



Title	Demographic, behavioral, dietary, and socioeconomic characteristics related to persistent organic pollutants and mercury levels in pregnant women in Japan
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Citation	Chemosphere, 133, 13-21 https://doi.org/10.1016/j.chemosphere.2015.02.062
Issue Date	2015-08
Doc URL	http://hdl.handle.net/2115/87376
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Type	article (author version)
File Information	44_Chemosphere.pdf



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45 Abstract

46 Persistent organic pollutants and mercury are known environmental chemicals
47 that have been found to be ubiquitous in not only the environment but also in humans,
48 including women of reproductive age. The purpose of this study was to evaluate the
49 association between personal lifestyle characteristics and environmental chemical levels
50 during the perinatal period in the general Japanese population. This study targeted 322
51 pregnant women enrolled in the Hokkaido Study on Environment and Children's Health.
52 Each participant completed a self-administered questionnaire and a food-frequency
53 questionnaire to obtain relevant information on parental demographic, behavioral,
54 dietary, and socioeconomic characteristics. In total, 58 non-dioxin-like polychlorinated
55 biphenyls, 17 dibenzo-p-dioxins and -dibenzofuran, and 12 dioxin-like polychlorinated
56 biphenyls congeners, perfluorooctane sulfonate, perfluorooctanoic acid, and mercury
57 were measured in maternal samples taken during the perinatal period. Linear regression
58 models were constructed against potential related factors for each chemical
59 concentration. Most concentrations of environmental chemicals were correlated with the
60 presence of other environmental chemicals, especially in the case of non-dioxin-like
61 polychlorinated biphenyls and, polychlorinated dibenzo-p-dioxins and -dibenzofurans
62 and dioxin-like polychlorinated biphenyls which had similar exposure sources and
63 persistence in the body. Maternal smoking and alcohol habits, fish and beef intake and
64 household income were significantly associated with concentrations of environmental
65 chemicals. These results suggest that different lifestyle patterns relate to varying
66 exposure to environmental chemicals.

67

68 Keywords: persistent organic pollutants, mercury, pregnant women, biomarkers, POPs

69

70 1. Introduction

71 Polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and
72 -dibenzofurans (PCDDs/PCDFs), perfluorooctane sulfonate (PFOS), perfluorooctanoic
73 acid (PFOA)—categorized as persistent organic pollutants—and mercury (Hg) are
74 known environmental chemicals that have been detected ubiquitously in animal samples
75 and the environment. Exposure to environmental chemicals during prenatal and
76 neonatal periods, which are considered windows of vulnerability for fetuses, may cause
77 various toxicities including carcinogenicity, teratogenicity, endocrine, immune, and
78 reproductive disruption, and neurobehavioral effects (Clarkson and Magos, 2006; Olsen
79 et al., 2009; Todaka et al., 2010; Wigle et al., 2008).

80 Epidemiological studies of Asian, European, and US populations have revealed
81 that environmental chemical levels measured in maternal samples were associated with
82 demographic, behavioral, dietary, and socioeconomic characteristics. Fish and seafood
83 are the main dietary sources of PCB and PCDDs/PCDFs exposure in Japan, Taiwan,
84 Nordic countries, and Italy (Arisawa et al., 2011); whereas, meat products, dairy
85 products and fish are the main dietary sources in the US, The Netherlands, and Germany
86 (Larsen, 2006). Potential exposure sources of PFOS and PFOA were reported to be fish
87 and marine mammals, red meat, animal fat, tap (drinking) water, and household dust in
88 Spain, Norway, and Denmark (D'Hollander et al., 2010; Haug et al., 2011). Many
89 reports to date have also found fish/seafood consumption responsible for
90 bio-accumulated methylmercury in humans (Clarkson and Magos, 2006; Kim et al.,
91 2008; Ramon et al., 2008). Consequently, it is plausible that the presence of exposure
92 sources and their contribution to whole body burden levels of environmental chemicals
93 would vary according to the specific characteristics of populations in different countries
94 or regions (Glynn et al., 2007; Halldorsson et al., 2008; Kim et al., 2008; Ramon et al.,
95 2008; Sonneborn et al., 2008; Brauner et al., 2011; Ibarluzea et al., 2011).

96 The elimination rate of toxic substances as a reflection of internal metabolism
97 is an effective way to detect body burden levels of environmental chemicals..Tobacco
98 smoking and alcohol habits are considered behavioral factors related to altered
99 elimination rates of environmental chemicals. For example, tobacco smoking induces
100 increased expression of dioxin-metabolizing enzymes, such as cytochrome P450 (CYP)
101 1A2, leading to enhanced elimination of PCDDs/PCDFs and dioxin-like PCBs
102 (DL-PCBs) (Milbrath et al., 2009). Animal and human studies have also demonstrated
103 that fluorinated organic compounds can regulate CYP enzymes (Ishibashi et al., 2008;
104 Narimatsu et al., 2011).

105 To date, limited epidemiological studies have been conducted in Japan among

106 pregnant women with no history of accidental poisoning in Japan. Some studies found
107 that PCBs and PCDDs/PCDFs in maternal samples increased with maternal age, alanine
108 aminotransferase levels and alcohol intake, as well as decreased with maternal history
109 of delivery and smoking (Arisawa et al., 2011; Tajimi et al., 2005; Nakamura et al.,
110 2008). However, no study has assessed maternal smoking and alcohol habits during the
111 pre-pregnancy periods, which is considered an important period because chemicals that
112 have a long half-life could be influenced by lifestyle factors during the entire perinatal
113 period. There have also been no current studies to evaluate associations between
114 background exposure levels of environmental chemicals even though certain chemical
115 levels could be correlated with the presence of other chemicals in the human body. This
116 information could help in estimating the magnitude of body burden levels after
117 exposure to various chemicals.

118 Thus, the purpose of this study was to evaluate associations between
119 concentrations of individual chemicals including non-dioxin-like PCBs (NDL-PCBs),
120 PCDDs/PCDFs and DL-PCBs, PFOS, PFOA and Hg and the potential factors
121 responsible for their varied elimination rates and exposure sources in the general
122 Japanese population.

123

124 2. Materials and Methods

125

126 2.1 Study population

127 We enrolled 514 Japanese women at 23–35 weeks gestation who were visiting
128 the Sapporo Toho Hospital to take part in the Hokkaido Study on Environment and
129 Children’s Health Study (Kishi et al., 2011) between July 2002 and September 2005
130 (Supplementary Figure 1). In their last trimester, the subjects filled out a
131 self-administered questionnaire regarding the following parental information: tobacco
132 smoking and alcohol habits during pre- and post-pregnancy; frequency of food
133 consumption during pregnancy of items such as shoreline fish (e.g., saury, Pacific
134 herring, mackerel), pelagic fish (e.g., tuna, bonito, salmon), beef, pork, chicken, milk,
135 and eggs; education level; and household income. Estimated intake value for alcohol
136 (g/day) was calculated from a modified self-administered questionnaire about frequency
137 and type of alcohol consumption (Washino et al., 2009).

138 From enrollment to delivery, 10 subjects dropped out because of intrauterine
139 growth retardation (2), hospital transfer (1), or voluntary withdrawal (7). The medical
140 records for the remaining 504 mother–newborn pairs were used to obtain data on
141 maternal height and weight before pregnancy. To obtain information on maternal fish

142 intake throughout pregnancy, we contacted subjects within 5 days of delivery. They
143 completed part of a food-frequency questionnaire (FFQ) and provided information
144 about intake frequency and portion size for 28 fish and seafood items and their
145 estimated total fish intake (g/day) was calculated as previously described (Yasutake et
146 al., 2003) (Supplementary Table 1). We were not able to contact 74 subjects because of
147 poor health conditions immediately after delivery. Subjects also provided a sample of
148 their hair for Hg measurements and information on their past history of having their hair
149 permed. This study was conducted with written informed consent from all subjects and
150 was approved by the institutional ethics board for epidemiological studies at the
151 Hokkaido University Graduate School of Medicine.

152 153 2.2 Experimental and exposure assessment

154 A 40-mL blood sample was taken from the maternal peripheral vein in the last
155 trimester, except in those subjects with pregnancy-related anemia, from whom blood
156 samples were taken immediately after delivery. All blood samples were stored at -80°C .
157 NDL-PCBs (Supplementary Table 2) and, PCDDs/PCDFs and DL-PCBs levels
158 (Supplementary Table 3) in maternal blood were detected by high-resolution gas
159 chromatography/high-resolution mass spectrometry equipped with a solvent-cut
160 large-volume injection system at the Fukuoka Institute of Health and Environmental
161 Sciences as previously described (Iida and Todaka, 2003; Todaka et al., 2003; Todaka et
162 al., 2008). NDL-PCBs and, PCDDs/PCDFs and DL-PCBs levels were adjusted by total
163 lipid content (pg/g lipid)(Todaka et al., 2003). Toxic equivalent (TEQ) values were
164 calculated by multiplying the concentration of each individual congener of
165 PCDDs/PCDFs and DL-PCBs by its specific toxic equivalency factor value (Van den
166 Berg et al., 2006). PFOS and PFOA levels in maternal serum were detected by
167 column-switching liquid chromatography–tandem mass spectrometry at Hoshi
168 University as previously described (Inoue et al., 2004a; Inoue et al., 2004b; Nakata et al.,
169 2005a; Nakata et al., 2005b). Values below the detection limit were assigned as 50% of
170 the detection limit. The remaining samples were not analyzed owing to unavailable or
171 insufficient sample volumes for measurement. Total Hg levels were detected in the 1-cm
172 hair fiber closest to the scalp (0.7–1.2 mg) by the oxygen combustion-gold
173 amalgamation method using an MD-1 atomic absorption detector (Nippon Institute, Co.,
174 Ltd., Osaka) at the National Institute for Minamata Disease as previously described
175 (Yasutake et al., 2003). Total hair Hg concentration is a convenient biomarker for
176 methylmercury exposure because >90% of total hair Hg is methylmercury, which
177 covalently binds to cysteines in hair proteins (Clarkson and Magos, 2006). Finally, 58

178 NDL-PCBs, 12 DL-PCBs and 17 PCDDs/PCDFs congeners—were detected in 426
179 blood samples. PFOS and PFOA, and total Hg were detected in 447 sera samples and
180 430 hair samples, respectively.

181

182 2.3 Statistical analysis

183 In total, 322 subjects that had complete data about concentration of
184 environmental chemicals and personal characteristics were included in the statistical
185 analyses. Subjects were divided into four categories for each of maternal age, BMI,
186 blood sampling period, and fish intake during pregnancy as shown in Table 1.
187 Spearman's rank test was used to determine correlations between concentrations of
188 environmental chemicals. The Mann–Whitney U-test and Kruskal–Wallis test were used
189 to evaluate simple associations between subject characteristics and the concentrations of
190 each environmental chemical. Linear regression analyses were performed to evaluate
191 associations between concentrations of environmental chemicals and subject
192 characteristics. Because of skewed distributions in these concentrations,
193 log₁₀-transformed values were used for linear regression analysis. Linear regression
194 models were constructed for explanatory variables that had previously been reported as
195 related to concentrations of environmental chemicals or that were significantly
196 associated with these concentrations by bivariate analysis in this study. Backward
197 stepwise regression was used to eliminate those variables with a p-value >0.1.

198 Subgroup analyses were performed to confirm significant associations between
199 maternal smoking history and alcohol consumption during pregnancy and
200 concentrations of each environmental chemical. Duration of maternal smoking (years)
201 was used as a continuous explanatory variable in subgroup analyses of subjects with a
202 history of smoking. Alcohol intake levels (g/day), after categorization into four groups
203 according to their quartile distribution, were used in subgroup analyses of a subjects'
204 alcohol consumption during pregnancy. Presence of NDL-PCBs and, PCDDs/PCDFs
205 and DL-PCBs congeners were examined in subgroup analyses among alcohol drinkers
206 during pregnancy. Statistical significance was defined as p <0.05. Statistical analyses
207 were performed using SPSS for Windows version 19.0J (SPSS, Inc., USA).

208

209 3. Results

210 Parental characteristics based on the self-administered questionnaire and the
211 FFQ are shown in Table 1. Approximately half (52.2%) of the mothers had a history of
212 tobacco smoking; 17.1% of mothers smoked during the pregnancy. There was a history
213 of alcohol consumption in 73.6% of the mothers, and 30.1% of the mothers consumed

214 alcohol during pregnancy. Median concentrations of NDL-PCBs, PCDDs/PCDFs and
215 DL-PCBs, PFOS, PFOA, and hair Hg were 95.1 ng/mL lipid, 13.8 TEQ pg/g lipid, 5.00
216 ng/mL, 1.30 ng/mL, and 1.39 μ g/g, respectively (Table 2). Table 3 shows the
217 correlations between concentrations of individual environmental chemicals. The
218 strongest correlation was found between NDL-PCBs and, PCDDs/PCDFs and DL-PCBs
219 ($r = 0.80$, $p < 0.01$). In univariate analyses of maternal levels of environmental chemicals
220 in relation to maternal characteristics, levels of environmental chemicals were
221 significantly associated with maternal age at delivery, parity, blood sampling period,
222 education level, smoking and alcohol habits, fish intake, frequency of food consumption,
223 and annual household income ($p < 0.05$; Table 4).

224 The linear regression models in Figure 1 show the potential relationship
225 between various factors and maternal blood concentrations of NDL-PCBs and,
226 PCDDs/PCDFs and DL-PCBs, PFOS, and PFOA, and total hair Hg. Significant positive
227 associations with each \log_{10} -transformed concentration of environmental chemicals
228 were observed for maternal age, maternal alcohol consumption during pregnancy, fish
229 intake, pelagic fish intake, beef intake, and household income (Supplementary Table 4).
230 Significant negative associations with each \log_{10} -transformed concentration of
231 environmental chemicals were observed for multiparous subjects, smoking history, and
232 blood sampling period (Supplementary Table 4). In the subgroup analyses of the 168
233 subjects with a history of smoking, the duration of tobacco smoking was inversely
234 associated with \log_{10} -transformed PFOS values [Figure 2; -0.08 (-0.15 , -0.02)]. With
235 additional adjustment for maternal age, statistical significance remained. In the
236 subgroup analysis of the 97 subjects who reported drinking alcohol during their
237 pregnancy, there was no significant association between NDL-PCBs and,
238 PCDDs/PCDFs and DL-PCBs and their congeners with alcohol intake for any quartile
239 as well as across quartiles (Table 5; Supplementary Table 5).

240

241 4. Discussion

242

243 4.1 Correlations between concentrations of environmental chemicals

244 NDL-PCBs and, PCDDs/PCDFs and DL-PCBs are reported to have high
245 lipophilicities and the biological half-life of most their congeners ranges from a few
246 years to approximately 20 years (Todaka et al., 2010). Perfluoroalkyl acids (PFAAs) are
247 reported to distribute mainly in blood serum and the liver as a result of protein fraction
248 binding (Karrman et al., 2010), and the half-lives of PFOS and PFOA are estimated to
249 be 3.8 and 5.4 years, respectively (Olsen et al., 2009). Methylmercury binds to

250 hemoglobin in the blood, and its half-life is estimated to be 2 months (Clarkson and
251 Magos, 2006). The degrees of correlations between environmental chemicals could
252 correspond to differences in their exposure sources as well as their individual
253 pharmacokinetics. Almost all of the environmental chemicals, with the exception of
254 relationships between PFOS and PCBs, PFOA and PCBs, and Hg and PFOA, were
255 significantly correlated, implying that concentrations of certain environmental
256 chemicals could be used to estimate the magnitude of exposure among the general
257 population in Japan to other environmental chemicals, especially those from similar
258 exposure sources and with similar persistence in the body.

259

260 4.2 Tobacco smoking

261 Maternal smoking history was significantly related to a decline in
262 concentrations of PCDDs/PCDFs and DL-PCBs, and PFOS in this study. A previous
263 study reported that tobacco smoking lead to a decrease in PCDDs/PCDFs and DL-PCBs
264 levels because of increased expression of dioxin-metabolizing enzymes after activation
265 of the aryl hydrocarbon receptor (Milbrath et al., 2009). PFAAs are known to activate
266 peroxisome proliferator-activator receptor (PPAR), and a study in wild animals
267 suggested the possibility that a signaling pathway exists between receptor PPA α and
268 CYP that promotes elimination of PFAAs from the body after PFAA exposure (Ishibashi
269 et al., 2008). Previous epidemiological studies reported inconsistent relationships
270 between PFAA levels and smoking status among pregnant women when categorized by
271 history or current smoking status (Halldorsson et al., 2008; Halldorsson et al., 2012;
272 Jain, 2013; Ode et al., 2013). In a Swedish study, maternal cotinine levels among
273 current smokers were not associated with PFOA and PFOS plasma levels; in fact, PFOA
274 and PFOS plasma levels were significantly lower than those of subjects who had never
275 smoked (Ode et al., 2013). Ode discussed that these results could reflect differences in
276 lifestyle patterns between smokers and non-smokers that were associated with sources
277 of PFOA and PFOS exposure or an enhanced elimination rate of these environmental
278 chemicals in smokers. Our study is the first study to indicate an inverse association
279 between PFOS concentrations and the duration of tobacco smoking using a linear
280 regression model adjusted for confounding factors. This result supports previous studies
281 and suggests that a smoking habit may lead to enhanced elimination rate of not only
282 PCDDs/PCDFs and DL-PCBs but also PFOS through activation of PPA α and CYP.

283

284 4.3 Alcohol consumption

285 Our results showed that mothers who drank alcohol during pregnancy had

286 higher blood concentrations of NDL-PCBs and, PCDDs/PCDFs and DL-PCBs. In a
287 previous Japanese study that included women of reproductive age, two possible
288 explanations for this positive association between PCDDs/PCDFs and DL-PCBs and
289 alcohol consumption were proposed. The first is that alcohol intake likely affects
290 hepatic drug-metabolizing enzymes, which could result in slowed elimination of these
291 environmental chemicals. The second is that alcohol intake may indicate a greater
292 likelihood of rich, fatty food consumption, which may result in increased
293 PCDDs/PCDFs and DL-PCBs levels (Arisawa et al., 2011). In this study, no significant
294 associations were found across alcohol-intake quartiles and concentrations of
295 NDL-PCBs and, PCDDs/PCDFs and DL-PCBs, and congeners among alcohol drinkers
296 during pregnancy. However, concentrations NDL-PCBs and, PCDDs/PCDFs and
297 DL-PCBs in maternal drinkers were higher than those of women who did not drink
298 during pregnancy. Because of their long half-lives, NDL-PCBs and, PCDDs/PCDFs and
299 DL-PCBs could be influenced by drinking in the pre-pregnancy period as well as that of
300 pregnancy, on the assumption that maternal alcohol intake could affect the elimination
301 rate of these chemicals. However, in this study, history of alcohol consumption had no
302 association with NDL-PCBs and, PCDDs/PCDFs and DL-PCBs levels. Therefore,
303 maternal alcohol consumption may reflect subsequent lifestyle patterns during
304 pregnancy that increase concentrations of NDL-PCBs and, PCDDs/PCDFs and
305 DL-PCBs, rather than indicating an effect on hepatic drug-metabolizing enzymes.

306

307 4.4 Food intake

308 We found that meat, especially beef intake, may be an important exposure
309 source of NDL-PCBs in Japan, similar to that in the US and the Europe (Larsen, 2006).
310 In a Japanese food market study, meat provided the second highest contribution to total
311 daily dietary intake of PCDDs/PCDFs and DL-PCBs (Sasamoto et al., 2006). Our
312 results indicated that fish/seafood, especially pelagic fish, may be an important exposure
313 source for Hg. This is supported by another study that showed that large predatory fish
314 were the largest contributor to total hair Hg among pregnant women in Japan
315 (Yaginuma-Sakurai et al., 2009).

316

317 4.5 Other related factors

318 In agreement with previous studies, a history of parity was associated with
319 decreasing concentrations of NDL-PCBs and, PCDDs/PCDFs and DL-PCBs, PFOS and
320 PFOA, suggesting that reproductive events could play a role in elimination of
321 environmental chemicals from the maternal body (Milbrath et al., 2009; Olsen et al.,

2009). PFOS and PFOA were inversely associated with gestational age at the time of blood sampling, possibly due to the dilutional effect of plasma volume expansion, especially after the last trimester (Glynn et al., 2007). NDL-PCBs and, PCDDs/PCDFs and DL-PCBs increased with maternal age, which could be explained by previous reports that maternal age might be a good marker for the estimated duration of exposure to chemicals with long half-lives (Milbrath et al., 2009). Hg in hair increased with household income in our study, which is supported by a previous report indicating that high socioeconomic status is related to increased fish consumption, dental amalgams and vaccines, which are all associated with increased exposure to Hg (Tyrrell et al., 2013).

332

333 4.6 Strengths and limitations

334 This study provides useful information on associations between demographic, behavioral, dietary, and socioeconomic characteristics and background concentrations of individual chemicals including NDL-PCBs and, PCDDs/PCDFs and DL-PCBs, PFOS, PFOA and Hg during the perinatal period by linear regression models. These characteristics may also influence the level of fetal exposure to environmental chemicals through effects on maternal exposure levels. However, we did not collect data during maternal breast-feeding despite indications that this is an important determinant in the body burden of environmental chemicals (Milbrath et al., 2009). Further studies are also needed to evaluate etiological mechanisms of maternal smoking on the elimination rate of PFOS mediated by PPA α and CYP activation.

344 In conclusion, most concentrations of individual NDL-PCBs, PCDDs/PCDFs and DL-PCBs, PFOS, PFOA and Hg were correlated, especially the association between NDL-PCBs and, PCDDs/PCDFs and DL-PCBs, which had similar exposure sources and persistence in the body. PCDDs/PCDFs and DL-PCBs and PFOS decreased with maternal smoking history. NDL-PCBs and, PCDDs/PCDFs and DL-PCBs increased with maternal alcohol consumption during pregnancy. Total hair Hg increased with household income. Beef and fish/seafood intake may be important exposure sources of NDL-PCBs. These results may reflect various lifestyle patterns associated with exposure sources and elimination rates of these environmental chemicals.

353

354

355 **Conflicts of Interest**

356 The authors declare they have no competing financial interests. This study was supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of

358 Health, Labour and Welfare, and from the Japanese Ministry of Education, Culture,
359 Sports, Science & Technology. The funding sources did not play a role in the study
360 design; the collection, analysis and interpretation of the data; the writing of the report;
361 or the decision to submit the article for publication.

362

363 **Acknowledgements**

364 We would like to express our gratitude to all study participants and staff members at
365 Sapporo Toho Hospital for their generous collaboration.

366

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1 Table 1. Subject characteristics (n = 322).

		Mean ± SD	n (%)
Maternal characteristics			
Age at delivery (years)		30.63 ± 4.70	
BMI before pregnancy (kg/m ²)		21.12 ± 3.21	
Parity	≥1		170 (52.8)
Blood sampling period	<28 weeks		19 (5.9)
	28 to <36 weeks		144 (44.7)
	≥36 weeks		70 (21.2)
	After delivery		99 (30.7)
Education level (years)	>12		191 (59.3)
Tobacco smoking history	Yes		168 (52.2)
Tobacco smoking during pregnancy	Smoker		55 (17.1)
Alcohol consumption history	Yes		237 (73.6)
Alcohol consumption during pregnancy	Drinker		97 (30.1)
Alcohol intake (g/day) during pregnancy		0.00 (0.00, 0.46) ^a	
Fish intake (g/day) during pregnancy		50.00 (30.00, 50.00) ^a	
Frequency of food consumption during pregnancy			
Shoreline fish	≥once/week		155 (48.1)
Pelagic fish	≥once/week		178 (55.3)
Beef	≥once/week		86 (26.7)
Eggs	≥once/week		322 (100)
Milk	≥once/week		285 (88.5)
Paternal characteristics			
Tobacco smoking history	Yes		279 (86.7)
Tobacco smoking during their partner's pregnancy	Smoker		225 (69.9)
Annual household income (million yen)	≥5		110 (34.2)

2 BMI: body mass index

3 ^aMedian (minimum, maximum)

1 Table 2. Concentrations of environmental chemicals in maternal samples (n = 322)

	Geometric mean	Minimum	Percentile			
			25th	50th	75th	Maximum
NL-PCBs (ng/g lipid)	94.0	16.0	66.0	95.1	130	445
DL-PCBs and PCDDs/PCDFs (TEQ pg/g lipid) ^a	13.5	3.17	9.86	13.8	18.3	42.9
PFOS (ng/mL)	4.78	1.30	3.20	5.00	6.98	14.7
PFOA (ng/mL)	1.20	0.25	0.80	1.30	1.80	5.30
Hair Hg (µg/g) ^b	1.35	0.24	0.96	1.39	1.89	7.55

2 ^aTEQs were calculated from the individual congener toxic equivalency factor values (Van den Berg et al., 2006).

3 ^b>90% methylmercury

1 Table 3. Correlation coefficients between individual environmental chemicals (n = 322)

	PCDDs/PCDFs and DL-PCBs	PFOS	PFOA	Hair Hg
NDL-PCBs	0.80**	0.07	0.10	0.38**
PCDDs/PCDFs and DL-PCBs		0.24**	0.14*	0.30**
PFOS			0.25**	0.12*
PFOA				0.03

2 *p <0.05, **p <0.01 by Spearman's rank correlation

1 Table 4. Maternal environmental chemical levels in relation to characteristics (n = 322)

Characteristics		PCDDs/PCDFs					Hair Hg (µg/g)
		NDL-PCBs (ng/g lipid)	and DL-PCBs (TEQ pg/g lipd)	PFOS (ng/mL)	PFOA (ng/mL)		
Age at delivery (years)	<25	60.78**	9.80**	5.0	1.4	1.4	
	25 to <30	86.0	13.7	5.3	1.2	1.4	
	30 to <35	101.3	14.4	5.0	1.4	1.3	
	≥35	136.4	16.9	4.2	1.2	1.7	
BMI at delivery (kg/m ²)	<18.5	89.7	13.7	5.4	1.4	1.3	
	18.5 to <25	97.2	13.9	5.0	1.3	1.4	
	25 to <30	108.4	13.4	4.4	1.4	1.2	
	≥30	77.3	12.8	4.3	1.2	1.1	
Parity	0	101.0	14.6**	5.50**	1.50**	1.4	
	≥1	90.8	13.3	4.6	1.0	1.4	
Timing of blood sampling	<28 weeks	114.6	16.7	6.4**	1.8**		
	28 to <36 weeks	108.6	13.9	5.6	1.5		
	≥36 weeks	102.1	13.8	4.6	1.2		
	After delivery	108.2	13.5	3.8	1.2		
Education level (years)	≤12	89.4	12.8	4.8	1.3	1.4	
	>12	99.2	14.1	5.3	1.4	1.4	
Tobacco smoking history	No	101.0	15.2**	5.30**	1.4	1.5	
	Yes	87.8	12.6	4.7	1.2	1.3	
Tobacco smoking during pregnancy	Nonsmoker	96.9	14.1	5.0	1.3	1.4	
	Smoker	84.8	12.2	4.8	1.2	1.4	
Alcohol consumption history	No	86.0	13.3	4.8	1.2	1.3	
	Yes	101.0	14.0	5.0	1.4	1.4	
Alcohol consumption during pregnancy	Non-drinker	92.1	13.8	5.0	1.3	1.4	
	Drinker	101.0	13.8	5.1	1.3	1.4	
Alcohol intake (g/day) during pregnancy	Quartile 1 (<0.73)	0.1	0.0	0.0	0.0	0.1	
	Quartile 2 (0.73 to <1.52)	96.0	12.3	4.1	1.2	1.3	
	Quartile 3 (1.52 to <3.52)	117.3	13.7	5.6	1.2	1.6	
	Quartile 4 (≥3.52)	100.4	15.7	6.2	1.4	1.4	
Fish intake (g/day) during pregnancy	Quartile 1 (<25)	101.5*	14.0	4.6	1.3	1.5**	
	Quartile 2 (25 to <38.75)	84.8	13.0	5.3	1.4	1.3	
	Quartile 3 (38.75 to <50)	101.3	13.8	4.9	1.4	1.4	
	Quartile 4 (≥50)	104.1	14.3	5.0	1.2	1.7	
Frequency of food consumption during pregnancy							
Shoreline fish	<once/week	87.2	13.3	4.7	1.2	1.28*	
	≥once/week	101.0	14.5	5.3	1.3	1.5	

Pelagic fish	<once/week	94.8	13.4	4.7	1.3	1.25**
	≥once/week	95.6	14.2	5.4	1.3	1.5
Beef	<once/week	95.0	13.7	5.0	1.3	1.34*
	≥once/week	100.0	14.1	5.2	1.3	1.5
Egg	<once/week	92.6	13.6	4.10**	1.3	1.28**
	≥once/week	95.1	13.8	5.0	1.3	1.4
Milk	<once/week	66.2	11.0*	4.3	1.2	1.3
	≥once/week	99.2	14.1	5.0	1.3	1.4
Paternal characteristics						
Tobacco smoking history	No	97.5	15.1	5.4	1.3	1.3
	Yes	94.7	13.7	5.0	1.3	1.4
Tobacco smoking during their partner's pregnancy	Non-smoker	102.0	15.1*	5.4	1.4	1.4
	Smoker	92.5	13.1	4.8	1.3	1.4
Annual household income (million yen)	<5	91.8**	13.3**	4.7	1.2	1.27*
	≥5	113.0	15.8	5.5	1.5	1.5

1 Values shown are medians. BMI: body-mass index

2 *p < 0.05, **p < 0.01 by the Mann–Whitney U-test and Kruskal–Wallis test

3

1 Table 5. Partial regression coefficients (95%CI) for environmental chemical concentrations from mothers
 2 who consumed alcohol during pregnancy (n = 97)

Quartiles by alcohol intake (n, range in g/day)	NDL-PCBs		PCDDs/PCDFs and DL-PCBs	
	B (95%CI)	p for trend ^a	B (95% CI)	p for trend ^a
Quartile 1 (n = 26, <0.73)	reference	0.802	reference	0.404
Quartile 2 (n = 27, 0.73–1.52)	0.07 (–0.05, 0.18)		0.06 (–0.03, 0.16)	
Quartile 3 (n = 24, 1.52–3.52)	0.07 (–0.05, 0.18)		0.08 (–0.02, 0.17)	
Quartile 4 (n = 20, ≥3.52)	0.00 (–0.12, 0.13)		0.04 (–0.07, 0.14)	

3 B: partial regression coefficient provides the expected change in the log₁₀-transformed environmental chemical
 4 concentrations between quartiles in the regression linear model, adjusted for maternal age, parity, smoking history,
 5 fish intake, shoreline fish intake, and beef intake.

6 ^aQuartiles are represented as ordinal variables.

7











