maternal level of LCPUFAs. Similar results were obtained when examining only subjects with a normal birth weight and gestation period.

Median concentrations of PCB 153 have been reported with a wide range, from 10.7 ng/g lipid weight in a Poland cohort to 450 ng/g lipid in the maternal serum of a Faroe Island cohort (Grandjean et al., 2001; Hertz-Picciotto et al., 2005; Sonneborn et al., 2008; Wojtyniak et al., 2010). Concerning the exposure levels among the general population in Japan, the maternal PCB 153 level of 21.0 ng/g lipid in this study seemed to be comparable to that of 15.9 ng/g lipid (Nakamura et al., 2008) and 16.0 ng/g lipid (Hisada et al., 2014) measured in pregnant women in previous studies. Hisada (2014) described that no association was observed between prenatal exposure to PCBs and birth size, and the levels of PCB exposure among the general population in this study was considerably lower than that among European (Wojtyniak et al., 2010) and American populations (Hertz-Picciotto et al., 2005), in which a significant negative association with prenatal exposure to PCBs and birth size was found. Therefore, one of the reasons for the inconsistent results may be the difference in PCB exposure level. Murphy (2010) reported no association between prenatal exposure to antiestrogenic PCBs and birth weight of newborns of fish anglers, which is consistent with our findings. The estrogenic/antiestrogenic activities of PCBs have been demonstrated in *in vitro* and *in vivo* models; however, their affinity for estrogens and xenoestrogens are two to five times lower than that of natural hormones (Decastro et al. 2006). This suggests that the concentrations of estrogenic/antiestrogenic PCBs in our study may not be at levels too low to see any adverse effects on birth size but rather indicate a true biological effect.

A European meta-analysis with a pooled dataset including populations with a low PCB exposure described that birth weight reduced because of PCB 153 in cord serum (El Majidi et al., 2012; Casas et al., 2015). However, a systematic analysis of 20 epidemiological studies described that the observed discrepancies in the concentration–response relation between prenatal PCB exposure and birth weight could not be attributed conclusively to a difference in biological PCB levels (El Majidi et al., 2012). In fact, in Inuit children exposed to high concentrations of PCBs, a lack of association between PCB 153 in cord blood and birth size was observed (Dallaire et al., 2014). As one of the possible explanations, the beneficial nutrients from fish/seafood intake may have an opposite action to the toxic effects of PCBs (Mahaffey, 2004; Halldorsson et al., 2008; Ramon et al., 2009). In the Danish National Birth Cohort of subjects with 70 ng/g lipid of the median PCB 153 and 5 g/day of median fatty fish intake from the FFQ, inverse associations were observed between maternal PCB levels and birth weight (Halldorsson et al., 2008). In a Faroe Island cohort of subjects, higher concentrations of PCB 153 and PUFAs than that in our study were found, and a negative effect of maternal EPA and no effect of PCB exposure on birth weight were observed (Grandjean et al., 2001). We found no association between maternal levels of LCPUFAs and birth weight, birth length, chest circumference, and head circumference or SGA risk in this study. However, our previous study on the same cohort suggested that maternal EPA might affect infant chest circumference (Jia et al., 2014). It is difficult to compare our results with those of other studies because of substantial differences in the exposure levels, profiles of fish/seafood intake, and contribution rate of fish/seafood to the overall PCB exposure level. However, we have provided additional data to support the finding that low exposure to PCBs is likely insufficient to cause a negative effect on fetal growth taking into account maternal LCPUFAs.

Prenatal exposure to MeHg and birth size

Our findings suggest that prenatal exposure to MeHg has no association with newborn anthropometric measurements, although the incidence of babies born SGA by weight may reduce with higher concentrations of Hg in hair. The maternal hair Hg level of 1.41 μg/g at delivery in our population was comparable to that of 1.96 μg/g (Suzuki et al., 2010) and 1.62 μg/g in pregnant women (Sakamoto et al., 2012), and that of 1.43 μg/g in nonpregnant women from the general population in Japan (Yasutake et al., 2003), in which the effect on birth size was not evaluated.

Our finding is consistent with the results of several epidemiological studies that also showed a lack of significant association between parental exposure to MeHg and birth weight (Drouillet-Pinard et al., 2010; Gundacker et al., 2010; Ramirez et al., 2000; Ramon et al., 2009; van Wijngaarden et al., 2014). However, two different studies described adverse effects from prenatal exposure to MeHg in relation to birth size taking into account maternal fish intake (Ramon et al., 2009; Vejrup et al., 2014). In a study in Spain in which the subjects had a mean total Hg of 9.4 μg/L in cord blood and a mean fish intake of 36 g/day, the concentrations of total Hg increased with reduced birth weight and increased the risk of being born SGA for length but not SGA for weight (Ramon et al., 2009). One possible explanation for the inconsistent findings is that the subjects of our study more frequently consumed fatty fish than the subjects of the Spanish study. Fatty fish is known to be the main source of PUFAs (Leventakou et al., 2014). In study in the Republic of Seychelles on subjects with a mean hair MeHg of 5.9 μg/g and a median omega-3 fatty acid level of 30 μg/mL, no association was observed between MeHg or PUFAs and birth weight (van Wijngaarden et al., 2014). In a Norwegian study of subjects with 1.45 μg/day median estimated dietary Hg and 6 g/day fatty fish intake, a positive effect of maternal fish/seafood intake and a negative effect of Hg exposure on birth weight were observed (Vejrup et al., 2014). Our study subjects had 23.3 g/day median fatty fish intake and 43 μg/mL median omega-3 fatty acids, which were higher than that found in the Seychelles and Norwegian studies. The beneficial effect of essential nutrition in our study may mask the adverse effects of MeHg on birth size, as observed in the Norwegian study.

On the other hand, our finding that the risk of SGA by weight reduced at higher concentrations of Hg in hair remained significant after adjustment for the concentrations of LCPUFAs. A plausible physiological mechanism underlying our findings should be investigated. To our knowledge, no previous studies have reported a reasonable assumption about the direct protective role of low MeHg exposure *in utero* on fetal growth. As another possible explanation, the association between higher Hg in hair and reduced risk of SGA by weight may be confounded by an unobserved common factor. In fact, biochemical observations showed that selenium, one of the essential micronutrients for fetal growth, plays a protective role against Hg toxicity (Zahir et al., 2005; Chen et al., 2006). Because our findings of the impact of prenatal MeHg exposure on fetal growth even at low levels are not conclusive, we consider continuous risk assessment as important among our population in which the fourth quartile included subjects (n = 59) with hair Hg concentrations >2.2 μg/g, which corresponds to the provisionally tolerable MeHg intake level as set by the Food and Agriculture Organization and the World Health Organization in 2006 (1.6 μg/kg body weight/week) (FAO/WHO, 2006).

Strengths and limitations

The strengths of this study are as follows: (1) the assessment of biomarkers of LCPUFAs; (2) the detection of 70 congeners of PCBs that were reported as the most predominant congeners in the Japanese population (Todaka et al., 2008ab); (3) a high PCB detection rate of 98.8%, and the ability to group and analyze them based on bioactivities such as estrogen/antiestrogen, and dioxin-like effects; (4) various demographic, socioeconomic, behavioral, and dietary data were collected prospectively, minimizing recall error; and (4) evaluation with multiple linear models adjusted for confounding effects between demographic characteristics, socioeconomic status, maternal diet, and PCB or Hg contamination in fish/seafood. We propose that additional studies be conducted to assess whether exposure to PCBs and MeHg in the general population is at levels insufficient to cause impaired fetal growth in humans. The mothers included in this study were older at delivery, had heavier weight at prepregnancy, had lower smoking rate during pregnancy, and had a later sampling period than mothers who were not included in analysis. However, we considered that the potential selection bias was limited because we found no difference in PCB and Hg exposure levels between the mothers included and those not included in this study. The children included in this study had a higher gestational age, weight, length, chest circumference, and head circumference, and lower SGA for length at birth than those children who were not included in the analysis. A potential selection bias may have resulted from the effect on healthy children, in whom the influence of contaminants on birth size may have been underestimated. We cannot exclude the possibility that our findings occur by chance because of the small number of babies born SGA. A further study with a larger sample size is needed to evaluate the effects of prenatal exposure to PCBs and MeHg on the later growth of children.

Conclusion

No overall association was found between mercury concentrations and birth weight, length, chest circumference, and head circumference. We observed that the risk of SGA by weight reduced with increasing mercury concentration in hair in regression analyses with adjustment for polyunsaturated fatty acids. In Japanese pregnant women, who are known to have a high frequency of fish consumption, the beneficial effect of essential nutrition may mask the adverse effects of MeHg on birth size, as was observed in a previous European study. On the other hand, we cannot exclude the possibility that prenatal MeHg exposure may adversely influence fetal growth even at low levels; therefore, a follow-up study is needed to evaluate the effect of prenatal MeHg exposure on the later growth of children. The concentrations of estrogenic, antiestrogenic, dioxin-like, and NDL PCBs had no association with birth weight, length, chest circumference, head circumference, and SGA risk.

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Table 1. Maternal and infant characteristics (n = 367).

Characteristics	n (%)
Maternal characteristics	
Age at delivery (years)	30.8 ± 4.8[a]
Height (cm)	158 ± 5.4[a]
Prepregnancy maternal weight (kg)	52.5 ± 8.0[a]
Parity	
0	180 (49.0)
1	146 (39.8)
2	35 (9.5)
3	6 (1.6)
Blood sampling period	
<28 weeks	21 (5.7)
28 to <36 weeks	148 (40.3)
≥36 weeks	78 (21.3)
After delivery	120 (32.7)
History of chemical hair waving	
No	260 (70.8)
Yes	107 (29.2)
Education level (years)	
≤9	7 (1.9)
10–12	147 (40.1)
13–16	208 (56.7)
≥17	5 (1.4)
Annual household income (million yen)	
<3	61 (16.6)
3 to <5	183 (49.9)
5 to <7	78 (21.3)
≥7	45 (12.3)
Tobacco smoking during pregnancy	
Nonsmoker	305 (83.1)
Smoker	62 (16.9)
Alcohol consumption during pregnancy	
No	255 (69.5)
Yes	112 (30.5)
Caffeine intake during pregnancy (mg/day)	120 (1.50, 646)[b]
Frequency of food consumption during pregnancy	
Shoreline fish	
<Once/week	198 (54.0)
≥Once/week	169 (46.0)
Pelagic fish	
<Once/week	171 (46.6)
≥Once/week	196 (53.4)
Beef	
<Once/week	274 (75.3)
≥Once/week	90 (24.7)
Pork	
<Once/week	274 (75.3)
≥Once/week	90 (24.7)
Chicken	
<Once/week	30 (8.2)
≥Once/week	337 (91.8)
Egg	
<Once/week	53 (14.4)
≥Once/week	314 (85.6)
Milk	
<Once/week	10 (2.7)
≥Once/week	356 (97.3)
Fish intake from food frequency questionnaires	
Fish intake (g/day)	38.8 (0.0, 400)[b]
Fatty fish	23.3 (0.0, 160)[b]
Lean fish	0.0 (0.0, 66.7)[b]
Shellfish	11.1 (0.0, 200)[b]
Whale	0.0 (0.0, 6.70)[b]
Infant characteristics	
Sex	

Male	173 (47.1)
Female	194 (52.9)
Type of delivery	
Vaginal birth	292 (79.3)
Cesarean section	76 (20.7)
Gestational age at birth (weeks)	39.0 ± 1.4[a]
Birth weight (g)	3073 ± 37[a]
Length (cm)	48.1 ± 1.9[a]
Chest circumference (cm)	31.5 ± 1.6[a]
Head circumference (cm)	33.3 ± 1.3[a]
SGA by weight	18 (4.9)
SGA by length	43 (11.7)

[a]Mean ± SD.
[b]Median (minimum, maximum).
SGA: small for gestational age.

Table 2. Concentrations of LCPUFA (μg/mL) in maternal blood (n = 367).

		Percentile			
	Minimum	25th	50th	75th	Maximum
EPA + DHA	3.0	20.5	32.2	47.8	163
AA	2.8	43.5	61.2	89.7	219
Omega-3 fatty acids	4.1	28.2	43.4	63.9	188
Omega-6 fatty acids	16.1	581	798	1030	2840

LCPUFA: long-chain polyunsaturated fatty acids; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; AA: arachidonic acid; Omega-3 fatty acids: EPA, DHA, α-linolenic acid (ALA); Omega-6 fatty acids: AA, linoleic acid (LA).

Table 3. Concentrations of polychlorinated biphenyls in maternal blood (PCBs; ng/g lipid) and hair mercury (μg/g) in maternal samples (n = 367).

	Minimum	Percentile			Maximum
		25th	50th	75th	
Estrogenic PCBs[a]	3.88	19.5	28.7	40.0	147
Antiestrogenic PCBs[b]	0.63	2.75	4.13	5.60	21.7
Dioxin-like PCBs[c]	1.74	7.51	11.2	15.6	49.8
Non-dioxin-like PCBs	16.0	64.8	95.7	133	445
Hair Hg	0.24	0.96	1.41	1.89	4.73

[a]PCB 52, 49, 47, 44, 70, 95, 101, 99, 110, and 153 (Cooke, 2001).
[b]PCB 37, 77, 81, 126, 169, 114, 105, and 156 (Cooke, 2001).
[c]PCB 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189 (Van den Berg et al., 2006).

Table 4. Total polychlorinated biphenyls (PCBs) and hair mercury (Hg) levels in relation to maternal and infant characteristics and polyunsaturated fatty acids (n = 367)

Characteristics		Total PCBs (ng/g lipid)		Hair Hg (μg/g)	
		r	Median (min, max)	r	Median (min, max)
Maternal characteristics					
Age at delivery (years)		0.415[a]**		0.094[a]	
Height (cm)		0.079[a]		-0.055[a]	
Prepregnancy weight (kg)		0.016[a]		-0.029[a]	
Parity	0		115 (19.6, 495)*		1.41 (0.30, 3.73)*
	≥1		102 (17.8, 354)		1.38 (0.24, 4.73)
Blood sampling period	During pregnancy		110 (17.8, 363)		1.41 (0.24, 4.73)
	After delivery		104 (27.4, 495)		1.40 (0.30, 4.30)
History of chemical hair waving	No		108 (17.8, 495)		1.37 (0.24, 4.35)
	Yes		109 (19.6, 362)		1.46 (0.30, 4.73)
Education level (years)	≤12		99.0 (17.8, 363)		1.33 (0.24, 4.35)
	>12		111 (19.6, 495)		1.42 (0.30, 4.73)
Annual household income (million yen)	<5		102 (17.8, 362)*		1.28 (0.24, 4.73)*
	≥5		123 (27.4, 495)		1.47 (0.30, 4.33)
Tobacco smoking during pregnancy	Nonsmoker		110 (17.8, 362)		1.41 (0.30, 4.35)
	Smoker		95.4 (19.6, 495)		1.39 (0.24, 4.73)
Alcohol consumption during pregnancy	No		100 (17.8, 354)		1.33 (0.31, 4.03)
	Yes		113 (27.8, 495)		1.42 (0.24, 4.73)
Caffeine intake (mg/day)		0.017[a]		-0.005[a]	
Frequency of food consumption during pregnancy					
Shoreline fish	<Once/week		101 (17.8, 362)		1.31 (0.31, 4.35)
	≥Once/week		113 (19.6, 495)		1.46 (0.24, 4.73)
Pelagic fish	<Once/week		106 (17.8, 362)		1.24 (0.24, 4.03)**
	≥Once/week		109 (27.4, 495)		1.49 (0.32, 4.73)
Beef	<Once/week		108 (17.8, 363)		1.34 (0.24, 4.73)*
	≥Once/week		107 (19.6, 495)		1.51 (0.30, 3.69)
Pork	<Once/week		85.9 (19.6, 302)		1.54 (0.66, 4.03)
	≥Once/week		109 (17.8, 495)		1.39 (0.24, 4.73)
Chicken	<Once/week		108 (31.3, 362)		1.30 (0.37, 4.03)
	≥Once/week		108 (17.8, 495)		1.41 (0.24, 4.73)
Egg	<Once/week		102 (59.0, 213)		1.28 (1.19, 1.49)
	≥Once/week		108 (17.8, 495)		1.41 (0.24, 4.73)
Milk	<Once/week		74.9 (30.2, 354)**		1.24 (0.45, 3.09)
	≥Once/week		111 (17.8, 495)		1.42 (0.24, 4.73)
Food frequency questionnaires at delivery					
Fish intake (g/day)		0.187[a]**		0.215[a]**	
Fatty fish (g/day)		0.141[a]**		0.210[a]**	
Shellfish (g/day)		0.087[a]		0.084[a]	
LCPUFA in maternal blood					
EPA + DHA		0.182[a]**		0.056[a]	
AA		0.048[a]		-0.077[a]	
Omega-3 fatty acids		0.155[a]**		0.022[a]	
Omega-6 fatty acids		0.073[a]		-0.018[a]	
Infant characteristics					
Sex	Male		111 (27.4, 362)		1.41 (0.24, 4.35)
	Female		104 (17.8, 495)		1.39 (0.30, 4.73)
Type of delivery	Vaginal birth		109 (17.8, 363)		1.43 (0.24, 4.73)
	Cesarean section		97.0 (19.6, 495)		1.24 (0.30, 4.35)
Gestational age (weeks)		0.025[a]		0.017[a]	
SGA by weight	No		108 (17.8, 495)		1.42 (0.30, 4.73)*
	Yes		98.7 (51.0, 223)		0.92 (0.24, 2.62)
SGA by length	No		109 (17.8, 495)		1.41 (0.24, 4.73)
	Yes		97 (19.6, 247)		1.24 (0.46, 3.55)

[a]r: Spearman's rank correlation coefficient.

*$p<0.05$, **$p<0.01$ by Mann–Whitney U-test and Spearman's rank correlation test.
LCPUFA: long-chain polyunsaturated fatty acids, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, AA: arachidonic acid.

Table 5. Odds ratios for babies born small for gestational age (n = 367).

		SGA by weight			SGA by length		
		Crude	Adjusted 1[a]	Adjusted 2[a]	Crude	Adjusted 1[b]	Adjusted 2[b]
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)		OR (95% CI)
Estrogenic PCBs	Quartile 1	1	1	1	1	1	1
	Quartile 2	0.41 (0.10–1.65)	0.51 (0.11–2.30)	0.56 (0.12–2.58)	1.21 (0.53–2.79)	1.48 (0.60–3.67)	1.57 (0.62–4.00)
	Quartile 3	0.27 (0.05–1.34)	0.40 (0.07–2.24)	0.42 (0.07–2.41)	0.38 (0.13–1.14)	0.36 (0.11–1.17)	0.37 (0.11–1.22)
	Quartile 4	0.85 (0.27–2.62)	1.95 (0.46–8.18)	1.88 (0.45–7.83)	1.00 (0.42–2.36)	0.81 (0.28–2.29)	0.68 (0.23–2.03)
	p for trend	0.662	0.694	0.696	0.509	0.334	0.197
	P for interaction			0.335			0.211
Antiestrogenic PCBs	Quartile 1	1	1	1	1	1	1
	Quartile 2	0.99 (0.28–3.54)	1.16 (0.29–4.68)	1.31 (0.32–5.34)	1.31 (0.56–3.05)	1.44 (0.58–3.57)	1.53 (0.61–3.84)
	Quartile 3	0.57 (0.13–2.47)	0.99 (0.20–4.87)	1.07 (0.21–5.47)	0.50 (0.18–1.42)	0.52 (0.17–1.63)	0.50 (0.16–1.57)
	Quartile 4	1.00 (0.28–3.58)	1.95 (0.44–8.55)	1.89 (0.43–8.29)	1.10 (0.46–2.65)	1.07 (0.38–2.98)	0.94 (0.32–2.73)
	p for trend	0.824	0.523	0.511	0.71	0.718	0.550
	P for interaction			0.317			0.249
Dioxin-like PCBs	Quartile 1	1	1	1	1	1	1
	Quartile 2	1.23 (0.36–4.18)	1.54 (0.39–6.05)	1.93 (0.48–7.77)	1.34 (0.57–3.13)	1.62 (0.64–4.09)	1.79 (0.70–4.56)
	Quartile 3	0.38 (0.07–2.02)	0.66 (0.11–4.06)	0.64 (0.10–4.01)	0.60 (0.22–1.62)	0.68 (0.22–2.07)	0.62 (0.20–1.94)
	Quartile 4	1.01 (0.28–3.62)	2.20 (0.48–10.1)	2.01 (0.44–9.19)	1.01 (0.42–2.47)	1.01 (0.35–2.90)	0.83 (0.28–2.48)
	p for trend	0.669	0.570	0.560	0.617	0.714	0.260
	P for interaction			0.155			0.096
Non-dioxin like PCBs	Quartile 1	1	1	1	1	1	1
	Quartile 2	0.48 (0.12–1.97)	0.56 (0.12–2.59)	0.57 (0.12–2.65)	1.71 (0.73–3.99)	1.99 (0.79–5.01)	2.02 (0.79–5.17)
	Quartile 3	0.81 (0.24–2.77)	1.36 (0.32–5.69)	1.47 (0.34–6.42)	0.47 (0.15–1.42)	0.49 (0.15–1.64)	0.49 (0.14–1.66)
	Quartile 4	0.64 (0.18–2.36)	1.21 (0.24–6.22)	1.18 (0.23–5.96)	1.21 (0.50–2.97)	1.00 (0.33–3.05)	0.88 (0.28–2.76)
	p for trend	0.654	0.759	0.752	0.697	0.483	0.345
	P for interaction			0.417			0.461
Hair Hg	Quartile 1	1	1	1	1	1	1
	Quartile 2	0.28 (0.07–1.04)	0.24 (0.06–1.00)	0.22 (0.05–0.94)*	0.68 (0.29–1.62)	0.69 (0.27–1.76)	0.71 (0.27–1.84)
	Quartile 3	0.18 (0.04–0.86)*	0.12 (0.02–0.68)*	0.11 (0.02–0.64)*	0.60 (0.25–1.47)	0.58 (0.22–1.54)	0.57 (0.21–1.55)
	Quartile 4	0.28 (0.07–1.05)	0.17 (0.04–0.79)*	0.16 (0.03–0.77)*	0.69 (0.29–1.64)	0.65 (0.24–1.76)	0.61 (0.22–1.73)
	p for trend	0.023	0.014	0.014	0.359	0.362	0.324
	P for interaction			0.965			0.562

The odds ratios (OR) and 95% confidence intervals (95% CI) for babies born small for gestational age (SGA) were calculated by using the first quartile as the reference category.

p for trend: linear trend across quartiles.

Adjusted 1[a]: adjusted for maternal age, maternal height, prepregnancy maternal weight, tobacco smoking during pregnancy, alcohol consumption during pregnancy, household income, blood sampling period, and total PCBs or hair Hg.

Adjusted 2[a]: adjusted for omega-3 fatty acids in addition to the adjusted factors in Adjusted 1[a]

Adjusted 1[b]: adjusted for maternal age, maternal height, prepregnancy maternal weight, tobacco smoking during pregnancy, alcohol consumption during pregnancy, household income, blood sampling period, parity, infant sex, and total PCBs or hair Hg.

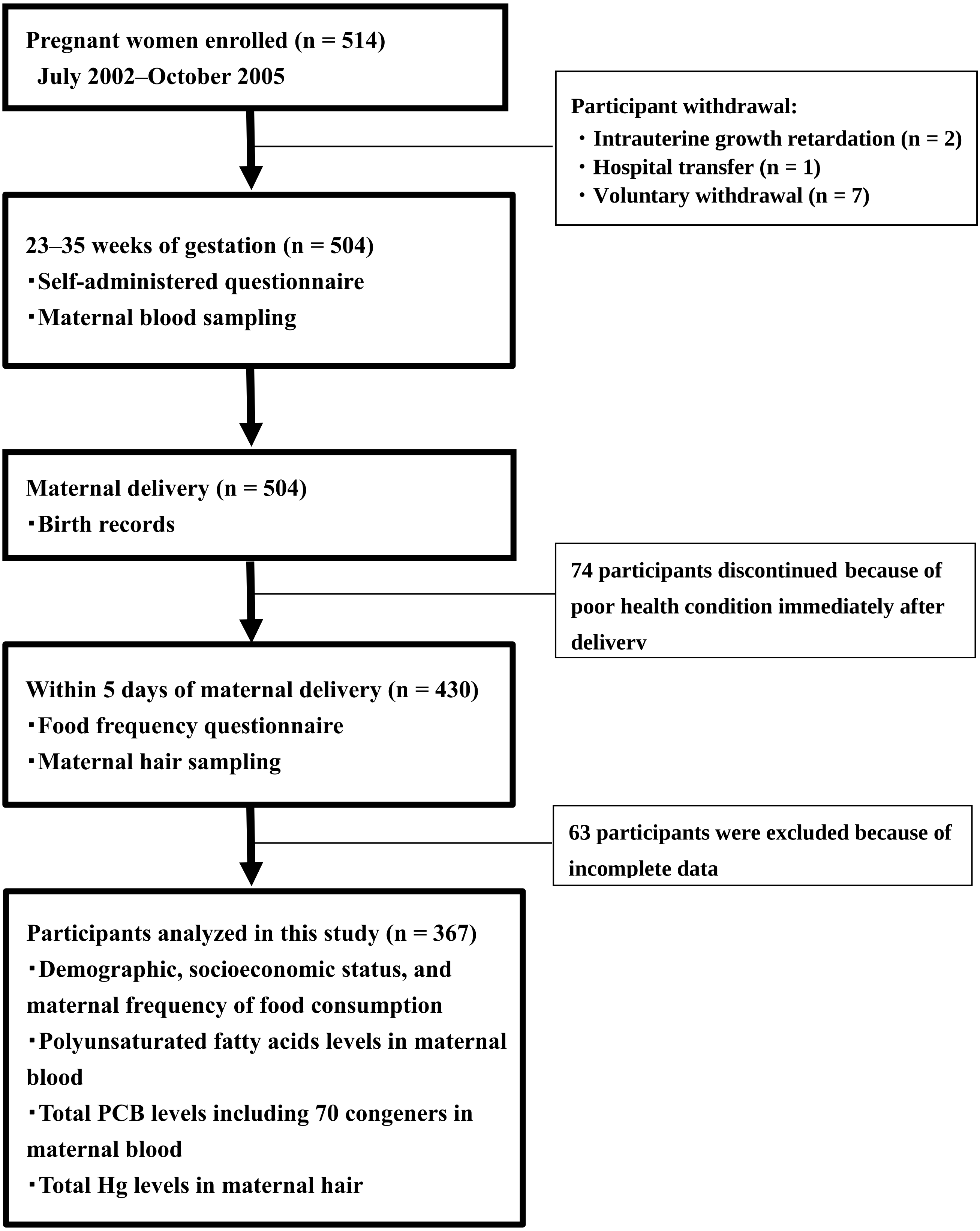
Adjusted 2[b]: adjusted for omega-3 fatty acids in addition to the adjusted factors in Adjusted 1[b]

P for interaction: introduced for interaction terms of quartile PCBs or quartile Hg, and quartile omega-3 fatty acids, in addition to the adjusted factors in Adjusted 2[a] or Adjusted 2[b].

*p<0.05.

Figure legends

Figure 1. Research overview.



Supplementary Material

Supplementary Table 1. Food frequency questionnaire.

Question 1: Frequency of fish consumption (choose one of the following options)

1. Often. Please indicate the number of fish servings per day.
2. Sometimes. Please provide an estimated frequency of fish consumption.
3. Rarely
4. Never

Question 2: Portion size of fish in one serving (choose one of the following options)

1. <50 g
2. 50–100 g
3. 100–150 g
4. 150–200 g
5. >200 g
6. Unknown

Question 3: Type of fish frequently consumed (choose as many of the following as applicable)

Tuna, bonito, salmon, yellowtail, sea bream, flatfish, flounder, sardine, mackerel, saury, horse mackerel, eel, carp, sweetfish, crucian carp, cuttlefish, octopus, crab, shrimp, shellfish, whale, fish products, Atka mackerel, shishamo smelt, Pacific cod, Pacific herring, trout

Supplementary Table 2. Analysis results of 70 PCB congeners (ng/g lipid) (n = 367).

PCB congeners	Detection limit	Detection rate (%)	Median (25th–75th)	22′44′55′-HexaCB(#153)	Total 70 PCBs	
				r	r	Contribution rate (%)
245-TriCB(#29)	0.01	32.4	0.01 (0.01–0.03)	0.090	0.075	0.02
244′-TriCB(#28)	0.01	99.7	1.08 (0.78–1.50)	0.373**	0.393**	0.99
344′-TriCB(#37)	0.01	21.8	0.01 (0.01–0.01)	0.070	0.126*	0.27
22′55′-TetraCB(#52)	0.01	94.8	0.62 (0.36–0.90)	0.179**	0.225**	0.58
22′45′-TetraCB(#49)	0.01	92.1	0.17 (0.09–0.26)	0.122*	0.197**	0.16
22′44′-TetraCB(#47)	0.01	91.3	0.34 (0.20–0.50)	0.186**	0.263**	0.31
22′35′-TetraCB(#44)	0.01	84.2	0.27 (0.10–0.38)	0.113*	0.149**	0.22
23′4′6-TetraCB(#71)	0.01	86.4	0.10 (0.04–0.17)	-0.017	0.022	0.10
234′5-TetraCB(#63)	0.01	93.5	0.05 (0.03–0.07)	0.542**	0.572**	0.05
244′5-TetraCB(#74)	0.01	100	3.12 (2.20–4.55)	0.867**	0.862**	3.03
23′4′5-TetraCB(#70)	0.01	87.5	0.15 (0.09–0.20)	0.207**	0.205**	0.13
23′44′-TetraCB(#66)	0.01	100	0.64 (0.43–0.98)	0.643**	0.652**	0.63
233′4′-/2344′TetraCBs(#56/60)	0.01	98.1	0.27 (0.18–0.40)	0.568**	0.577**	0.25
22′35′6-PentaCB(#95)	0.01	96.7	0.37 (0.22–0.56)	0.318**	0.341**	0.35
22′355′-PentaCB(#92)	0.01	97.0	0.26 (0.16–0.41)	0.578**	0.599**	0.27
22′455′-PentaCB(#101)	0.01	99.5	0.64 (0.42–0.91)	0.552**	0.588**	0.62
22′44′5-PentaCB(#99)	0.01	100	3.97 (2.66–5.51)	0.893**	0.888**	3.72
234′56-PentaCB(#117)	0.01	98.9	0.25 (0.17–0.36)	0.714**	0.769**	0.24
22′345′-PentaCB(#87)	0.01	97.5	0.25 (0.18–0.35)	0.529**	0.590**	0.24
22′344′-PentaCB(#85)	0.01	93.5	0.09 (0.05–0.13)	0.411**	0.458**	0.09
233′4′6-PentaCB(#110)	0.01	89.9	0.17 (0.10–0.26)	0.338**	0.379**	0.16
233′4′5-PentaCB(#107)	0.01	99.2	0.28 (0.17–0.43)	0.734**	0.776**	0.28
22′355′6-HexaCB(#151)	0.01	99.2	0.32 (0.22–0.53)	0.641**	0.659**	0.36
22′33′56′-HexaCB(#135)	0.01	97.0	0.14 (0.09–0.23)	0.628**	0.628**	0.15
22′34′56-HexaCB(#147)	0.01	93.7	0.12 (0.07–0.18)	0.693**	0.694**	0.12
22′344′6-/22′34′5′6-HexaCB(#139/149)	0.01	91.3	0.24 (0.13–0.37)	0.486**	0.479**	0.23
22′33′56-HexaCB(#134)	0.01	35.7	0.01 (0.01–0.02)	0.030	0.044	0.01
233'55′6-HexaCB(#165)	0.01	0				
22′34′55′-HexaCB(#146)	0.01	100	2.99 (2.02–4.32)	0.973**	0.960**	2.89
22′33′46′-HexaCB(#132)	0.01	85.0	0.11 (0.06–0.17)	0.376**	0.354**	0.11
22′44′55′-HexaCB(#153)	0.01	100	21.4 (14.5–31.2)		0.982**	20.3
22′3455′-HexaCB(#141)	0.01	83.1	0.09 (0.05–0.16)	0.471**	0.495**	0.10
22′344′5-HexaCB(#137)	0.01	100	0.77 (0.52–1.06)	0.949**	0.949**	0.70
22′33′45′-HexaCB(#130)	0.01	100	0.66 (0.44–0.96)	0.909**	0.917**	0.63
233′4′5′6-HexaCB(#164)	0.01	100	3.99 (2.63–5.91)	0.842**	0.872**	3.91
22′344′5′-HexaCB(#138)	0.01	100	11.9 (7.84–16.7)	0.974**	0.967**	11.1
22′33′44′-HexaCB(#128)	0.01	99.5	0.32 (0.20–0.48)	0.662**	0.714**	0.33
22′33′566′-HptaCB(#179)	0.01	86.4	0.07 (0.04–0.13)	0.503**	0.515**	0.08
22′33′55′6-HptaCB(#178)	0.01	99.7	1.31 (0.90–1.90)	0.915**	0.935**	1.32
22′344′56-HptaCB(#182)	0.01	100	6.06 (3.86–8.52)	0.940**	0.965**	5.93
22′344′5′6-HptaCB(#183)	0.01	99.7	1.65 (1.09–2.42)	0.928**	0.945**	1.64
22′344′56-HptaCB(#181)	0.01	71.1	0.03 (0.01–0.05)	0.379**	0.399**	0.03
22′33′4′56-HptaCB(#177)	0.01	99.7	1.45 (0.99–2.13)	0.907**	0.938**	1.45
22′33′455′-HptaCB(#172)	0.01	98.9	0.68 (0.44–1.01)	0.858**	0.904**	0.68
22′344′55′-HptaCB(#180)	0.01	100	13.0 (8.63–19.4)	0.892**	0.935**	12.9
233′44′5′6-HptaCB(#191)	0.01	90.7	0.16 (0.11–0.25)	0.727**	0.759**	0.16
22′33′44′5-HptaCB(#170)	0.01	100	4.57 (3.05–6.42)	0.879**	0.923**	4.46
22′33′55′66′-OctaCB(#202)	0.01	99.7	0.49 (0.31–0.73)	0.828**	0.810**	0.48
22′33′45′66′-OctaCB(#200)	0.01	91.6	0.09 (0.06–0.15)	0.714**	0.708**	0.10
22′33′45′66′-/22′33′455′6-OctaCB(#201/198)	0.01	100	1.88 (1.22–2.71)	0.881**	0.880**	1.82

22′344′55′6-OctaCB(#203)	0.01	100	1.63 (1.07–2.37)	0.859**	0.850**	1.58
22′33′44′56-OctaCB(#195)	0.01	100	0.42 (0.30–0.63)	0.877**	0.857**	0.41
22′33′44′55′-OctaCB(#194)	0.01	100	1.64 (1.11–2.37)	0.866**	0.871**	1.57
233′44′55′6-OctaCB(#205)	0.01	87.2	0.07 (0.05–0.10)	0.619**	0.631**	0.07
22′33′455′66′-NonaCB(#208)	0.01	98.1	0.22 (0.14–0.33)	0.737**	0.707**	0.22
22′33′44′566′-NonaCB(#207)	0.01	96.2	0.11 (0.07–0.17)	0.694**	0.656**	0.11
22′33′44′55′6-NonaCB(#206)	0.01	99.7	0.54 (0.38–0.75)	0.827**	0.816**	0.51
22′33′44′55′66′-DecaCB(#209)	0.01	100	0.46 (0.33–0.61)	0.776**	0.763**	0.42
344′5-TeCB(#81)	0.01	0				
33′44′-TeCB(#77)	0.01	64.3	0.01 (0.01–0.01)	0.266**	0.328**	0.01
33′44′5-PentaCB(#126)	0.01	96.5	0.03 (0.02–0.05)	0.726**	0.763**	0.03
33′44′55′-HexaCB(#169)	0.01	94.6	0.02 (0.02–0.03)	0.777**	0.817**	0.02
2′344′5-PetaCB(#123)	0.01	98.1	0.11 (0.07–0.15)	0.727**	0.750**	0.10
23′44′5-PetaCB(#118)	0.01	100	5.78 (3.82–8.22)	0.896**	0.902**	5.43
2344′5-PetaCB(#114)	0.01	98.9	0.34 (0.23–0.48)	0.880**	0.887**	0.32
233′44′-PetaCB(#105)	0.01	100	1.40 (0.98–2.06)	0.847**	0.859**	1.35
23′44′55′-HexaCB(#167)	0.01	99.7	0.69 (0.46–0.98)	0.945**	0.950**	0.65
233′44′5-HexaCB(#156)	0.01	100	1.95 (1.32–2.72)	0.931**	0.932**	1.82
233′44′5′-HexaCB(#157)	0.01	99.7	0.48 (0.33–0.66)	0.916**	0.910**	0.45
233′44′55′-HptaCB(#189)	0.01	99.2	0.24 (0.17–0.34)	0.811**	0.803**	0.22
Total PCBs			108 (72.7–149)			

r was calculated by using Spearman's rank correlation coefficient.
*p < 0.05, **p < 0.01.

Supplementary Table 3. Relation between newborn anthropometric measurements and subject characteristics (n = 367).

Characteristics		Birth weight (g)		Length (cm)		Chest circumference (cm)		Head circumference (cm)	
		r	Mean ± SD	r	Mean ± SD	r	Mean ± SD	r	Mean ± SD
Maternal characteristics									
Age at delivery (years)		–0.010[a]		–0.033[a]		0.021[a]		0.023[a]	
Height (cm)		0.127[a]*		0.153[a]**		0.123[a]*		0.116[a]*	
Pre-pregnancy weight (kg)		0.162[a]*		0.140[a]**		0.119[a]*		0.106[a]*	
Parity	0		3055 ± 375		48.1 ± 2.0		31.4 ± 1.7		33.2 ± 1.4
	≥1		3092 ± 368		48.2 ± 1.9		31.6 ± 1.4		33.4 ± 1.3
Education level (years)	≤12		3056 ± 384		48.0 ± 1.8		31.4 ± 1.6		33.2 ± 1.3
	>12		3086 ± 363		48.2 ± 2.0		31.6 ± 1.5		33.4 ± 1.3
Annual household income (million yen)	<5		3084 ± 365		48.1 ± 2.0		31.5 ± 1.5		33.3 ± 1.3
	≥5		3052 ± 385		48.2 ± 1.7		31.5 ± 1.7		33.3 ± 1.3
Tobacco smoking during pregnancy	Nonsmoker		3085 ± 384		48.2 ± 2.0		31.5 ± 1.6		33.4 ± 1.4
	Smoker		3019 ± 299		47.9 ± 1.5		31.4 ± 1.2		33.1 ± 1.2
Alcohol consumption during pregnancy	No		3059 ± 386		48.0 ± 2.0		31.4 ± 1.7		33.3 ± 1.4
	Yes		3107 ± 335		48.3 ± 1.7		31.7 ± 1.2		33.4 ± 1.3
Caffeine intake (mg/day)		–0.072[b]		–0.043[b]		–0.091[b]		–0.001[b]	
Frequency of food consumption during pregnancy									
Shoreline fish	<Once/week		3096 ± 341		48.2 ± 1.7		31.5 ± 1.4		33.4 ± 1.3
	≥Once/week		3047 ± 404		48.0 ± 2.2		31.5 ± 1.7		33.2 ± 1.3
Pelagic fish	<Once/week		3060 ± 380		48.0 ± 1.8		31.4 ± 1.5		33.3 ± 1.3
	≥Once/week		3086 ± 365		48.2 ± 2.0		31.6 ± 1.6		33.3 ± 1.4
Beef	<Once/week		3061 ± 384		48.0 ± 1.8		31.5 ± 1.6		33.3 ± 1.3
	≥Once/week		3112 ± 334		48.3 ± 2.4		31.6 ± 1.4		33.3 ± 1.3
Pork	<Once/week		3061 ± 384		48.0 ± 1.8		31.5 ± 1.6		33.3 ± 1.3
	≥Once/week		3112 ± 334		48.3 ± 2.4		31.6 ± 1.4		33.3 ± 1.3
Chicken	<Once/week		2969 ± 325		47.5 ± 1.5*		31.2 ± 1.5		33.1 ± 1.2
	≥Once/week		3083 ± 374		48.2 ± 2.0		31.5 ± 1.6		33.3 ± 1.3
Egg	<Once/week		3002 ± 384		47.5 ± 2.9		31.3 ± 1.7		33.2 ± 1.4
	≥Once/week		3085 ± 369		48.2 ± 1.7		31.5 ± 1.5		33.3 ± 1.3
Milk	<Once/week		3094 ± 410		48.3 ± 1.7		31.5 ± 1.2		33.3 ± 1.4
	≥Once/week		3074 ± 370		48.1 ± 1.9		31.5 ± 1.6		33.3 ± 1.3
Food frequency questionnaire at delivery[b]									
Fish intake (g/day)		0.000		0.025		–0.021		–0.087	
Fatty fish intake (g/day)		0.057		0.056		0.067		-0.018	
Shellfish (g/day)		0.013		0.043		-0.030		-0.074	
Fatty acid in maternal blood[b]									
EPA + DHA		-0.063		-0.034		-0.048		-0.048	
AA		-0.101		-0.078		-0.103		-0.075	
Omega-3 fatty acids		-0.075		-0.053		-0.063		-0.047	
Omega-6 fatty acids		-0.089		-0.094		-0.068		-0.046	
Infant characteristics									
Sex	Male		3132 ± 362**		48.4 ± 2.1**		31.7 ± 1.4*		33.7 ± 1.3**
	Female		3022 ± 373		47.8 ± 1.8		31.3 ± 1.7		32.9 ± 1.3
Type of delivery	Vaginal birth		3119 ± 343**		48.3 ± 1.9**		31.7 ± 1.4**		33.3 ± 1.3
	Cesarean section		2900 ± 423		47.2 ± 1.9		30.9 ± 1.9		33.3 ± 1.6
Gestational age (weeks)		0.467[a]**		0.395[a]**		0.421[a]**		0.221[a]**	

[a]r: Pearson correlation coefficient.
[b]r: Spearman's rank correlation coefficient.
*p < 0.05, **p < 0.01 by the Pearson correlation and Spearman's rank correlation test and one-way analysis of variance.

Supplementary Table 4. Relation between small for gestational age (SGA) status and subject characteristics (n = 367).

Characteristics		SGA by weight		SGA by length	
		No n (%)	Yes n (%)	No n (%)	Yes n (%)
Maternal characteristics					
Age at delivery (years)		30.8 ± 4.8[a]	30.9 ± 4.5[a]	30.8 ± 4.8[a]	30.8 ± 4.8[a]
Height (cm)		158 ± 5.3[a]	157 ± 6.7[a]	158 ± 5.2[a]	158 ± 6.5[a]
Pre-pregnancy weight (kg)		52.7 ± 8.0[a]	47.9 ± 5.7[a]*	52.9 ± 7.9[a]	49.7 ± 7.8[a]*
Parity	0	169 (93.9)	11 (6.1)	156 (86.7)	24 (13.3)
	≥1	180 (96.3)	7 (3.7)	168 (89.8)	19 (10.2)
Education level (years)	≤12	143 (92.9)	11 (7.1)	129 (83.8)	25 (16.2)*
	>12	206 (96.7)	7 (3.3)	195 (91.5)	18 (8.5)
Annual household income (million yen)	<5	232 (95.1)	12 (4.9)	212 (86.9)	32 (13.1)
	≥5	117 (95.1)	6 (4.9)	112 (91.1)	11 (8.9)
Tobacco smoking during pregnancy	Nonsmoker	289 (94.8)	16 (5.2)	270 (88.5)	35 (11.5)
	Smoker	60 (96.8)	2 (3.2)	54 (87.1)	8 (12.9)
Alcohol consumption during pregnancy	No	243 (95.3)	12 (4.7)	229 (89.8)	26 (10.2)
	Yes	106 (94.6)	6 (5.4)	95 (84.8)	17 (15.2)
Caffeine intake (mg/day)		117 (1.50, 646)[b]	128 (2.00, 395)[b]	115 (1.50, 646)[b]	135 (2.00, 427)[b]
Frequency of food consumption during pregnancy					
Shoreline fish	<Once/week	191 (96.5)	7 (3.5)	178 (89.9)	20 (10.1)
	≥Once/week	158 (93.5)	11 (6.5)	146 (86.4)	23 (13.6)
Pelagic fish	<Once/week	161 (94.2)	10 (5.8)	147 (86.0)	24 (14.0)
	≥Once/week	188 (95.9)	8 (4.1)	177 (90.3)	19 (9.7)
Beef	<Once/week	259 (94.5)	15 (5.5)	242 (88.3)	32 (11.7)
	≥Once/week	87 (96.7)	3 (3.3)	79 (87.8)	11 (12.2)
Pork	<Once/week	30 (100)	0 (0)	27 (90.0)	3 (10.0)
	≥Once/week	319 (94.7)	18 (5.3)	297 (88.1)	40 (11.9)
Chicken	<Once/week	52 (98.1)	1 (1.9)	48 (90.6)	5 (9.4)
	≥Once/week	297 (94.6)	17 (5.4)	276 (87.9)	38 (12.1)
Egg	<Once/week	10 (100)	0 (0)	9 (90.0)	1 (10.0)
	≥Once/week	338 (94.9)	18 (5.1)	314 (88.2)	42 (11.8)
Milk	<Once/week	48 (94.1)	3 (5.9)	43 (84.3)	8 (15.7)
	≥Once/week	301 (95.3)	15 (4.7)	281 (88.9)	35 (11.1)
Food frequency questionnaire at delivery[b]					
Fish intake (g/day)		40.0 (0.83, 400)	41.7 (1.67, 100)	37.5 (0.83, 250)	50.0 (1.67, 400)
Fatty fish intake (g/day)		25.0 (0.00, 160)	20.0 (1.11, 75.0)	25.0 (0.00, 160)	20.0 (0.00, 133)
Shellfish (g/day)		11.1 (0.00, 200)	12.2 (0.00, 32)	11.1 (0.00, 100)	7.5 (0.00, 200)
Fatty acid in maternal blood[b]					
EPA + DHA		31.8 (2.96, 163)	33.1 (9.44, 110)	31.7 (2.96, 163)	33.2 (5.47, 121)
AA		60.8 (2.77, 219)	90.2 (9.20, 158)	61.9 (2.77, 219)	57.8 (3.71, 187)

Omega-3 fatty acids		43.5 (4.09, 188)	41.4 (12.0, 144)	43.2 (4.09, 179)	45.2 (6.09, 188)
Omega-6 fatty acids		790 (16.1, 2836)	911 (68.2, 1552)	798 (16.1, 2836)	811 (36.7, 2104)
Infant characteristics					
Sex	Male	165 (95.4)	8 (4.6)	161 (93.1)	12 (6.9)**
	Female	184 (94.8)	10 (5.2)	163 (84.0)	31 (16.0)
Type of delivery	Vaginal birth	278 (95.5)	13 (4.5)	259 (89.0)	32 (11.0)
	Cesarean section	71 (93.4)	5 (6.6)	65 (85.5)	11 (14.5)
Gestational age (weeks)		39.0 ± 1.4[a]	39.2 ± 1.6[a]	39.0 ± 1.4[a]	39.2 ± 1.1[a]

[a]Mean ± SD.

[b]Median (minimum, maximum).

*p<0.05, **p<0.01 by the t-test and χ^2 test.

SGA: small for gestational age, LCPUFA: long-chain polyunsaturated fatty acids, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, AA: arachidonic acid.

Supplementary Table 5. Regression coefficients between newborn anthropometric measurements and concentrations of PCBs and Hg (n = 367).

		Birth weight (g)	Length (cm)	Chest circumference (cm)	Head circumference (cm)
		B (95% CI)	B (95% CI)	B (95% CI)	B (95% CI)
Estrogenic PCBs	Crude	0.68 (-175–176)	0.33 (-0.58–1.24)	0.55 (-0.18–1.28)	-0.24 (-0.87–0.38)
	Adjusted 1	-95.6 (-273–82.2)	0.18 (-0.80–1.17)	0.32 (-0.46–1.11)	-0.24 (-0.94–0.46)
	Adjusted 2	-80.1 (-259–98.8)	0.28 (-0.72–1.28)	0.38 (-0.40–1.17)	-0.27 (-0.98–0.43)
Antiestrogenic PCBs	Crude	-8.61 (-178–161)	0.08 (-0.80–0.96)	0.30 (-0.40–1.00)	-0.17 (-0.78–0.43)
	Adjusted 1	-86.8 (-258–84.1)	-0.09 (-1.04–0.85)	0.05 (-0.71–0.80)	-0.16 (-0.83–0.51)
	Adjusted 2	-87.3 (-258–83.5)	-0.07 (-1.02–0.89)	0.04 (-0.72–0.79)	-0.23 (-0.90–0.45)
Dioxin-like PCBs	Crude	-3.83 (-172–164)	0.31 (-0.56–1.18)	0.43 (-0.26–1.13)	-0.06 (-0.66–0.54)
	Adjusted 1	-131 (-301–38.5)	0.01 (-0.93–0.95)	0.09 (-0.66–0.84)	-0.10 (-0.77–0.57)
	Adjusted 2	-119 (-290–52.1)	0.09 (-0.87–1.05)	0.14 (-0.62–0.89)	-0.16 (-0.84–0.51)
Non-dioxin-like PCBs	Crude	-23.8 (-197–149)	0.19 (-0.71–1.08)	0.40 (-0.32–1.12)	-0.27 (-0.89–0.35)
	Adjusted 1	-122 (-305–61.8)	0.07 (-0.95–1.08)	0.15 (-0.66–0.96)	-0.33 (-1.05–0.40)
	Adjusted 2	-104 (-289–81.4)	0.17 (-0.86–1.21)	0.22 (-0.60–1.04)	-0.36 (-1.09–0.38)
Hair Hg	Crude	121 (-53.8–296)	0.36 (-0.55–1.27)	0.46 (-0.27–1.19)	-0.26 (-0.88–0.37)
	Adjusted 1	160 (-3.34–323)	0.27 (-0.64–1.17)	0.32 (-0.41–1.04)	-0.19 (-0.83–0.46)
	Adjusted 2	140 (-24.2–304)	0.24 (-0.68–1.16)	0.24 (-0.48–0.97)	-0.24 (-0.89–0.41)

Adjusted 1: adjusted for maternal age, maternal height, pre-pregnancy maternal weight, tobacco smoking during pregnancy, alcohol consumption during pregnancy, household income, blood sampling period, parity, gestational age, infant sex and $\log_{10}$-transformed PCBs or $\log_{10}$-transformed Hg.

Adjusted 2: adjusted for omega-3 fatty acids in addition to adjustment factors in Adjusted 1

B: partial regression coefficient, CI: confidence interval,