



Title	Effects of prenatal exposure to perfluoroalkyl acids on prevalence of allergic diseases among 4-year-old children
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1 Effects of prenatal exposure to perfluoroalkyl acids on prevalence of  
2 allergic diseases among 4-year-old children

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20 Abstract

21 Perfluoroalkyl acids (PFAAs) are ubiquitous chemicals extremely resistant and widespread  
22 throughout the environment, frequently being detected in human blood samples. Animal  
23 studies have revealed that exposure to PFAAs results in immunotoxicity. However, the  
24 association between PFAAs, especially long-chain PFAAs, and allergies in humans is not well  
25 established. We examined whether prenatal exposure to PFAAs is associated with allergic  
26 diseases among 4-year-old children in a large-scale prospective birth cohort in Hokkaido, Japan.  
27 1558 Mother-child pairs were included in this study and prenatal levels of eleven PFAAs were  
28 measured in maternal plasma samples obtained between 28 and 32 weeks of pregnancy by  
29 using ultra-performance liquid chromatography-tandem mass spectrometry. Participant  
30 demographic and characteristic information were obtained from self-administered pre- and  
31 postnatal questionnaires and medical birth records. Infant allergies were assessed using the  
32 Japanese version of the International Study of Asthma and Allergies in Childhood (ISAAC)  
33 Phase Three questionnaire, which was administered 4 years post-delivery including eczema,  
34 wheezing and rhinoconjunctivitis with prevalence of 19.0%, 18.7%, and 5.4%, respectively.  
35 Associations of PFAA quartiles with allergic outcomes were examined using logistic models.  
36 Adjusted odds ratios (ORs) in the 4<sup>th</sup> quartile vs. 1<sup>st</sup> quartile (Q4 vs. Q1) for total allergic  
37 diseases (including at least one allergic outcome) significantly decreased for  
38 perfluorododecanoic acid (PFDoDa) (Q4 vs. Q1 OR: 0.621; 95% confidence interval (CI):

39 0.454, 0.847) and perfluorotridecanoic acid (PFTrDA) (Q4 vs. Q1 OR: 0.712; 95% CI: 0.524,  
40 0.966) in all children. We found similar results when examining the association between  
41 PFAAs and eczema. The adjusted OR (Q4 vs. Q1) for wheezing in relation to higher maternal  
42 PFHxS levels was 0.728 (95% CI: 0.497, 1.06) in all children. In conclusion, prenatal exposure  
43 to long-chain PFAAs, such as PFDoDa and PFTrDA, may have immunosuppressive effects on  
44 allergic diseases in 4-year-old children.

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58 1. Introduction

59 Perfluoroalkyl acids (PFAAs) are ubiquitous chemicals with widespread occurrence in the  
60 environment, animals, and humans. PFAAs are synthetic chemicals with varying carbon  
61 lengths (4 to 14 carbons) that exhibit high thermal and chemical stability owing to fluorine-  
62 carbon covalent bonding. PFAAs have extensive industrial applications, such as in textile  
63 impregnation, furnishings, non-stick housewares, and food packaging (Kannan et al., 2004;  
64 Butenhoff et al., 2006; Fromme et al., 2009). The major routes of exposure to PFAAs are  
65 through contaminated food, water, and house dust (Kato et al., 2009; Vestergren et al., 2012).  
66 The most commonly used PFAAs are perfluorooctane sulfonic acid (PFOS) and  
67 perfluorooctanoic acid (PFOA). While PFOS and PFOA are being actively phased out by  
68 several industries, they are still present in older products. PFAAs are resistant to metabolism,  
69 with long elimination half-lives of 3.8, 5.4, and 8.5 years in humans for PFOA, PFOS, and  
70 perfluorohexane sulfonate (PFHxS), respectively (Olsen et al., 2007). The stability and long  
71 half-lives of PFAAs contribute to their continued presence in the environment and to human  
72 exposure.

73 Animal studies revealed the resulting endocrine disruption, growth, and neuro- and  
74 hepatotoxic properties of PFOS and PFOA (Lau et al., 2003; Leubker et al., 2005; Seacat et al.,  
75 2003). Exposure to PFOS and PFOA in animals decreased lymphoid organ weights, and  
76 reduced the number of lymphoid cells and antibody production (Yang et al. 2001, 2002; Peden-

77 Adams et al., 2007). In animals, PFOS and PFOA inhibit the T-cell-dependent immunoglobulin  
78 M (IgM) antibody response (TDAR), which is an essential mediator of immune system  
79 function. For appropriate TDAR, proper function of B cells, T cells, and antigen presenting  
80 cells is necessary to produce antibodies. Immune system development starts during the fetal  
81 period, and in utero exposure of fetuses to chemicals can alter the development of immune  
82 cells, contributing to a modified risk of developing allergic diseases in postnatal life (Luster et  
83 al., 1992; DeWitt et al., 2012). Keil et al. (2008) examined the effects of gestational exposure  
84 to PFOS and PFOA on the immune function of mice, 4 and 8 weeks following birth and found  
85 that functional immune system deficits were not evident until 8 weeks of age when natural  
86 killer cell function and IgM production were significantly reduced. This suggests an age-related  
87 immune response effect during postnatal life following in utero exposure to PFAAs.

88 PFAAs can pass through the placental barrier during pregnancy and infants are exposed to  
89 these chemicals through lactation and indoor house dust (Inoue et al., 2004; Vestergren et al.,  
90 2012; Cariou et al., 2015). Exposure to PFAAs during this critical window of susceptibility  
91 may affect several aspects of health later in life, including immune function. Previous  
92 epidemiological studies proposing immunomodulatory effects of PFAAs indicated that prenatal  
93 exposure to PFOS and PFOA was associated with levels of IgE in cord blood with conflicting  
94 results (Wang et al., 2011; Okada et al., 2012). Additionally, pre- and post-natal exposure to  
95 PFOS and PFOA are related to reduced antibody levels of tetanus and diphtheria (Grandjean et

96 al., 2012), and rubella (Granum et al., 2013) in children, and influenza in adults (Looker et al.,  
97 2014). In addition, Fletcher et al. (2009) reported an inverse association between immunologic  
98 biomarkers such as IgA, IgE, and C-reactive protein with high PFOA exposure levels in mid-  
99 Ohio Valley residents living near a plant that contaminated the water supply with PFOA.

100 Recent studies revealed a trend of decreasing PFOA and PFOS levels in the general  
101 population of the United States (Kato et al., 2009; Olsen et al., 2012) and European countries  
102 such as Sweden and Germany (Glyn et al., 2012; Schroder-Kermani et al., 2012). Although we  
103 previously reported a declining trend of PFOS and PFOA, we observed an increasing trend for  
104 perfluorononanoic acid (PFNA, C9) and perfluorodecanoic acid (PFDA, C10) levels among  
105 pregnant women between 2003 and 2011 in Hokkaido, Japan (Okada et al. 2013). We  
106 previously reported negative association of PFTrDA and eczema in infancy in the same cohort  
107 (Okada et al. 2014). Although some animal experiments suggest prenatal PFAA exposure  
108 modifies the postnatal immune response throughout the period of early childhood (Keil et al.  
109 2008), the long-term effects of PFAAs, including long-chain PFAAs, on allergic diseases in  
110 childhood are not well understood thus far. In this study, we examined the association of 11  
111 prenatal PFAA levels with allergic diseases among 4-year-old children assessed by ISAAC  
112 questionnaires in a prospective birth cohort.

113

114 2. Methods

## 115 2.1. Study population

116 The current work is a part of a large ongoing birth cohort of over 20,000 mother-infant pairs  
117 that were recruited through the Hokkaido Study on Environment and Children's health. The  
118 details of this study have previously been described (Kishi et al. 2011 and 2013). This study  
119 was initiated in February 2003 with native Japanese mother-child pairs. Pregnant women who  
120 attended prenatal visits in early pregnancy (>13 weeks of gestational age) at any of the 37  
121 participating hospitals and clinics in the Hokkaido prefecture were eligible for this study.  
122 Health care personnel approached the pregnant women during these visits to explain the study.  
123 Among 33,500 eligible women from 2003 to 2009, 17,869 mothers agreed to participate in this  
124 study. Of these, we selected 12,847 who had submitted a baseline questionnaire with available  
125 third trimester blood samples and hospital birth records. After exclusion of cases with  
126 miscarriage and stillbirth (n = 19), congenital malformation (n = 143), and multiple births (n =  
127 162), we selected 6335 participants who had completed all three postnatal questionnaires at 4,  
128 12, and 24 months after birth. Among these, we randomly selected 300 participants per year  
129 from 2003 to 2008 and 295 participants in 2009 (n=2095) for PFAA measurement in maternal  
130 plasma samples (Okada et al., 2014). After exclusion of cases with congenital malformations  
131 that became apparent following completion of the follow-up questionnaire at 12 months (n=17),  
132 maternal blood samples obtained before 26 weeks of gestation (n=15), withdrawal (n=6), and  
133 an extremely high PFOS level (n=1); among remaining 2056 mother child pairs, 1558 mother-



134 child pairs sent us questionnaires pertaining to their 4-year-old children for inclusion in the  
135 current study (Supplementary data, Figure S1).

136 This study was conducted after obtaining all of the participants' written informed consent  
137 from the time of pregnancy up to two years following delivery. Informed consent was also  
138 obtained when the children reached 4 years of age. The institutional ethics board for  
139 epidemiological studies at Hokkaido University Center for Environmental and Health Sciences  
140 and Hokkaido University Graduate School of Medicine approved the study protocol.

## 141 2.2. Data collection

142 Mothers completed a self-administered baseline questionnaire during the first trimester of  
143 pregnancy, which included parental information related to age, prepregnancy BMI, previous  
144 medical history, educational level, annual household income, parity, alcohol consumption,  
145 medication, and smoking during pregnancy. We extracted information on gestational age, infant  
146 gender, and birth weight, as well as miscarriage, stillbirth, multiple births, and congenital  
147 anomalies from medical birth records. We collected a self-administered questionnaire at 4  
148 months following delivery, including information about birth size, maternal complications  
149 during pregnancy, and maternal smoking status in the third trimester, as reported by mothers.  
150 At 4 years post-delivery, participants completed another self-administered questionnaire  
151 collecting information related to infant size, breast feeding, smoking status of parents, parental  
152 history of allergic diseases, pets in the home, cooling/heating system at homes, environmental

153 tobacco smoke (ETS) exposure and day care attendance. ETS exposure was defined as a self-  
154 reported positive response to the presence of a smoker in the environment where children lived  
155 daily life at 4 years of age. At this time point, mothers reported any previous or current medical  
156 history of infant allergic diseases including eczema, wheezing, and rhinoconjunctivitis  
157 symptoms.

### 158 2.3. Exposure assessment

159 Detailed sample preparation and PFAAs measurement methods have been previously described  
160 (Okada et al., 2013). In brief, we collected a 10-mL blood sample from the maternal peripheral  
161 vein between 28 and 32 weeks of pregnancy with samples being stored at -80°C until analysis.  
162 We used maternal plasma for exposure assessment using ultra-performance liquid  
163 chromatography, coupled with triple quadrupole tandem mass spectrometry instrumentation  
164 (UPLC-MS/MS) (Waters, USA). We measured concentrations of 11 PFAAs: PFASs  
165 (perfluoroalkane sulfonates) including PFHxS, PFOS; and PFCAs (perfluorinated carboxylic  
166 acids) including perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), PFOA,  
167 PFNA, PFDA, PFUnDA, PFDoDA, PFTTrDA, perfluorotetradecanoic acid (PFTeDA) in  
168 maternal plasma samples obtained during the third trimester of pregnancy.

### 169 2.4. Outcome assessment

170 12 months prevalence of three allergic diseases were assessed based on the mothers' self-  
171 administered questionnaires, obtained 4 years post-delivery. Allergic diseases were defined

172 using a modified section of the Japanese version of the International Study of Asthma and  
173 Allergies in Childhood (ISAAC) Phase Three questionnaire (Asher et al., 2006). Eczema was  
174 defined based on positive answers to all three of the following questions: “Has your child had  
175 this itchy rash at any time in the past 12 months?”, “Has your child ever had a recurrent skin  
176 rash for at least 6 months?”, and “Has this itchy rash at any time affected any of the following  
177 places: the folds of the elbows; behind the knees; in front of the ankles; under the buttocks; or  
178 around the neck, ears, or eyes?”. Wheezing was defined as a positive answer to the question:  
179 “Has your child had wheezing or whistling in the chest in the past 12 months?” Current  
180 rhinoconjunctivitis symptoms were assessed based on positive answers to both of the following  
181 questions: “In the past 12 months, has your child had a problem sneezing or a runny or blocked  
182 nose when he or she did not have a cold or the flu?” and if the answer is positive, “In the past  
183 12 months, has this nose problem been accompanied by itchy watery eyes?” (Asher et al., 2006).  
184 We also defined total allergic diseases as cases with at least one of the symptoms associated  
185 with eczema, wheezing, or rhinoconjunctivitis.

## 186 2.5. Data analysis

187 We performed all of the statistical analyses using JMP pro 10 (SAS Institute Inc., NC, USA).  
188 The results were considered statistically significant if  $p < 0.05$ . For participants with PFAA  
189 levels less than MDL, a value equal to half of the MDL was substituted. We divided participants  
190 into four groups according to quartiles (Q) of prenatal PFAA levels. In crude and adjusted

191 logistic regression analyses, we examined associations between prenatal PFAA concentrations  
192 and prevalence of allergic diseases. Odds ratios (ORs) for the risk of allergic diseases were  
193 evaluated for PFAA levels in the second through fourth quartiles and compared to those in the  
194 lowest quartiles. For calculation of p for trend, we used the linear contrast coefficients -3, -1,  
195 +1, +3 assigned to quartiles 1, 2, 3, and 4, respectively (Kishi et al., 2015; Goudarzi et al.,  
196 2015). We examined the effects on total allergic diseases and on each allergic symptom  
197 separately. We selected study confounders according to a review of the literature and based on  
198 the change in estimate criteria, which was set to a value of greater than 10%. Potential  
199 confounding variables considered in the analysis were maternal age (continuous), number of  
200 older siblings (0,  $\geq 1$ ), maternal education ( $\leq 12$ ,  $> 13$  years), parental allergic history (yes/no),  
201 infant gender, breast-feeding period ( $< 6$ ,  $\geq 6$  months), day care attendance (yes/no), and  
202 environmental tobacco smoke (ETS) exposure at the age of 4 years (yes/no). The number of  
203 older siblings was obtained from parity information. Due to potential sex differences of PFAA  
204 health effects, we also stratified the results by sex.

205

### 206 3. Results.

207 In total, 1,558 mother-child pairs were included in this study. The average maternal age  
208 (SD) was 31.1 (4.4), and prepregnancy BMI (SD) was 20.9 (2.9). 45.7 % of mothers were  
209 nulliparous and 6.2% were smoking during pregnancy. 50.9% of infants were male (Table 1).

210 Due to a low detection rate, we excluded PFHxA, PFHpA and PFTeDA prior to data  
211 analysis. Among the eight remaining PFAAs, PFHxS and PFDoDA had detection rates of 82.6  
212 and 90.6%, respectively. Other PFAAs had detection rates greater than 97%. PFOS had the  
213 highest median exposure levels (4.92 ng/mL) followed by PFOA (2.01 ng/mL), PFUnDA (1.43  
214 ng/mL), and PFNA (1.18 ng/mL).

215 The number and percentage of children who developed allergic diseases in the preceding  
216 12 months were as follows: wheezing 291 (18.7%), eczema 296 (19.0%), and  
217 rhinoconjunctivitis 84 (5.4%). In total, 536 (34.4%) had at least one of the indicated allergic  
218 symptoms. Incidence of allergic symptoms was higher among boys than among girls, but this  
219 trend was not found to be statistically significant (Table 3).

220 We analyzed the association of PFAAs with total allergic diseases (Table 4, Figure 1),  
221 eczema (Figure 2, Supplementary Table S1), wheezing (Table 5), and rhinoconjunctivitis  
222 (Supplementary data, Table S2) using logistic regression models. Although not statistically  
223 significant (p-for trend= 0.085), we observed a negative association between total allergic  
224 diseases across PFUnDA quartiles (Q4 vs. Q1 adjusted OR: 0.736, 95% CI: 0.538, 1.00) in all  
225 children. In addition, adjusted ORs in the highest quartile versus that in the lowest quartile for  
226 total allergic diseases significantly decreased for PFDoDA (Q4 vs. Q1 OR: 0.621; 95% CI:  
227 0.454, 0.847; p for trend= 0.008) and PFTrDA (Q4 vs. Q1 OR: 0.712; 95% CI: 0.524, 0.966; p  
228 for trend= 0.013). Following sex stratification, we observed a significant association between

229 total allergic diseases and PFDoDA or PFTrDA only within the male population (Table 4, figure  
230 2).

231 As shown in Figure 2 and Supplemental Table S1, among males, the adjusted ORs for  
232 eczema and PFOA decreased significantly for the three highest quartiles when compared with  
233 that in the lowest quartile (Q4 vs. Q1 OR: 0.592; 95% CI: 0.319, 1.08, p for trend= 0.022) .  
234 The adjusted ORs for the highest vs. lowest quartiles were 0.566 (95% CI: 0.383, 0.831) for  
235 PFDoDA, and 0.672 (95% CI: 0.465, 0.968) for PFTrDA in all children. Effects of these long  
236 chain PFAAs were highly prominent among boys. This is further supported by the adjusted  
237 ORs of eczema in boys across the second to fourth quartiles compared with the lowest quartile  
238 of PFDoDA; the adjusted ORs were 0.877 (95% CI: 0.536, 1.43), 0.828 (95% CI: 0.500, 1.36),  
239 and 0.451 (95% CI: 0.253, 0.785), respectively, indicating a dose-response relationship (p for  
240 trend= 0.008).

241 Among PFAAs, PFHxS was found to be significantly associated with prevalence of  
242 wheezing (Table 5); the adjusted OR of PFHxS in the fourth quartile versus the first quartile  
243 was 0.728 (95% CI: 0.497, 1.06, p for trend= 0.038) in all children. Following sex stratification,  
244 this association was more pronounced among boys (Q4 vs. Q1 OR: 0.650; 95% CI: 0.391, 1.07;  
245 p for trend= 0.063).

246 PFNA exhibited a significant association with monotonic reduced prevalence of  
247 rhinoconjunctivitis (Q4 vs. Q1 OR: 0.409; 95% CI: 0.192, 0.825; p for trend= 0.019). After sex

248 stratification, the adjusted OR of the fourth quartile compared to the first quartile was reduced  
249 for PFNA across both sexes. However, the p value for the trend was not statistically significant  
250 (Supplementary data, Table S2). In addition, adjusted ORs for rhinoconjunctivitis decreased  
251 among the three highest quartiles of PFUnDA (Q4 vs. Q1 OR: 0.285; 95% CI: 0.099, 0.714; p  
252 for trend= 0.030) and PFDoDA (Q4 vs. Q1 OR: 0.430; 95% CI: 0.176, 0.985; p for trend=  
253 0.045) when compared with that in the lowest quartile in a male population. Although we found  
254 some associations between PFAAs and rhinoconjunctivitis, these results should be interpreted  
255 cautiously due to the small number of participants with rhinoconjunctivitis in our study.

256

257

#### 258 4. Discussion.

259 In this prospective birth cohort study, we focused on the effects of prenatal exposure to  
260 eleven PFAAs, including long-chain molecules, on the prevalence of allergic diseases in  
261 children at 4 years of age. We found that prenatal exposure to long-chain PFAAs, including  
262 PFDoDA and PFTrDA, was inversely associated with prevalence of total allergic diseases in  
263 4-year-old children. We observed that PFDoDA and PFTrDA were associated with a reduction  
264 in prevalence of eczema. Additionally, exposure to PFHxS was negatively associated with  
265 prevalence of wheezing. Within the female population, almost all adjusted ORs of allergic  
266 diseases across second to fourth quartiles of PFAAs were less than one when compared with

267 the first quartile values as a reference. However, we observed the association between PFAAs  
268 and allergic diseases in 4-year-old children to be statistically significant only in boys.

269 In this study, median values of PFAAs with C6-C8 chains, including PFHxS, PFOS, and  
270 PFOA, were low during pregnancy compared to those in the U.S. (Stein et al., 2012), Denmark  
271 (Halldorsson et al., 2012), Korea (Lee et al., 2013), and China (Jiang et al., 2014). However,  
272 longer chain PFAA concentrations ( $C \geq 9$ ) were higher than those reported in western countries  
273 such as Spain, Denmark, Sweden, and the U.S. (Harada et al. 2011).

274 We developed several adjusted models using different potential confounding factors to  
275 examine the consistency of the results. In addition to the confounders mentioned in the methods  
276 section, we included annual household income in the adjusted models, but this had no effect  
277 on the results. We also included smoking, alcohol consumption during pregnancy and maternal  
278 prepregnancy BMI in the adjusted models and found that the results remained consistent.  
279 Furthermore, owing to the importance of home environment in relation to allergic diseases  
280 (Araki et al., 2012; Cong et al. 2014), we collected information on owning pets and having  
281 carpets, heating/cooling systems (electrical systems vs. fuel systems), and the presence of mold  
282 and dew condensation in homes in 4 years post-delivery questionnaires. We included these  
283 covariates one by one and collectively in the adjusted model, with no significant changes in the  
284 results. This suggests that our results regarding the association between prenatal exposure to  
285 PFAAs and allergic diseases at 4 years of age may not be confounded by the noted covariates.



286 One of sources of exposure to environmental chemicals including PFAAs is sea food, however  
287 sea foods contain omega 3 fatty acids which are anti-inflammatory nutrients and linked with  
288 better respiratory function (Miata and Arita2015). We have not assessed sources of the mother's  
289 intake of PFAAs such as diet in this study, and it might be a confounder in the current analysis.

290 Several previous animal studies suggest that PFAAs have immunotoxic effects, including  
291 suppression of cytokine production affecting TNF- $\alpha$ , IL4, and IFN- $\gamma$  (Qazi et al., 2010), and  
292 reduced IgM production and humoral immunity (Dewitt et al., 2009; Peden-Adams et al., 2007).  
293 In animal studies, PFAAs changed T-helper (Th)-1 and -2 cell cytokine balance and shift  
294 toward a more Th2 cytokine pathway, lead to suppression of their cellular response and  
295 enhancement of their humoral response (Dong et al. 2011; Zheng et al. 2011). However,  
296 epidemiological studies have reported the suppression of antibody production in individuals  
297 exposed to higher PFAA levels. Prenatal exposure to PFAAs was negatively associated with  
298 the presence of the anti-rubella antibody among 3-year-old children (Granum et al., 2013).  
299 They examined four specific PFAAs and the strength of the inverse association between PFAA  
300 and antibody levels were ranked as follows: PFNA>PFOA>PFHxS>PFOS, indicating that  
301 PFCAs have a stronger influence on antibody production than PFSAs. Similarly, we also  
302 observed a stronger association between PFCAs with longer carbon chains and allergic  
303 outcomes. In another study, higher pre- and postnatal exposure to PFOS and PFOA were  
304 inversely associated with tetanus and diphtheria antibody concentrations at 5 and 7 years of

305 age (Grandjean et al., 2012). Results of these birth cohorts are consistent with our results. In  
306 contrast, a Taiwanese case-control study reported a positive association between serum levels  
307 of PFHxS, PFOS, PFOA, PFNA, and PFDA and juvenile asthma and IgE levels among 10- to  
308 15-year-old children. In a cross sectional, National Health and Nutrition Examination Survey  
309 (NHANES), Humblet et al. (2014) reported an association between PFOA levels and an  
310 increased likelihood of asthma diagnosis. An inverse association between PFOS levels and  
311 both asthma and wheezing among children aged 12-19 years was noted. In both studies,  
312 prenatal exposures were not assessed and serum childhood PFAAs levels were several times  
313 higher than the levels in our study. This lack of consistency may be attributable to several  
314 factors such as different timing of blood sampling, PFAA exposure levels, age of examined  
315 children, and different study designs.

316 The most likely target of PFAAs has been shown to be the peroxisome proliferator-activated  
317 receptor (PPAR)- $\alpha$  (Vanden Heuvel et al., 2006; Takacs and Abbott 2007). Experimental  
318 studies suggested that PFOA is a stronger agonist than PFOS for the transactivation of PPAR-  
319  $\alpha$  and PPAR- $\gamma$  in mouse and human cells (Vanden Heuvel et al., 2006). PFOA and PFNA  
320 (PFCAs) also have higher transplacental passage efficiencies than PFOS (Gustzkow et al.,  
321 2012; Lee et al., 2013). Moreover, laboratory studies suggest that PFAAs with longer carbon  
322 chains have a greater toxic potential and lower EC50 values (Kleszczynski et al. 2007; Buhrke  
323 et al., 2013). Therefore, these conclusions may partially explain why PFCAs, especially those

324 with longer carbon chains such as PFTrDA and PFDODA, display a strong association with  
325 allergic outcomes in this study.

326 We previously reported the relation of PFAAs to infant allergic diseases at 12 and 24  
327 months of age using ISAAC questionnaires (Okada et al., 2014), illustrating a link between  
328 prenatal exposure to PFTrDA and a reduced risk of eczema among female infants (n=2,062).  
329 In the current study, we followed those infants to 4 years of age and found that other PFAAs  
330 that contain shorter carbon chains, including PFHxS, PFNA, PFUnDA, PFDODA, are also  
331 negatively associated with prevalence of allergic outcomes. Taken together, although we  
332 observed some differences in characteristics between the two studies, these two reports suggest  
333 consistent dyshomeostasis of immune function in infant and early childhood after prenatal  
334 exposure to PFAAs.

335 Some previous animal studies showed sex differences of PFAA effects on immune system  
336 functions. Gestational exposure to PFOS induced suppression of innate and humoral immunity  
337 in next generation male, but not female offspring, in a mouse model (Keil et al., 2008). Another  
338 study that only examined female pups concluded that in utero exposure to PFOA did not  
339 suppress IgM production (Hu, Strynar, and DeWitt 2010). Most of the previously conducted  
340 epidemiological studies concerning the association between PFAAs and allergic outcomes  
341 included sex as a confounder in the adjusted models (Grandjean et al., 2012; Dong et al. 2013;  
342 Humblet et al., 2014) but they did not stratify the results according to sex. As explained, the

343 adjusted OR values of allergic diseases across the second to fourth quartiles of prenatal PFAAs  
344 were less than those in the first quartile, which was used as a reference for both sexes. In current  
345 study, we found p for trend in the association between PFAAs and prevalence of allergic  
346 diseases were significant among boys but not girls; however, p values for exposure-sex  
347 interaction did not show significant interaction.

348 We applied logistic regression to estimate association of PFAAs and allergic outcomes in  
349 current study. However, some previous studies suggest that applying logistic regression when  
350 outcome is not rare may result in overestimation of odds ratios and using Poisson regression  
351 and log-binomial regression may provide more correct estimates (Barros and Hirakata, 2003).  
352 Therefore, we performed data analysis using log-binominal regression and Poisson regression.  
353 However, the results did not change.

354 In this study, we examined several types of PFAAs with varying carbon chain lengths. We  
355 assessed PFAA exposure levels using UPLC-MS-MS, which is a very sensitive method that  
356 involves a standard protocol. Owing to changing blood volume plasma concentration of  
357 environmental chemicals during pregnancy, plasma samples were only collected within a  
358 narrow blood sampling period (between 28 and 32 weeks of pregnancy) to maintain the  
359 standardization and consistency of results. In this study, information was collected relating to  
360 a wide variety of demographic characteristics and possible confounding variables through  
361 questionnaires completed during and following pregnancy. The prospective design of this study

362 permits the suggestion of a strong causal relationship between exposure and outcome  
363 assessment when compared with case-control or cross-sectional studies. However, this study  
364 has some limitations. We collected information on allergic diseases based on maternal reports,  
365 which is prone to recall bias. We measured PFAA levels once, however due to long half-life  
366 and moderate-to-high reliability of PFAA measurements in maternal samples during pregnancy,  
367 single measurement of PFAAs is probably enough and has minimal influence on the results.  
368 We did not measure postnatal exposure levels of PFAAs and immunological biomarkers in  
369 children including IgE, and Th1/Th2 cytokines. In addition, when compared to the original  
370 cohort (n>20,000) participants in the current study have higher socioeconomic status as  
371 indicated by higher maternal education levels, lower maternal smoking rates during pregnancy  
372 and lower postnatal ETS exposure. In addition, we missed around 25% of participants in the  
373 current study compare with our previous study regarding the effects of PFAAs on allergic  
374 diseases in first 24 months of life (Okada et al. 2014). These suggest the possibility of selection  
375 bias. In report of Okada et al. (2014), mothers had higher smoking rate during pregnancy, but  
376 other demographic characteristics of these two studies such as maternal age, parity, child  
377 gender and parental allergy history were similar (Supplementary data, Table S3). In current  
378 study, we examined 12 months prevalence of three allergic diseases using ISAAC  
379 questionnaires. Allergic diseases have turnover such as onset, remission and relapse during  
380 infancy and childhood. Therefore, further studies with longer observations are needed for

381 finding remission, relapse and persistence of allergic diseases associated with prenatal  
382 chemical exposure in later life.

383

384 Conclusion.

385 We found an inverse association between prenatal exposure to long-chain PFAAs and  
386 prevalence of allergic diseases in early childhood. This provides new evidence that PFAAs in  
387 humans may disrupt immune system balance consistent with animal studies. However, more  
388 studies with longer observation periods need to be conducted to further elucidate longer effects  
389 and the underlying mechanisms.

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564 Abbreviations:

565 PFAAs, perfluoroalkyl acids; PFSAs, perfluoroalkane sulfonates; PFCAs, perfluorinated

566 carboxylic acids; PFHxA, perfluorohexanoic acid; PFHpA, perfluoroheptanoic acid; PFOA,

567 perfluorooctanoic acid; PFNA, perfluorononanoic acid; PFDA, perfluorodecanoic acid;

568 PFUnDA, perfluoroundecanoic acid; PFDoDA, perfluorododecanoic acid; PFTrDA,

569 perfluorotridecanoic acid; PFTeDA, perfluorotetradecanoic acid; PFHxS, perfluorohexane

570 sulfonate; PFOS, perfluorooctane sulfonate; MDL, method detection limits; CI, confidence

571 interval; OR, odds ratio; Ig, immunoglobulin; ETS, environmental tobacco smoke; ISAAC,  
572 International Study of Asthma and Allergies in Childhood.

573

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581 Table 1. Characteristics of the Hokkaido Study on Environment and Children's Health Study  
 582 Population, Japan (n=1558).

Characteristics	4-year postpartum assessment (n=1558), mean±SD or No. (%)
<b>Parental characteristics</b>	
Maternal age (years) (mean ± SD)	31.1±4.4
Prepregnancy BMI	20.9±2.9
Maternal educational level (years)	≤12
	>12
Parity (times) <sup>a</sup>	0
	≥1
Maternal smoking status during pregnancy	Nonsmoker
	Smoker
Maternal allergic history	Yes
Paternal allergic history	Yes
Annual household income (million yen) <sup>a</sup>	<5
	≥5
<b>Children characteristics</b>	
Gender	Male
	Female
Breast feeding (months)	<6
	≥6
Older siblings (numbers)	0
	≥1
Day care attendance at 4-year-old <sup>a</sup>	Yes
	No
ETS exposure at 4-year-old <sup>a, b</sup>	Yes
	No

614 <sup>a</sup>Missing data: parity (n=21), annual household income (n=183), day care attendance (N=37), and ETS  
 615 exposure (n=52).

616 <sup>b</sup>ETS: environmental tobacco smoke.

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619 Table 2. Concentrations of PFAAs in maternal plasma samples from the Hokkaido Study on  
 620 Environment and Children's Health, Japan, 2003–2013 (n=1558).

Compound	Detection			Concentration (ng/mL)							
	MDL <sup>a</sup>	No.	%	GM	Mean	SD	Minimum	25th	50th	75th	Maximum
PFHxS (C6)	0.2	1287	82.6	0.275	0.322	0.202	<0.2	0.221	0.296	0.395	3.386
PFHxA (C6)	0.1	721	46.2	0.085	0.103	0.071	<0.1	<0.1	<0.1	0.145	0.694
PFHpA (C7)	0.1	549	35.2	0.076	0.095	0.080	<0.1	<0.1	<0.1	0.125	0.757
PFOS (C8)	0.3	1558	100	4.932	5.456	2.61	1.003	3.667	4.925	6.654	30.283
PFOA (C8)	0.2	1557	99.9	2.105	2.713	2.30	<0.2	1.314	2.013	3.346	24.88
PFNA (C9)	0.3	1556	99.8	1.23	1.402	0.94	<0.3	0.908	1.183	1.589	13.189
PFDA (C10)	0.1	1551	99.5	0.514	0.575	0.282	<0.1	0.393	0.522	0.694	2.434
PFAUnDA (C11)	0.1	1555	99.8	1.368	1.534	0.722	<0.1	1.037	1.431	1.895	5.89
PFAoDA (C12)	0.1	1413	90.6	0.172	0.191	0.081	<0.1	0.14	0.186	0.233	0.729
PFATrDA (C13)	0.1	1524	97.8	0.316	0.350	0.154	<0.1	0.247	0.332	0.424	1.325
PFAteDA (C14)	0.1	238	15.2	0.057	0.061	0.029	<0.1	<0.1	<0.1	<0.1	0.303

621 <sup>a</sup>MDL: method detection limit , SD: standard deviation

Table 3. Prevalence of allergic diseases in 4 years of age in the Hokkaido Study on Environment and Children's Health, Japan, 2003–2013 (n = 1558).

Symptoms	Total		Male children		Female children		p <sup>a</sup>
	(n=1558)		(n=793)		(n=765)		
	n	(%)	n	(%)	n	(%)	
Total allergic diseases <sup>b</sup>	536	(34.4)	285	(35.9)	251	(32.8)	0.194
Wheezing	291	(18.7)	162	(20.4)	129	(16.8)	0.071
Eczema	296	(19.0)	153	(19.2)	143	(18.6)	0.762
Rhinoconjunctivitis symptoms	84	(5.4)	46	(5.8)	38	(4.9)	0.467

<sup>a</sup>Chi-square test.

<sup>b</sup>“Total allergic diseases” indicates cases with at least one of the listed symptoms.

Table 4. Prenatal PFAA concentrations and prevalence of total allergic diseases at 4 years of age in the Hokkaido Study on Environment and Children's Health, Japan, 2003–2013 (n= 1558).

Compound	Total (n = 1558)					Male children (n = 793)					Female children (n = 765)				
	n*	Crude		Adjusted <sup>a</sup>		n*	Crude		Adjusted <sup>a</sup>		n*	Crude		Adjusted <sup>a</sup>	
		OR	(95% CI)	OR	(95% CI)		OR	(95% CI)	OR	(95% CI)		OR	(95% CI)	OR	(95% CI)
<b>PFHxS</b>															
Quartile 1	140	1		1		76	1		1		64	1		1	
Quartile 2	143	0.981	(0.732, 1.31)	0.917	(0.675, 1.24)	75	0.995	(0.663, 1.49)	0.886	(0.577, 1.35)	68	0.973	(0.638, 1.48)	0.953 (0.608, 1.49)	
Quartile 3	123	0.799	(0.593, 1.07)	0.771	(0.563, 1.05)	65	0.791	(0.524, 1.19)	0.739	(0.479, 1.13)	58	0.811	(0.526, 1.25)	0.836 (0.525, 1.32)	
Quartile 4	130	0.858	(0.638, 1.15)	0.841	(0.615, 1.15)	69	0.859	(0.571, 1.29)	0.800	(0.519, 1.23)	61	0.859	(0.559, 1.32)	0.910 (0.572, 1.44)	
p for trend		0.163		0.177			0.300		0.223			0.357		0.588	
P for Sex interaction		0.999		0.999											
<b>PFOS</b>															
Quartile 1	148	1		1		78	1		1		70	1		1	
Quartile 2	121	0.727	(0.54, 0.978)	0.658	(0.481, 0.898)	61	0.73	(0.483, 1.10)	0.674	(0.433, 1.04)	60	0.724	(0.473, 1.108)	0.655 (0.416, 1.02)	
Quartile 3	131	0.823	(0.614, 1.10)	0.787	(0.577, 1.07)	71	0.948	(0.631, 1.42)	0.884	(0.572, 1.36)	60	0.713	(0.466, 1.091)	0.717 (0.457, 1.12)	
Quartile 4	136	0.868	(0.648, 1.16)	0.815	(0.596, 1.11)	75	0.962	(0.643, 1.43)	0.910	(0.590, 1.40)	61	0.776	(0.507, 1.188)	0.750 (0.474, 1.18)	
p for trend		0.513		0.391			0.852		0.975			0.257		0.308	

P for Sex interaction		0.708				0.700									
PFOA															
Quartile 1	133	1				1	66	1			1	67	1		1
Quartile 2	144	1.11	(0.833, 1.49)	1.07	(0.791, 1.47)		87	1.362	(0.907, 2.04)	1.24	(0.810, 1.92)	57	0.878	(0.571, 1.34)	0.950 (0.602, 1.49)
Quartile 3	138	1.05	(0.785, 1.41)	0.954	(0.695, 1.31)		73	1.089	(0.719, 1.64)	0.936	(0.601, 1.45)	65	1.017	(0.668, 1.54)	1.02 (0.646, 1.62)
Quartile 4	121	0.862	(0.639, 1.16)	0.830	(0.591, 1.16)		59	0.828	(0.54, 1.26)	0.746	(0.458, 1.20)	62	0.898	(0.589, 1.36)	0.989 (0.612, 1.59)
p for trend		0.300		0.208				0.236		0.125			0.785		0.955
P for Sex interaction		0.335		0.469											
PFNA															
Quartile 1	139	1				1	73	1			1	66	1		1
Quartile 2	143	1.05	(0.784, 1.40)	1.36	(0.891, 2.08)		83	1.278	(0.853, 1.91)	1.36	(0.891, 2.08)	60	0.847	(0.553, 1.29)	0.919 (0.589, 1.43)
Quartile 3	127	0.869	(0.646, 1.16)	0.694	(0.442, 1.08)		58	0.759	(0.498, 1.15)	0.694	(0.442, 1.08)	69	0.989	(0.651, 1.50)	1.00 (0.644, 1.56)
Quartile 4	127	0.865	(0.643, 1.16)	0.873	(0.562, 1.35)		71	0.915	(0.609, 1.37)	0.873	(0.562, 1.35)	56	0.809	(0.525, 1.24)	0.900 (0.562, 1.44)
p for trend		0.192		0.137				0.242		0.143			0.501		0.769
P for Sex interaction		0.159		0.224											
PFDA															
Quartile 1	140	1				1	74	1			1	66	1		1
Quartile 2	132	0.899	(0.669, 1.20)	0.886	(0.652, 1.20)		71	0.93	(0.619, 1.398)	0.867	(0.564, 1.33)	61	0.866	(0.565, 1.32)	0.930 (0.595, 1.45)

Quartile 3	133	0.913	(0.68, 1.22)	0.880	(0.646, 1.19)	76	1.08	(0.724, 1.633)	1.08	(0.709, 1.67)	57	0.756	(0.492, 1.16)	0.746	(0.473, 1.17)
Quartile 4	131	0.879	(0.654, 1.18)	0.906	(0.663, 1.23)	64	0.819	(0.542, 1.239)	0.812	(0.522, 1.25)	67	0.944	(0.62, 1.43)	1.06	(0.680, 1.67)
p for trend		0.433		0.544			0.516		0.590			0.667		0.978	
P for Sex interaction		0.399		0.280											
PFUnDA															
Quartile 1	145	1		1		75	1		1		70	1		1	
Quartile 2	130	0.838	(0.624, 1.12)	0.859	(0.631, 1.16)	82	0.986	(0.662, 1.47)	0.971	(0.637, 1.48)	48	0.664	(0.426, 1.03)	0.731	(0.459, 1.15)
Quartile 3	137	0.897	(0.669, 1.20)	0.905	(0.667, 1.22)	67	0.908	(0.599, 1.37)	0.919	(0.591, 1.42)	70	0.891	(0.590, 1.34)	0.928	(0.604, 1.42)
Quartile 4	124	0.778	(0.579, 1.04)	0.736	(0.538, 1.00)	61	0.712	(0.469, 1.08)	0.715	(0.460, 1.10)	63	0.851	(0.559, 1.29)	0.777	(0.496, 1.21)
p for trend		0.151		<b>0.085</b>			0.100		0.134			0.735		0.461	
P for Sex interaction		0.306		0.646											
PFDoDA															
Quartile 1	151	1		1		81	1		1		70	1		1	
Quartile 2	129	0.753	(0.561, 1.01)	0.735	(0.540, 0.999)	82	0.854	(0.573, 1.27)	0.810	(0.532, 1.23)	47	0.616	(0.396, 0.960)	0.630	(0.395, 0.998)
Quartile 3	140	0.853	(0.638, 1.14)	0.810	(0.597, 1.09)	68	0.714	(0.473, 1.07)	0.683	(0.443, 1.05)	72	1.02	(0.675, 1.54)	0.976	(0.629, 1.51)
Quartile 4	116	0.638	(0.474, 0.860)	0.621	(0.454, 0.847)	54	0.497	(0.325, 0.760)	0.492	(0.314, 0.766)	62	0.819	(0.538, 1.24)	0.805	(0.516, 1.25)
p for trend		<b>0.011</b>		<b>0.008</b>			<b>&lt;0.001</b>		<b>0.001</b>			0.840		0.750	
P for Sex interaction		0.033		0.084											

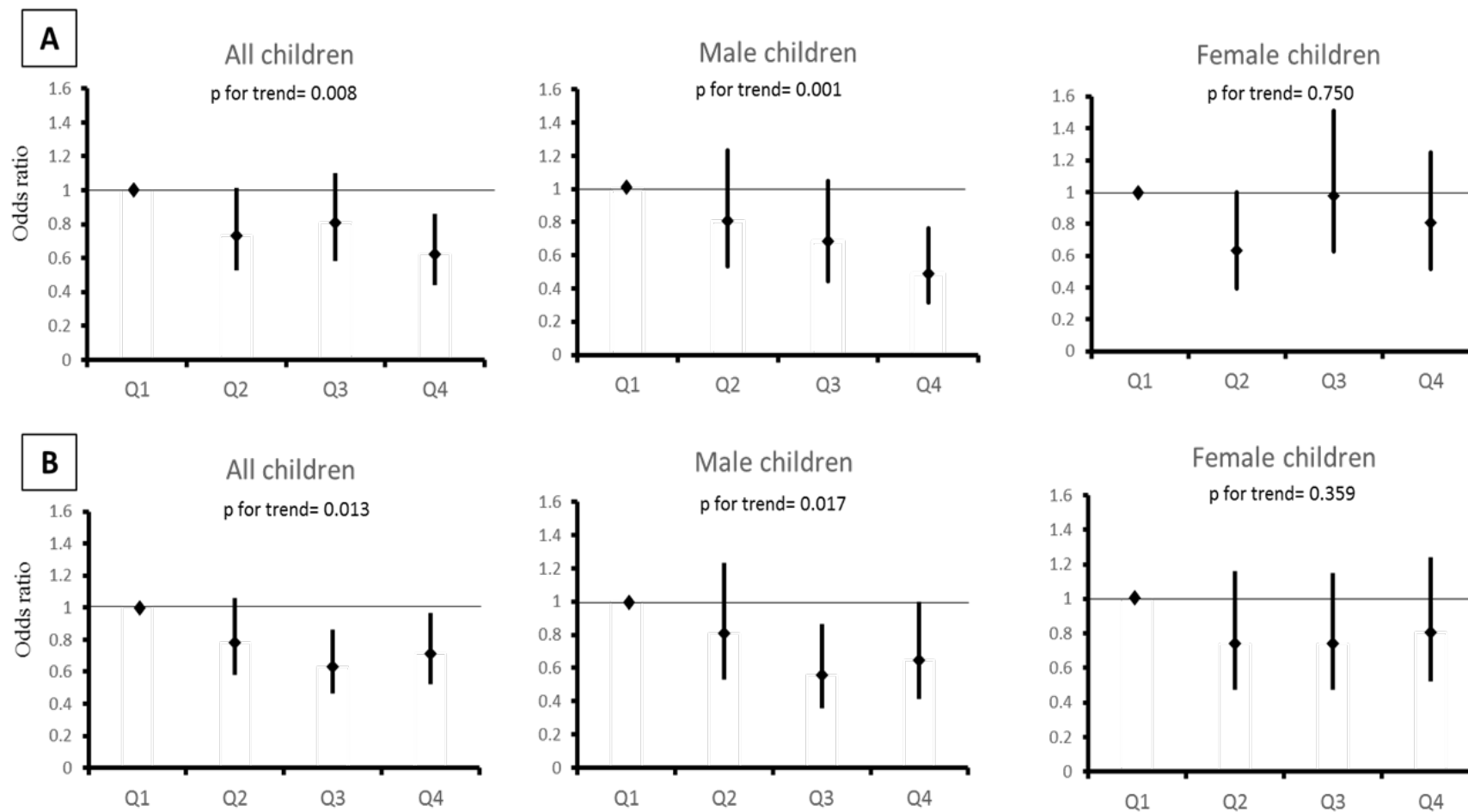
PFTrDA														
Quartile 1	155	1		1		82	1		1		73	1		1
Quartile 2	132	0.772	(0.576, 1.03)	0.784	(0.577, 1.06)	79	0.799	(0.535, 1.194)	0.807	(0.529, 1.23)	53	0.715	(0.464, 1.10)	0.742 (0.472, 1.16)
Quartile 3	118	0.657	(0.488, 0.884)	0.634	(0.464, 0.864)	61	0.565	(0.372, 0.856)	0.557	(0.358, 0.862)	57	0.763	(0.498, 1.16)	0.739 (0.472, 1.15)
Quartile 4	131	0.740	(0.553, 0.991)	0.712	(0.524, 0.966)	63	0.628	(0.414, 0.951)	0.647	(0.416, 1.00)	68	0.869	(0.576, 1.31)	0.806 (0.522, 1.24)
p for trend		<b>0.024</b>		<b>0.013</b>			<b>0.008</b>		<b>0.017</b>			0.575		0.359
P for Sex interaction		0.381		0.547										

<sup>a</sup> Adjusted for maternal age, maternal educational level, parental allergic history, number of older siblings, breast feeding, day care attendance, and ETS exposure at 4 years of age. In addition to the aforementioned confounders, we included child gender in adjusted models for total children prior to sex stratification.

\*Indicates number of cases with allergic symptoms.

Figure 1. The association between quartiles of PFDoDA (A), PFTrDA (B) and prevalence of total allergic diseases among 4-year old children. The total allergic diseases were defined as cases with at least one of the following symptoms: eczema, wheezing, or rhinoconjunctivitis. Data was adjusted for maternal age, maternal educational level, parental allergic history, number of older siblings, breast feeding, day care attendance, and ETS exposure at 4 years of age. In addition to the mentioned confounders, we included child gender in adjusted models for total children prior to sex stratification. Q: quartile.





Figure

2. The association between quartiles of PFDoDA (A) and PFTrDA (B) and prevalence of eczema among 4-year old children. Data was adjusted for maternal age, maternal educational level, parental allergic history, number of older siblings, breast feeding, day care attendance, and ETS exposure at 4 years of age. In addition to the mentioned confounders, we included child gender in adjusted models for total children prior to sex stratification. Q: quartile.

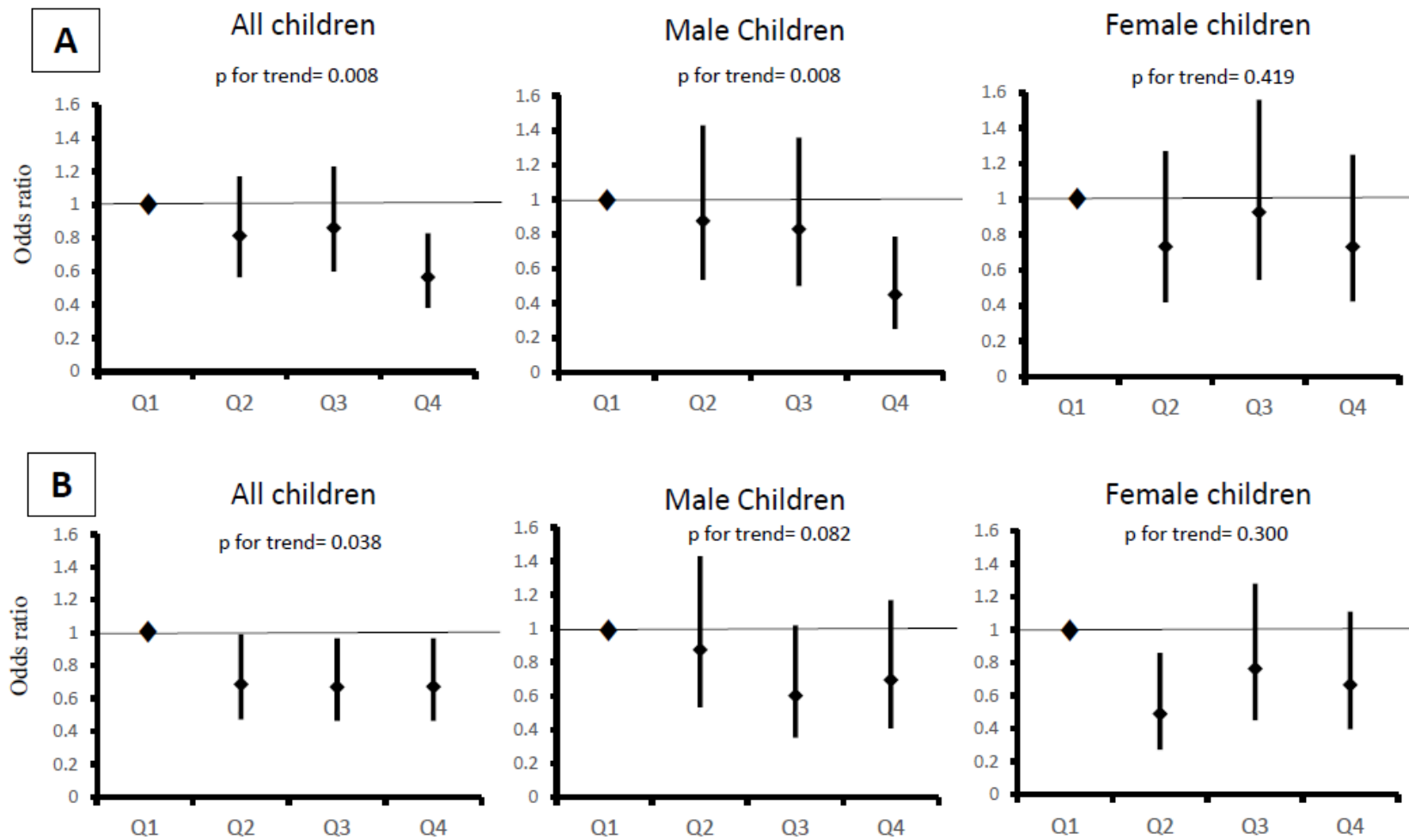


Table 5.

Prenatal PFPA concentrations and prevalence of wheezing in 4 years old in the Hokkaido Study on Environment and Children's Health, Japan, 2003–2013 (n=1558).

Compound	Total (n = 1558)				Male children (n = 793)				Female children (n = 765)						
	n*	Crude		Adjusted <sup>a</sup>		n*	Crude		Adjusted <sup>a</sup>		n*	Crude		Adjusted <sup>a</sup>	
		OR	(95% CI)	OR	(95% CI)		OR	(95% CI)	OR	(95% CI)		OR	(95% CI)	OR	(95% CI)
<b>PFHxS</b>															
Quartile 1	80	1		1		49	1		1		31	1		1	
Quartile 2	83	1.00	(0.711, 1.41)	0.895	(0.624, 1.28)	42	0.829	(0.519, 1.32)	0.705	(0.430, 1.15)	41	1.28	(0.764, 2.14)	1.21	(0.706, 2.10)
Quartile 3	61	0.702	(0.486, 1.01)	0.652	(0.443, 0.954)	33	0.612	(0.374, 1.00)	0.582	(0.346, 0.966)	28	0.842	(0.483, 1.47)	0.811	(0.448, 1.46)
Quartile 4	67	0.778	(0.543, 1.11)	0.728	(0.497, 1.06)	38	0.722	(0.448, 1.16)	0.650	(0.391, 1.07)	29	0.867	(0.499, 1.50)	0.889	(0.494, 1.59)
p for trend		0.056		<b>0.038</b>			0.097		0.063			0.320		0.398	
P for Sex interaction		0.654		0.670											
<b>PFOS</b>															
Quartile 1	78	1		1		43	1		1		35	1		1	
Quartile 2	67	0.822	(0.572, 1.18)	0.753	(0.514, 1.09)	33	0.758	(0.458, 1.25)	0.751	(0.439, 1.27)	34	0.899	(0.533, 1.51)	0.753	(0.433, 1.30)
Quartile 3	79	1.01	(0.714, 1.43)	0.980	(0.680, 1.41)	47	1.21	(0.758, 1.94)	1.18	(0.718, 1.94)	32	0.826	(0.487, 1.40)	0.809	(0.467, 1.39)
Quartile 4	67	0.824	(0.574, 1.18)	0.770	(0.526, 1.12)	39	0.901	(0.555, 1.46)	0.889	(0.530, 1.48)	28	0.740	(0.429, 1.27)	0.676	(0.379, 1.19)
p for trend		0.527		0.398			0.855		0.921			0.259		0.238	
P for Sex interaction		0.459		0.582											
<b>PFOA</b>															
Quartile 1	66	1		1		32	1		1		34	1		1	
Quartile 2	74	1.13	(0.79, 1.64)	1.09	(0.743, 1.60)	44	1.33	(0.805, 2.20)	1.22	(0.722, 2.09)	30	0.94	(0.549, 1.61)	0.982	(0.557, 1.72)
Quartile 3	76	1.18	(0.823, 1.70)	1.10	(0.749, 1.62)	44	1.40	(0.845, 2.32)	1.29	(0.762, 2.22)	32	0.977	(0.575, 1.66)	0.969	(0.544, 1.72)
Quartile 4	75	1.16	(0.806, 1.67)	1.09	(0.729, 1.65)	42	1.37	(0.824, 2.28)	1.25	(0.711, 2.22)	33	0.971	(0.574, 1.64)	1.00	(0.555, 1.82)

p for trend	0.411		0.699		0.235		0.427		0.948		0.992				
P for Sex interaction	0.727		0.741												
PFNA															
Quartile 1	70	1	1		36	1	1		34	1	1				
Quartile 2	78	1.14	(0.801, 1.64)	1.16	(0.803, 1.67)	47	1.43	(0.883, 2.34)	1.49	(0.908, 2.49)	31	0.878	(0.514, 1.49)	0.860	(0.493, 1.49)
Quartile 3	67	0.945	(0.654, 1.36)	0.910	(0.617, 1.33)	34	0.987	(0.588, 1.65)	0.911	(0.526, 1.57)	33	0.906	(0.535, 1.53)	0.918	(0.528, 1.59)
Quartile 4	76	1.10	(0.767, 1.57)	1.11	(0.760, 1.63)	45	1.27	(0.781, 2.07)	1.23	(0.732, 2.09)	31	0.918	(0.537, 1.56)	1.04	(0.587, 1.85)
p for trend	0.872		0.875		0.658		0.852		0.788		0.820				
P for Sex interaction	0.523		0.463												
PFDA															
Quartile 1	76	1	1		40	1	1		36	1	1				
Quartile 2	65	0.816	(0.566, 1.17)	0.785	(0.537, 1.14)	36	0.873	(0.529, 1.43)	0.794	(0.468, 1.34)	29	0.755	(0.441, 1.29)	0.785	(0.451, 1.35)
Quartile 3	82	1.09	(0.768, 1.54)	1.08	(0.756, 1.56)	52	1.45	(0.909, 2.32)	1.53	(0.943, 2.51)	30	0.762	(0.447, 1.29)	0.728	(0.415, 1.26)
Quartile 4	68	0.853	(0.594, 1.22)	0.879	(0.602, 1.28)	34	0.834	(0.503, 1.38)	0.859	(0.503, 1.45)	34	0.874	(0.521, 1.46)	0.918	(0.532, 1.58)
p for trend	0.755		0.917		0.966		0.743		0.637		0.702				
P for Sex interaction	0.203		0.119												
PFUnDA															
Quartile 1	72	1	1		37	1	1		35	1	1				
Quartile 2	70	0.96	(0.667, 1.38)	0.994	(0.682, 1.44)	45	1.13	(0.695, 1.83)	1.20	(0.725, 2.01)	25	0.753	(0.43, 1.31)	0.793	(0.444, 1.40)
Quartile 3	77	1.06	(0.748, 1.52)	1.10	(0.762, 1.60)	41	1.216	(0.739, 2.00)	1.32	(0.783, 2.25)	36	0.944	(0.565, 1.57)	0.918	(0.541, 1.56)
Quartile 4	72	0.991	(0.69, 1.42)	1.04	(0.714, 1.51)	39	1.047	(0.635, 1.72)	1.19	(0.709, 2.03)	33	0.931	(0.551, 1.57)	0.906	(0.522, 1.56)

p for trend	0.889		0.706			0.803		0.462			0.980		0.843		
P for Sex interaction	0.735		0.762												
PFDODA															
Quartile 1	71	1	1		34	1		1		37	1		1		
Quartile 2	71	0.972	(0.675, 1.39)	0.962	(0.659, 1.40)	50	1.42	(0.874, 2.32)	1.41	(0.851, 2.36)	21	0.553	(0.31, 0.987)	0.556	(0.303, 1.00)
Quartile 3	79	1.109	(0.776, 1.58)	1.12	(0.778, 1.63)	41	1.21	(0.731, 2.01)	1.22	(0.728, 2.08)	38	1.01	(0.613, 1.67)	1.02	(0.604, 1.73)
Quartile 4	70	0.946	(0.657, 1.36)	0.999	(0.684, 1.45)	37	1.04	(0.622, 1.74)	1.14	(0.668, 1.95)	33	0.859	(0.512, 1.44)	0.864	(0.502, 1.48)
p for trend	0.960		0.794			0.903		0.781			0.950		0.533		
P for Sex interaction	0.066		0.119												
PFTrDA															
Quartile 1	78	1	1		44	1		1		34	1		1		
Quartile 2	73	0.918	(0.643, 1.31)	0.966	(0.669, 1.39)	38	0.737	(0.452, 1.199)	0.810	(0.487, 1.34)	35	1.16	(0.691, 1.96)	1.19	(0.696, 2.04)
Quartile 3	65	0.800	(0.556, 1.15)	0.805	(0.550, 1.17)	39	0.788	(0.485, 1.281)	0.813	(0.486, 1.35)	26	0.789	(0.453, 1.37)	0.801	(0.449, 1.41)
Quartile 4	75	0.926	(0.650, 1.31)	0.944	(0.653, 1.36)	41	0.883	(0.545, 1.43)	0.978	(0.590, 1.61)	34	0.976	(0.579, 1.64)	0.919	(0.531, 1.58)
p for trend	0.526		0.565			0.694		0.931			0.614		0.474		
P for Sex interaction	0.558		0.597												

<sup>a</sup> Adjusted for maternal age, maternal educational level, parental allergic history, number of older siblings, breast feeding, day care attendance, and ETS exposure at 4 years of age. In addition to the aforementioned confounders, we included child gender in adjusted models for total children prior to sex stratification.

\*Indicates number of cases with wheezing.