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## The associations of prenatal exposure to dioxins and polychlorinated biphenyls with neurodevelopment at 6 Months of age: Multi-pollutant approaches

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### Abstract

**Background:** Prenatal exposure to persistent organic pollutants, including polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), dioxin-like polychlorinated biphenyls (DL-PCBs), and nondioxin-like PCBs (NDL-PCBs), has been hypothesized to have a detrimental impact on neurodevelopment. However, the association of prenatal exposure to a dioxin and PCB mixture with neurodevelopment remains largely inconclusive partly because these chemical levels are correlated.

**Objectives:** We aimed to elucidate the association of *in utero* exposure to a mixture of dioxins and PCBs with neurodevelopment measured at 6 months of age by applying multipollutant methods.

**Methods:** A total of 514 pregnant women were recruited between July 2002 and October 2005 in the Sapporo cohort, Hokkaido Study on Environment and Children's Health. The concentrations of individual dioxin and PCB isomers were assessed in maternal peripheral blood during pregnancy. The mental and psychomotor development of the study participants' infants was evaluated using the Bayley Scales of Infant Development-2nd Edition (n = 259). To determine

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#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2022.112757>.

both the joint and individual associations of prenatal exposure to a dioxin and PCB mixture with infant neurodevelopment, Bayesian kernel machine regression (BKMR) and quantile-based g-computation were employed.

**Results:** Suggestive inverse associations were observed between *in utero* exposure to a dioxin and PCB mixture and infant psychomotor development in both the BKMR and quantile g-computation models. In contrast, we found no association of a dioxin and PCB mixture with mental development. When group-specific posterior inclusion probabilities were estimated, BKMR suggested prenatal exposure to mono-ortho PCBs as the more important contributing factors to early psychomotor development compared with the other dioxin or PCB groups. No evidence of nonlinear exposure-outcome relationships or interactions among the chemical mixtures was detected.

**Conclusions:** Applying the two complementary statistical methods for chemical mixture analysis, we demonstrated limited evidence of inverse associations of prenatal exposure to dioxins and PCBs with infant psychomotor development.

### Keywords

Child health; Prenatal exposure; Chemical mixtures; Persistent organic pollutants; Dioxins; Polychlorinated biphenyls; Polychlorinated dibenzo-p-dioxins; Polychlorinated dibenzofurans; Neurological development; Bayley scales of infant development; Bayesian kernel machine regression; Quantile g-computation; Birth cohort study

## 1. Introduction

Persistent organic pollutants (POPs) are a public health concern due to their resistance to environmental degradation, bioaccumulation, and long-range transport potential (Kodavanti, 2006). Types of POPs include dioxins, such as polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), dioxin-like polychlorinated biphenyls (DL-PCBs), and nondioxin-like PCBs (NDL-PCBs) (Jones and de Voogt, 1999; Van den Berg et al., 2006). These organic chemicals are mostly of anthropogenic origins, such as industrial and agriculture sectors (Guo et al., 2019; Jones and de Voogt, 1999). Despite a consistent decline in concentrations since the 1970s after being banned (Dopico and Gomez, 2015; Hansen, 2002; van den Berg et al., 2017), dioxins and PCBs are still found in the environment (Bettinetti et al., 2016; Hung et al., 2016; Jamieson et al., 2017; Letcher et al., 2010).

One of the key potential health risks of dioxins and PCBs to humans includes detrimental impacts on neurological outcomes (Goodman et al., 2010; Ribas-Fito et al., 2001; Starling et al., 2015). Moreover, accumulative evidence indicates that many of these dioxins and PCBs may cross the placental barrier, suggesting prenatal exposure (Jeong et al., 2018; National Children's Study Placenta et al., 2014; Porpora et al., 2013). Given that doses much lower than those affecting the adult brain could still cause perturbations of programmed neuronal development and network connectivity of the developing fetus, the impact of *in utero* exposure to dioxins or PCBs during this critical period of susceptibility could be even more substantial than later in life (Gore et al., 2014; Grandjean and Landrigan, 2006; Peterson et al., 1993).

Previous epidemiological studies have reported the associations of early-life exposure to dioxins and PCBs with neurodevelopmental outcomes mostly using traditional statistical methods such as multivariable regression approaches, typically adjusting for all chemicals in the same model (Ames et al., 2019; Caspersen et al., 2016a, 2016b; Daniels et al., 2003; Gladen et al., 1988; Ikeno et al., 2018; Koopman-Esseboom et al., 1996; Nakajima et al., 2006, 2017; Neugebauer et al., 2015; Nghiem et al., 2019; Nowack et al., 2015; Pham et al., 2015, 2019; Pham The et al., 2020; Tai et al., 2013; Tran et al., 2016; Walkowiak et al., 2001; Wilhelm et al., 2008; Winneke et al., 1998). For instance, Nakajima et al. examined the associations of *in utero* exposure to dioxins and PCBs with neurodevelopment at 6 months old in the Hokkaido Study Sapporo Cohort data, showing a stronger association in motor development compared with mental development (Nakajima et al., 2006). However, traditional approaches are limited due to several possible issues, including multicollinearity and model misspecification (Braun et al., 2016; Claus Henn et al., 2014; Gibson et al., 2019; Taylor et al., 2016). To address these issues, multiple advanced statistical methods, such as the Bayesian Kernel Machine Regression (BKMR) (Bobb et al., 2015) and quantile-based g-computation (Keil et al., 2020), have been introduced to the field. However, few previous studies have applied such newly developed methods to evaluate the effect of prenatal exposure to POPs on neurodevelopment (Lenters et al., 2019; Vuong et al., 2020).

Recognizing this research gap, the present study aimed to investigate overall and individual associations of prenatal exposure to multiple dioxins and PCBs with infant neurodevelopment using statistical mixture approaches—BKMR and quantile g-computation. BKMR is a semi-parametric statistical method that can be employed to estimate the overall mixture effect and individual chemical impact within a mixture on health outcomes. Without prior knowledge requirements, the BKMR is well suited to flexibly model a dose–response relationship between a chemical mixture and health outcomes, exploring potential nonlinearity and nonadditivity (Bobb et al., 2015, 2018). Quantile-based g-computation is a relatively new method that adapts the weighted quantile regression approach and enhances causal inferential aspects by g-computation. In comparison to BKMR, quantile g-computation can generate a single interpretable slope estimate for the overall effect (i.e., a single coefficient per unit increase in all chemical congeners within a mixture as a joint exposure for the change in the outcome) and is insensitive to outliers because of quantization. However, prior knowledge on nonlinearity and interactions must be known for accurate model specification, as in general for other traditional methods (Keil et al., 2020). These two methods take different statistical approaches to answer the similar research questions (i.e., joint and individual associations in a context of mixture), and we thus aimed to corroborate our findings by comparing the results from these two models. More specifically, our research objectives were to (i) estimate the joint associations of prenatal exposure to multiple dioxins and PCBs with infant neurodevelopment, which is accurately addressed by quantile-based g-computation, (ii) identify the most influential dioxin or PCB congener in relation to infant neurodevelopment, which is addressed by both methods, and (iii) explore nonlinear relationships or nonadditivity within a mixture in association with infant neurodevelopment, which is accurately addressed by BKMR. To our knowledge, our study is the first to assess

the influence of *in utero* exposure to a dioxin and PCB mixture on neurodevelopment using both BKMR and quantile g-computation.

## 2. Materials and methods

### 2.1. Study population

The Sapporo Cohort, the Hokkaido Study on Environment and Children's Health, was conducted between July 2002 and October 2005, with a total of 514 pregnant women who were recruited from the Sapporo Toho Hospital in Hokkaido, Japan. Of these, 10 pregnant women withdrew from the study before delivery, and among those who remained in the study, seven mothers delivered twins for a total of 511 children. All subjects were native Japanese residing in Sapporo and its surrounding areas. The study subjects completed a self-administered questionnaire after the second trimester during their last pregnancy, inquiring about their dietary habits, exposure to chemicals during daily life and at their work site, home environment, smoking habits, and medical histories of themselves and their partners. Additional information of the participating mothers and their children was obtained from medical records and face-to-face interviews. Details have been described elsewhere (Kishi et al., 2011, 2013, 2017, 2021). Of 511 infants, 271 (53.0%) completed the neurodevelopment test at 6 months of age. We further excluded any children who were not born singletons ( $n = 2$ ) or any mothers who were missing the exposure ( $n = 10$ ), leaving 259 mother-infant pairs for the final analytic sample (Figure S1). All study subjects' written informed consent was obtained. The study was approved by the Institutional Ethical Board for Epidemiologic Studies of the Center for Environmental and Health Sciences, Hokkaido University Graduate School of Medicine.

### 2.2. Exposure measures

We collected a 40 ml venous blood sample from all participating mothers after the second trimester during their last pregnancy. Details regarding blood sampling and specimen storage have been previously described (Nakajima et al., 2006). All samples were stored at  $-80^{\circ}\text{C}$  until analysis. Dioxin and PCB concentrations in the maternal blood were measured using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) equipped with a solvent-cut large-volume injection system (SGE Ltd, Victoria, Australia) at Fukuoka Institute of Health and Environmental Sciences. The gas chromatograph was an Agilent 6890 (Agilent Technologies Inc, Palo Alto, CA, USA) equipped with an AutoSpec-Ultima NT (Micromass Ltd, Manchester, UK). The dioxins and PCB levels were measured in each isomer (7 PCDDs, 10 PCDFs, 4 non-ortho DL-PCBs, 7 mono-ortho DL-PCBs, and 2 di-ortho NDL-PCBs). In cases where the values were below the detection limit, a value equal to half the detection limit was inputted for each isomer (Table S1) (Longnecker et al., 2003). Congeners with  $<50\%$  of the detection rate were excluded from the analysis (1,2,3,7,8,9-HxCDF, 1,2,3,4,7,8,9-HpCDF, and 3,4,4'-TCB(#81)) (Ikeno et al., 2018), leaving a total of 27 dioxin and PCB isomers for analysis.

In addition, the toxic equivalent (TEQ) was calculated by the sum of the concentration of each dioxin or PCB congener multiplied by its toxic equivalency factor (TEF) value (Van den Berg et al., 2006). The concept of TEF was developed as a risk assessment

tool, recognizing that most of the biological effects of dioxins and PCBs are mediated through the aryl hydrocarbon receptor (AhR), a cytosolic receptor protein found in most vertebrate tissues. The relative effect potency for individual PCDD, PCDF, DL-PCBs, and NDL-PCB compounds was determined for producing toxic effects relative to 2,3,7,8-TCDD as a reference congener (Van den Berg et al., 1998).

### 2.3. Developmental measures

The Bayley Scales of Infant Development second edition (BSID-II) was used to measure the infants' mental and psychomotor (motor) development at 6 months of age (Bayley, 1993). Because the BSID-II items are different by the assessment age, the evaluation period was strictly limited to 5 months 16 days to 6 months 15 days. The BSID-II consists of the Mental Developmental Index (MDI), which assesses cognitive, receptive, and expressive language development, and the Psychomotor Developmental Index (PDI), which evaluates fine and gross motor development (Johnson and Marlow, 2006). The standardized mean score for the MDI and PDI was 100 (standard deviation 15). While BSID-II is limited in that the test is time-consuming, representing mostly the U.S. population and requires appropriate training for administration (Balasundaram and Avulakunta, 2021; Gollenberg et al., 2010), it has been one of the most widely used development evaluation tools and is often regarded as "the gold standard in the assessment of early child development" (Walder D. J., 2012).

Each participating child was brought to the community center in Sapporo and tested in a quiet, private room in the presence of the parent (s). The developmental assessment was performed by the three occupational therapists who had clinical experience in developmental disabilities and were blinded to the children's dioxin and PCB exposure levels. For all study participants, one examiner implemented the evaluation and scored each child's performance, which was double-checked by two other examiners based on video recordings of each session. Because the BSID-II had not been standardized in Japan, the tool was translated in consultation with a manual for BSID (Kato T et al., 1987; Kato T et al., 1988).

### 2.4. Data analysis

We calculated and compared summary statistics of the baseline covariates for the final analytic sample versus those who were excluded from the analysis. Comparisons of continuous or categorical variables were performed using the Mann–Whitney *U* test or chi-square test, respectively. Geometric means and standard deviations were computed to show the distributions of the exposure concentrations and also compared between those included versus excluded from the analysis. Dioxin and PCB levels were natural-log transformed to approximate a normal distribution. To estimate the degree of correlation among dioxins and PCBs, Spearman rank correlation coefficients were calculated.

As a preliminary analysis, multiple linear regression models were used as a traditional approach to examine the association of prenatal exposure to dioxins and PCBs with infant neurodevelopment. First, each dioxin or PCB congener was evaluated separately in association with the BSID-II MDI and PDI for 6 months (single-congener model), followed by a single model with all dioxin and PCB congeners included together (multicongener model). All models were adjusted for maternal age at baseline, maternal

education level (high school graduate or less, some college graduate or more), annual household income (<3 million yen, 3–5 million yen, or >5 million yen), working during pregnancy (yes or no), smoking during pregnancy (yes or no), and fish intake during pregnancy (inshore and deep-sea fish; 2 times per month or less, 1–2 times per week, or more than 2 times per week or more). Potential confounding factors were selected a priori based on subject knowledge (Nakajima et al., 2006), with possible mediators, such as gestational age, not included. The possibility of multicollinearity was checked using the variation inflation factor (VIF). To minimize the amount of multicollinearity in our models, we excluded exposures with high (>10) VIF, such as 1,2,3,6,7,8-HxCDF, 33'44'55'-HxCB(#169), 23'44'5-PeCB(#118), 233'44'-PeCB(#105), 23'44'55'-HxCB(#167), 233'44'5-HxCB(#156), 233'44'5'-HxCB(#157), 22'33'44'5-HpCB(#170), and 22'344'55'-HpCB(#180), from the multicongener model.

In the main analysis, we used BKMR to assess overall and individual associations of dioxins and PCBs as a mixture with neurodevelopment (Bobb et al., 2015). To determine the joint association, we subtracted the mean value of the outcome when the dioxin and PCB mixture concentrations were at the 25th percentile from the mean value of the outcome when the mixture concentrations were at the 75th percentile while holding the covariates constant. Posterior inclusion probabilities (PIPs) were estimated to identify the relative importance levels of each dioxin and PCB congener within a mixture in association with the neurodevelopmental outcomes (Bobb et al., 2018). We also performed the hierarchical variable selection function, which is recommended in the presence of high within-group correlation but relatively lower across-group correlation. Given the moderate-to-high correlation within each cluster observed in our data, and distinguished neurodevelopmental toxicity according to different chemical structures (Fischer et al., 1998; Lee and Yang, 2012; Niemi et al., 1998; Shain et al., 1991), we categorized the dioxin and PCB congeners into five groups (PCDDs, PCDFs, non-ortho DL-PCBs, mono-ortho DL-PCBs, and di-ortho NDL-PCBs). To identify which dioxin or PCB group is more important in association with neurodevelopment, group importance scores, or group hierarchical PIPs were estimated. Possible nonlinearity and interactions were also examined among the mixture members. The model convergence was checked by visually inspecting trace plots. Models were run for 10,000 iterations using the Markov chain Monte Carlo sampler.

To investigate whether we observed consistent findings across different multipollutant approaches, we employed quantile g-computation as a complementary method (Keil et al., 2020). First, the combined effect estimate was computed for prenatal exposure to dioxins and PCBs in relation to neurodevelopment using a one-quartile change in mixture, assuming Gaussian distribution. The mixture slope and overall model confidence bounds were iterated by 500 bootstraps. Each dioxin or PCB was assigned a positive or negative weight. Prior knowledge from the BKMR, including possible nonlinearity or nonadditivity, was fed to the quantile g-computation if necessary. Additional details on these two methods are provided in the Supplemental Digital Content 2.

To test the robustness of our main findings, the following sensitivity analyses were conducted. First, given that almost half of the study population was excluded from our analysis, the issue of selection bias was raised. The potential structure of selection

bias is described in more detail in the Supplemental Digital Content 2. To account for potential selection bias, we combined the linear regression model with inverse probability of censoring weights (IPCW) (Hernan MA, 2020). IPCW is a statistical technique to assign study participants the inverse of the probability of being included in the analysis as a weight according to each individual's exposure and covariate values (Oppenheimer et al., 2021). To construct a stabilized IPCW, we included the dioxin and PCB mixture components and the following covariates that were associated with censoring in this study: maternal age, education level, annual household income, smoking during pregnancy, and fish intake during pregnancy (deep-sea fish) (Table 1). The IPCW was estimated based on the information from a total of 425 out of the original 511 mother-infant pairs, who were not missing the covariates selected. To address under-estimation of standard error calculated by IPCW due to clustering, a robust standard error was estimated (Hernan et al., 2000; van der Wal and Geskus, 2011). Second, we assessed how much other endocrine disrupting chemicals could affect our findings by adding mono (2-ethylhexyl) phthalate (MEHP), perfluorooctanoic acid (PFOA), and perfluorooctane sulfonate (PFOS) in the mixture of interest as these toxicants could share the same sources as our exposures of interest (Koch et al., 2006; Xiao et al., 2015). Third, given the nonnegative integer outcomes in the current study, we applied the Poisson distribution instead of the Gaussian distribution in quantile g-computation. Fourth, we investigated whether different numbers of quantiles could impact the results from quantile g-computation. Fifth, this study included 27 dioxin and PCB congeners and covariates in the final analytic sample size of 259 participants. To examine whether similar results are found after reducing the number of variables in the model, we repeated our analysis using TEQ values. As a secondary analysis, BKMR and quantile g-computation were repeat-stratified by sex, given the sex-specific relationships of endocrine disruptors on neurodevelopment (Dong et al., 2019; Lenters et al., 2019; Nakajima et al., 2017; Rebuli and Patisaul, 2016). To facilitate comparability across the different statistical approaches, we included the same set of covariates in all models. All analyses were conducted using R (version 4.0.4; R Development Core Team) with the packages "bkmr," "qgcomp," and "ipw" for BKMR, quantile g-computation, and IPCW, respectively.

### 3. Results

The characteristics of the participating mothers and infants are shown in Table 1. Overall, more than half of the participating mothers received more than 12 years of education and ate fish once or more per week, with an average childbirth age of 31 years old. The majority of the mothers did not work or smoke during pregnancy. Among those who were included in the final analytic sample, approximately half of the participating infants were first-born (48.8%), and the average scores of the BSID-II mental developmental index (MDI) and psychomotor developmental index (PDI) were  $90.7 \pm 5.9$  and  $90.8 \pm 10.8$ , respectively, both of which were lower than the mean standardized scores. The main reason for not being included in the analysis was missing information on the Bayley Scales of Infant Development 2nd Edition (BSID-II). Compared with those who were not included in the analysis, the final analytic participants were more likely to have higher socioeconomic status, such as education and household income, and less likely to smoke, eat fish, and use

alcohol during pregnancy. However, the values were similar between the two groups for most variables.

The dioxin and PCB concentrations are shown as geometric means and standard deviations in both final analytic sample and the participants who were not included in this study (Table S1). Overall, maternal exposure to dioxins and PCBs during pregnancy was higher among those included. However, the difference was not significant for most dioxin or PCB congeners. Additional explanations comparing the concentrations of dioxins in this study with those from a previous similar study are provided in the Supplemental Digital Content 2.

The Spearman rank correlation coefficients within a dioxin and PCB mixture varied by clustering. The correlation was the highest among the mono-ortho DL-PCBs (ranging from 0.64 to 0.98), followed by PCDDs (ranging from 0.42 to 0.82) and non-ortho DL-PCBs (ranging from 0.41 to 0.71). The correlation among the PCDFs showed a wide range, from -0.04 to 0.84. The correlation coefficient between the two di-ortho ND-PCBs was 0.95. While dioxins and PCBs were generally highly correlated among the same clusters, a few exceptions were detected. For instance, 2,3,4,7,8-PeCDF was also highly correlated with mono-ortho DL-PCBs (ranging from 0.66 to 0.79). Figure S2 displays how dioxins and PCBs cluster with each other, showing predominant clustering among the PCDD and PCB congeners compared with the weak to mild correlation across clusters. The correlation between the main mixture components (i.e. the 27 dioxins or PCBs) and other endocrine disrupting chemicals, i.e., mono (2-ethylhexyl) phthalate (MEHP), perfluorooctanoic acid (PFOA), and perfluorooctane sulfonate (PFOS), was weak, ranging between 0.2 and 0.4.

Figure S3 presents the associations of prenatal exposure to dioxins and PCBs with infant neurodevelopment using multiple linear regression models. In the adjusted single-congener models including one dioxin or PCB congener at a time, most of the mixture chemicals were negatively associated with neurodevelopment for both the BSID-II subscales, but particularly for the PDI (1,2,3,4,6,7,8-HpCDD, 1,2,3,7,8-PeCDF, 33'44'5-PeCB(#126), 23'44'5-PeCB(#118), 23'44'55'-HxCB(#167), 233'44'5-HxCB(#156), 233'44'5'-HxCB(#157), and 22'33'44'5-HpCB(#170)). In contrast, the results from the adjusted multicongener models showed that only a few congeners reached statistical significance ( $P < 0.05$ ) with the MDI (2,3,7,8-TCDD, 1,2,3,7,8,9-HxCDD, and 1,2,3,4,6,7,8-HpCDD) but not with the PDI after excluding all dioxins and PCBs with high VIF ( $>10$ ).

Fig. 1 shows the overall association of prenatal exposure to the mixture of dioxins and PCBs at each quantile with the expected differences in the BSID-II MDI and PDI scores by BKMR. The MDI score increased by 0.15 (95% credible interval [CI]: 1.13, 1.43), but PDI score decreased by 1.65 (95% CI: 4.59, 1.29) when concentrations of the dioxins and PCBs increased from 25th to 75th percentiles. However, the model yielded wide credible intervals, with a suggestive inverse relationship detected only for psychomotor development, not mental development.

The individual, group, and conditional PIPs from the BKMR model are summarized in Table 2. Overall, the estimated individual PIPs were small, with 33'44'-TCB(#77) showing the largest value (0.11) on infant mental development and 23'44'55'-HxCB(#167) on infant



psychomotor development (0.22). In the grouped analysis, the PCDD and mono-ortho DL-PCB groups showed the largest groupPIPs for mental and psychomotor development (0.23 and 0.50), respectively, indicating their relatively large influence within the mixture. Of the clusters with the highest groupPIPs, 1,2,3,7,8-PeCDD and 23'44'55'-HxCB (#167) were assigned the largest conditional PIPs for mental and psychomotor development, respectively, which are consistent with the individual PIPs. These findings suggest that different primary dioxins or PCBs may influence mental and psychomotor development at 6 months of age. The univariate exposure-response curves from BKMR are depicted in Figure S4. In both the mental and psychomotor developmental domains, individual dioxins and PCBs showed little evidence of associations with neurodevelopment, as shown in the estimated individual PIPs. Furthermore, no evidence of a nonlinear relationship was observed. The BKMR model also explored potential interactions among the 27 dioxin and PCB congeners (Figure S5). The associations of each dioxin or PCB isomer with neurodevelopmental outcomes were mainly unchanged while holding the other dioxin and PCB congeners within the mixture at fixed percentiles, indicating no synergistic or multiplicative interactions.

Quantile g-computation revealed a suggestive negative association of *in utero* exposure to dioxins and PCBs in relation to neurodevelopment, indicating its association with lower or delayed neurodevelopment at 6 months of age (Fig. 2). Given no evidence of nonlinearity or nonadditivity shown from BKMR, we did not include any polynomial or interaction terms of exposures in the model. Similar to the BKMR model, quantile g-computation also yielded wide confidence bands. Overall, as suggested in the BKMR models, the joint influence of dioxin and PCB exposure on neurodevelopment at 6 months of age was not statistically significant. Increasing one quartile in the dioxin and PCB mixture was associated with  $-2.61$  (95% CI:  $-5.70, 0.49$ ) and  $-4.71$  (95% CI:  $-10.35, 0.94$ ) changes in the BSID-II MDI and PDI scores, respectively (Table 3).

Using quantile g-computation, most dioxins and PCBs were assigned negative weights in association with neurodevelopment, which is consistent with the overall associations (Figure S6). For MDI, 1,2,3,7,8,9-HxCDD was assigned the largest positive weight (0.28), whereas 2,3,7,8-TCDD demonstrated the largest negative weight (0.30) (Table 3). For PDI, 2,3,4,7,8-PeCDF was the congener with the largest positive weight (0.19), whereas 2,3,7,8-TCDD had the greatest proportional negative contribution to the mixture effect (0.14).

In sensitivity analyses, results from traditional linear regression with IPCW (Figure S7) were similar to the models without IPCW (Figure S3). Further inclusion of MEHP, PFOA, and PFOS into the exposure mixture yielded significant, larger-in-magnitude effect estimates for psychomotor development (joint association estimate by quantile g-computation:  $-6.33$  [95% CI:  $12.32, -0.34$ ]), but not for mental development (Figure S8 and Table S2). The analysis using Poisson distribution with quantile g-computation indicated similar results in both the mental and psychomotor domains (Figure S9 and Table S2). The association with mental development was attenuated when the number of quantiles was changed, whereas stronger, consistent evidence of suggestive inverse association was found in the psychomotor developmental domain (Figure S10 and Table S2). However, both associations were not significant. In the TEQ-based analysis, associations slightly shifted away from the null in the quantile g-computation, particularly for psychomotor development (joint association

estimate:  $-1.40$ ; 95% CI:  $-2.73, -0.08$ ), while similar results were shown in BKMR models (Figure S11 and Table S2). In the sex-stratified analysis, the suggestive inverse association with mental development was detected among boys but not among girls (Figure S12). The association with psychomotor development was slightly attenuated in both infant sexes. However, as found in the main analysis, no association was significant in these sex-stratified analyses (Table S2).

#### 4. Discussion

In this prospective birth cohort study, we found limited evidence to indicate that prenatal exposure to a mixture of dioxins and PCBs had a negative trending association with psychomotor, not mental, neurodevelopment assessed by the BSID-II at 6 months of age, applying both the BKMR and quantile g-computation models. The overall mixture effect was spread across the 27 mixture components with weak individual effect estimates. With the feature of estimating group hierarchical importance in the BKMR models, PCDDs and mono-ortho DL-PCBs appeared to have a larger influence on mental and psychomotor development, respectively, compared with the other groups. However, neither of these groups was a strong driver of the associations. No evidence of nonlinear relationships or interactions among the individual dioxin or PCB isomers was found. While wide credible or confidence intervals from BKMR and quantile g-computation, respectively, might suggest possibly different underlying true relationships, the suggestive inverse associations of *in utero* exposure to dioxins and PCBs with infant psychomotor neurodevelopment remained robust in a series of sensitivity analyses. Particularly, statistically significant associations were found for psychomotor development when we included other endocrine disruptors in the models or repeated the analysis with fewer variables based on the TEQ values.

Animal studies suggest the inverse association of perinatal exposure to dioxins and PCBs with neurodevelopment (Endo et al., 2012; Haijima et al., 2010; Hojo et al., 2002; Kakeyama et al., 2014; Markowski et al., 2001; Mitsui et al., 2006; Schantz et al., 1996). Most of the existing studies in humans typically utilized multiple linear regression models to examine this association, showing inconsistent results (Boucher et al., 2014; Fornes et al., 2012; Gladen and Rogan, 1991; Gladen et al., 1988; Goodman et al., 2011; Koopman-Esseboom et al., 1996; Lai et al., 2001; Lynch et al., 2012; Park et al., 2010; Ribas-Fito et al., 2001; Rogan and Gladen, 1991; Ruel et al., 2019; Walkowiak et al., 2001; Wilhelm et al., 2008; Winneke et al., 1998). For instance, a 2006 Japanese study based on the same birth cohort found that one PCDD isomer, total PCDD concentration, and total PCDF concentration were associated with lower or delayed mental development, whereas two PCDD isomers and three PCDF isomers were inversely associated with psychomotor development (Nakajima et al., 2006). Findings from a 2017 Japanese study from the same birth cohort also reported sex-specific impact at 18 months old, with 10 dioxin or PCB isomers negatively related to psychomotor development among boys, but only one isomer showed a significant inverse relationship with psychomotor development among girls (Nakajima et al., 2017). In contrast, our study observed the groups of PCDDs and mono-ortho DL-PCBs as the most influential dioxin or PCB groups in association with mental and psychomotor development, respectively. Moreover, we found that the association of maternal exposure to dioxins and PCBs during pregnancy with neurodevelopment in the offspring was

more pronounced among boys in mental development domain. These discrepancies between the current study and previous studies from the same birth cohort could be attributable to the different statistical approaches and outcome measurement period.

However, some prior studies have similarly found a stronger inverse association between exposure to dioxins and PCBs with psychomotor development, rather than with mental development (Forns et al., 2012; Gladen et al., 1988; Koopman-Esseboom et al., 1996; Lynch et al., 2012; Park et al., 2010; Rogan and Gladen, 1991). For instance, a study of Vietnamese toddlers observed significant inverse associations between infant daily dioxin intake via breast feeding and motor development at 4 months (Tai et al., 2013). It should also be noted that these studies found inverse associations at younger age (<6 months) but null findings at older ages (>18 months). No information on nonlinearity in exposure-response relationships or additivity within a mixture has been reported in relation to neurodevelopment.

We applied two complementary multipollutant analytic methods, BKMR and quantile g-computation. Despite the different statistical approaches to mixtures, our findings from the BKMR models were generally in agreement with the results from the quantile g-computation models. For instance, the suggestive inverse association of prenatal exposure to multiple dioxins or PCBs with psychomotor development was consistently shown in both BKMR and quantile g-computation models. The discrepancy in the rank of the most influential dioxin or PCB component between BKMR and quantile g-computation could be attributable to the different way these techniques deal with the presence of highly correlated exposures, and small individual effects. First, in the presence of highly correlated chemicals within a mixture, BKMR is likely to exclude some covariates from the correlated clusters, while quantile g-computation is still subject to multicollinearity and might provide relevant weights in different directions to correlated exposures. In the current study, a moderate-to-high correlation was generally found within each cluster, among PCDDs, PCDFs, or PCBs in particular, but was also detected across clusters. Second, the deviation from the joint and individual trends of associations could be observed when the effect estimate is close to 0 (Lebeaux et al., 2020). In our study, the individual posterior inclusion probabilities from BKMR and weights from quantile g-computation were generally low, indicating the small, clinically negligible associations of individual dioxins and PCBs with infant neurodevelopment. However, even if individual chemicals might have small, clinically negligible effects, the joint effect could be significant and clinically relevant (Silva et al., 2002).

A significant inverse joint association was shown between the dioxin and PCB mixture and infant psychomotor development when the TEQ values were used. This finding could be due to the increased statistical power with the decreased number of exposures in the model. Another possibility suggests that the TEQ values are better indicators of the biological mechanisms of dioxins and PCBs. The common cellular mechanism of dioxin-like compounds is the action of the aryl hydrocarbon receptor (AhR) (Budinsky et al., 2014). Based on the potencies of dioxin-like compounds to activate various AhR-dependent endpoints, a toxic equivalence factor (TEF) approach for the risk assessment of mixtures was established, with the most toxic component (2,3,7, 8-TCDD, TEF = 1) as a reference

(Van den Berg et al., 1998). The TEQ is then computed as the sum of the concentrations of individual dioxin or PCB isomers multiplied by their TEFs (Safe, 1997; Van den Berg et al., 2006). Given the link between TEQ values and the AhR mechanism, the significant inverse association detected for infant psychomotor development could be reassuring the biological and toxicological pathways of dioxins and PCBs to neurodevelopment through the AhR.

There are several limitations of this study. First, given that log-transformed dioxins and PCBs were used for a better model fit, we cannot provide reference dose or toxicity equivalent of the exposures in relation to the outcomes of interest. Second, other distribution assumptions were only available in quantile g-computation, not in BKMR. However, the results utilizing the Poisson distribution were consistent with the results estimated with the Gaussian distribution. Third, the present study was based on local Japanese pregnant mothers and infant pairs, of which the findings could not be generalizable to other populations. However, the underlying biological mechanisms linking prenatal exposure to the dioxin and PCB mixture with neurodevelopment are unlikely to differ in other populations. Fourth, nonlinearity or interactions might have been nondetectable due to the relatively small sample size. Fifth, selection bias was likely given that almost half of the study population was not included in the analysis. However, the results with IPCW to account for selection bias materially unchanged from the models without IPCW, suggesting selection bias could not be a major concern in this study. Nevertheless, the interpretation is limited as inverse probability weighting analysis does not guarantee protection from unknown or unmeasured confounding factors (Nohr and Liew, 2018).

Several strengths are worth being noted. To the best of our knowledge, the present study is the first to implement mixture methods to estimate both the overall and individual effects of correlated dioxin and PCB exposure on infant neurodevelopment. Despite their different modeling techniques, both BKMR and quantile g-computation were largely similar to each other, corroborating our findings. Second, the prospective nature of the current study allowed us to determine the temporality of prenatal exposure to the dioxin and PCB mixture in relation to neurodevelopment. Third, we used the direct assessment of prenatal exposure to dioxins and PCBs rather than a proxy such as breastmilk or fish consumption, with good quality control of laboratory evaluation. Fourth, both the exposures and outcomes were measured during a strict time window, which could inform future public health intervention or prevention programs and facilitate comparisons with other studies. Fifth, we performed extensive sensitivity analyses to ensure the robustness of the results.

## 5. Conclusion

Using two complementary mixture analysis methods, BKMR and quantile g-computation, we found suggestive inverse associations of prenatal exposure to multiple dioxins and PCBs with psychomotor neurodevelopment at 6 months of age in a prospective birth cohort study. Our study contributes to the growing literature on the associations between multiple chemicals and neurodevelopmental outcomes. Further research is warranted to replicate our findings in other study settings.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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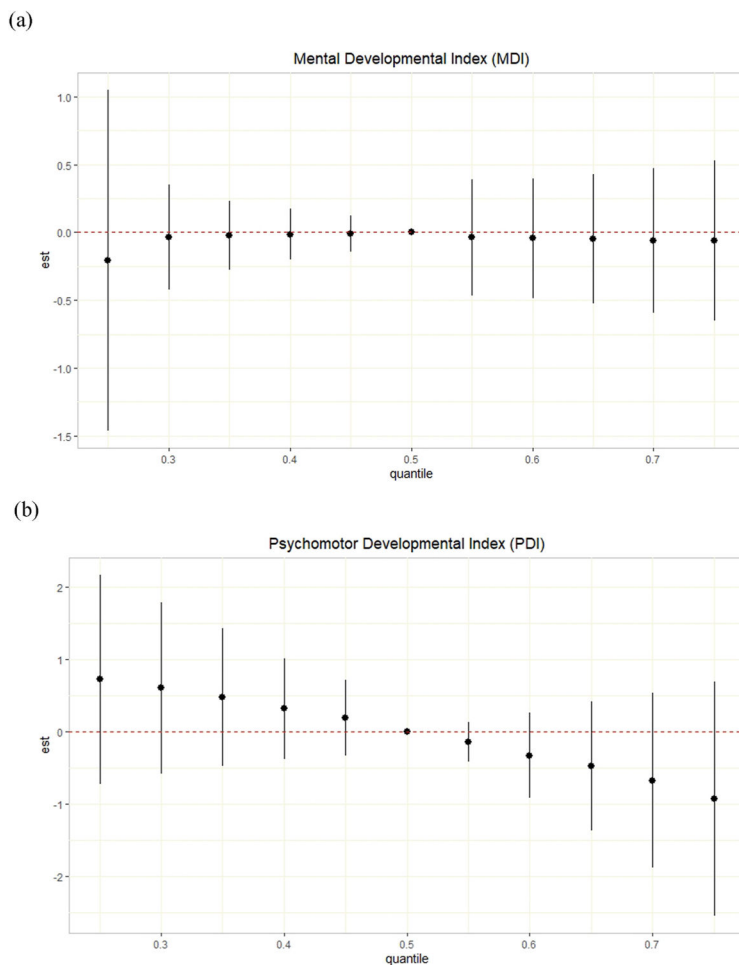
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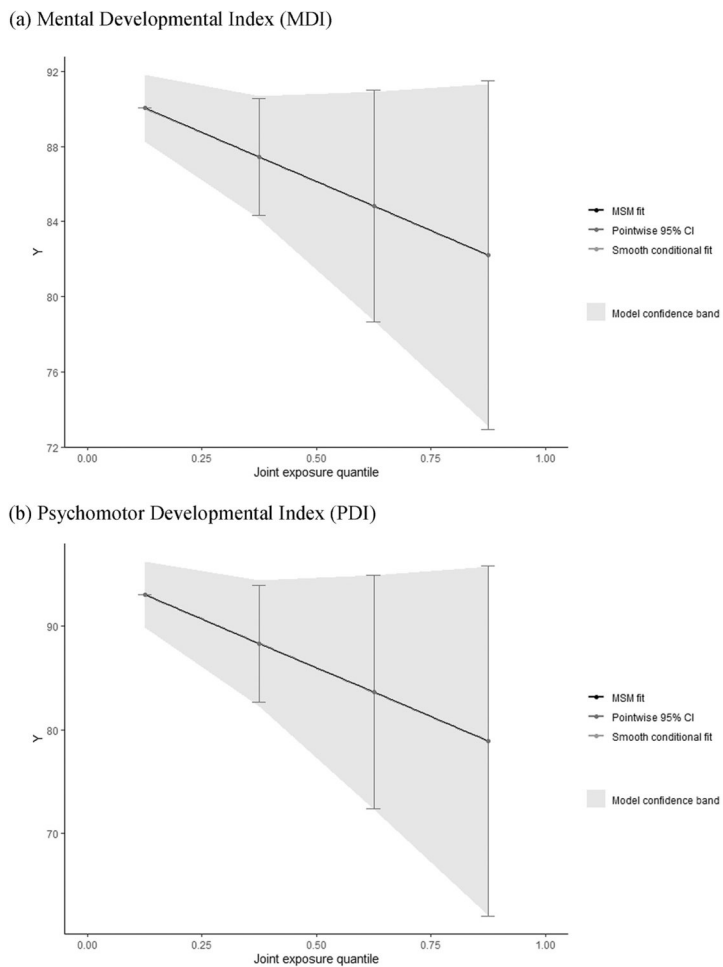
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**Fig. 1.** Joint effect (95% CI) of the log-transformed dioxin and PCB mixture on the Bayley Scales of Infant Development second edition (BSID-II) (a) Mental Developmental Index (MDI) and (b) Psychomotor Developmental Index (PDI) by Bayesian kernel machine regression (BKMR) model in the Hokkaido Study Small Cohort of the Hokkaido Study on Environment and Children’s Health (n = 259). The overall effect of prenatal exposure to dioxins and PCBs on infant neurodevelopment was plotted by comparing the impact when all dioxins and DL-PCBs are at a particular percentile with the effect when all of the congeners are at their medians. Models were adjusted for maternal age, education, household income, work status during pregnancy, smoking status during pregnancy, and fish intake during pregnancy (inshore and deep-sea fish).



**Fig. 2.** Smooth fit of the joint associations of the log-transformed dioxin and PCB mixture with infant neurodevelopment at 6 months using quantile g-computation for (a) MDI and (b) PDI in the Hokkaido Study Small Cohort of the Hokkaido Study on Environment and Children’s Health (n = 259). Models were adjusted for maternal age, education, household income, work status during pregnancy, smoking status during pregnancy, and fish intake during pregnancy (inshore and deep-sea fish).

**Table 1**

Characteristics of mothers and infants who were included in the final analytic sample (n = 259) and those who were not included (n = 252).

	The final analytic sample (n = 259)	The participants who were not included (n = 252)	<i>P</i> <sup>a</sup>
Characteristic	No. (%)		
<i>Maternal characteristics</i>			
Age (years) <sup>b</sup>	31.3 ± 4.9	30.2 ± 4.7	0.01
Education level (years)			0.03
12	101 (39.0)	124 (49.2)	
>12	158 (61.0)	128 (50.8)	
Economic status: annual income (yen)			0.04
< 3,000,000	43 (16.6)	51 (20.5)	
3,000,000–5,000,000	120 (46.3)	132 (53.0)	
>5,000,000	96 (37.1)	66 (26.5)	
Missing	0 (0)	3 (1.2)	
Worked during pregnancy	30 (11.6)	25 (9.9)	0.64
Smoked during pregnancy	32 (12.4)	56 (22.2)	<0.01
Alcohol intake during pregnancy	78 (30.1)	77 (30.6)	0.99
Fish intake during pregnancy			
Inshore fish			0.50
2 times/month; rarely/never	146 (56.4)	137 (54.4)	
1–2 times/week	100 (38.6)	96 (38.1)	
3+ times/week	13 (5.0)	19 (7.5)	
Deep-sea fish			0.05
2 times/month; rarely/never	125 (48.3)	109 (43.3)	
1–2 times/week	124 (47.9)	120 (47.6)	
3+ times/week	10 (3.9)	23 (9.1)	
Pregnancy-related complications	31 (12.1)	23 (9.2)	0.36
Missing	0 (0)	1 (0.4)	
Pre-pregnancy body mass index (kg/m <sup>2</sup> ) <sup>b</sup>	21.3 ± 3.2	21.17 ± 3.30	0.41
Missing	0 (0)	1 (0.4)	
<i>Child characteristics</i>			
Sex			1.00
Boy	125 (48.3)	121 (48.0)	
Girl	134 (51.7)	131 (52.0)	
First-born	126 (48.8)	116 (46.0)	0.84
Gestational age (days) <sup>b</sup>	275.6 ± 9.6	273.3 ± 12.0	0.14
Birth weight (g) <sup>b</sup>	3084.5 ± 374.8	2965.1 ± 456.9	0.01
Length (cm) <sup>b</sup>	48.1 ± 1.8	47.6 ± 2.5	0.05
Head circumference (cm) <sup>b</sup>	33.3 ± 1.3	33.1 ± 1.5	0.23

	The final analytic sample (n = 259)	The participants who were not included (n = 252)	<i>p</i> <sup>a</sup>
Characteristic	No. (%)		
BSID-II mental index score:	90.7 ± 5.9	86.2 ± 9.0	0.03
MDI <sup>b</sup>			
Missing	0 (0)	240 (95.2)	
BSID-II psychomotor index score: PDI <sup>b</sup>	90.8 ± 10.8	86.5 ± 12.3	0.09
Missing	0 (0)	240 (95.2)	

BSID-II: Bayley Scales of Infant Development second edition.

<sup>a</sup>Comparison of continuous data between those included and excluded was performed using Mann–Whitney *U* test. Categorical variables were compared using  $\chi^2$  test.

<sup>b</sup>Mean±SD.

**Table 2**

Posterior inclusion probabilities (PIPs) into infant neurodevelopment measured by the Bayley Scales of Infant Development second edition (BSID-II) by Bayesian kernel machine regression (BKMR) model in the Hokkaido Study Small Cohort (n = 259).

Dioxin and PCB congeners	Individual PIP	Group PIP	Conditional PIP
(a) Mental Developmental Index (MDI)			
PCDD			
2,3,7,8-TCDD	0.03	0.23	0.16
1,2,3,7,8-PeCDD	0.06		0.55
1,2,3,4,7,8-HxCDD	<0.01		0.04
1,2,3,6,7,8-HxCDD	<0.01		0.01
1,2,3,7,8,9-HxCDD	<0.01		0.02
1,2,3,4,6,7,8-HpCDD	0.08		0.16
OCDD	<0.01		0.06
PCDF			
2,3,7,8-TCDF	0.02	0.16	0.05
1,2,3,7,8-PeCDF	<0.01		0.07
2,3,4,7,8-PeCDF	0.01		0.05
0.0141,2,3,4,7,8-HxCDF	0.02		0.01
1,2,3,6,7,8-HxCDF	0.03		0.60
2,3,4,6,7,8-HxCDF	0.05		0.21
1,2,3,4,6,7,8-HpCDF	<0.01		<0.01
OCDF	<0.01		<0.01
Non-ortho DL-PCB			
33'44'-TCB(#77)	0.11	0.19	0.91
33'44'5-PeCB(#126)	<0.01		0.05
33'44'55'-HxCB(#169)	<0.01		0.04
Mono-ortho DL-PCB			
2'344'5-PeCB(#123)	0.02	0.03	0.22
23'44'5-PeCB(#118)	<0.01		0.02
2344'5-PeCB(#114)	<0.01		0.09
233'44'-PeCB(#105)	<0.01		0.04
23'44'55'-HxCB(#167)	<0.01		0.08
233'44'5-HxCB(#156)	<0.01		0.14
233'44'5'-HxCB(#157)	<0.01		0.41
Di-ortho DL-PCB			
22'33'44'5-HpCB(#170)	<0.01	<0.01	NA
22'344'55'-HpCB(#180)	<0.01		NA
(b) Psychomotor Developmental Index (PDI)			
PCDD			
2,3,7,8-TCDD	<0.01	0.20	0.06
1,2,3,7,8-PeCDD	<0.01		0.08

Dioxin and PCB congeners	Individual PIP	Group PIP	Conditional PIP
1,2,3,4,7,8-HxCDD	0.02		0.18
1,2,3,6,7,8-HxCDD	<0.01		0.05
1,2,3,7,8,9-HxCDD	0.03		0.08
1,2,3,4,6,7,8-HpCDD	0.15		0.47
OCDD	0.10		0.09
PCDF			
2,3,7,8-TCDF	<0.01	0.22	0.11
1,2,3,7,8-PeCDF	0.08		0.53
2,3,4,7,8-PeCDF	0.01		0.07
1,2,3,4,7,8-HxCDF	<0.01		0.06
1,2,3,6,7,8-HxCDF	0.02		0.14
2,3,4,6,7,8-HxCDF	0.05		0.09
1,2,3,4,6,7,8-HpCDF	<0.01		<0.01
OCDF	<0.01		<0.01
Non-ortho DL-PCB			
33'44'-TCB(#77)	0.01	0.18	0.25
33'44'5-PeCB(#126)	0.02		0.57
33'44'55'-HxCB(#169)	0.01		0.18
Mono-ortho DL-PCB			
2'344'5-PeCB(#123)	0.03	0.50	0.07
23'44'5-PeCB(#118)	0.06		0.10
2344'5-PeCB(#114)	0.04		0.08
233'44'-PeCB(#105)	0.06		0.08
23'44'55'-HxCB(#167)	0.22		0.37
233'44'5-HxCB(#156)	0.01		0.12
233'44'5'-HxCB(#157)	0.07		0.18
Di-ortho DL-PCB			
22'33'44'5-HpCB(#170)	0.02	0.03	0.70
22'344'55'-HpCB(#180)	<0.01		0.30

Models were adjusted for maternal age, education, household income, work status during pregnancy, smoking status during pregnancy, and fish intake during pregnancy (inshore and deep-sea fish).

**Table 3**

Quantile-based g-computation estimates per quantile increase in the mixture of log-transformed dioxins and DL-PCBs with congener-specific weights in relation to infant neurodevelopment measured by the Bayley Scales of Infant Development second edition (BSID-II) (n = 259).

<b>(a) Mental Developmental Index (MDI)</b>			
	<b>Estimate (95% CI)<sup>a</sup></b>	<b>P</b>	<b>Weights</b>
	<b>-2.61 (-5.70, 0.49)</b>	<b>0.10</b>	
Positive direction	Sum of positive coefficients = 7.45		
1,2,3,7,8,9-HxCDD			0.28
23'44'5-PeCB(#118)			0.16
33'44'-TCB(#77)			0.15
233'44'5-HxCB(#156)			0.15
33'44'55'-HxCB(#169)			0.11
2,3,4,7,8-PeCDF			0.08
2'344'5-PeCB(#123)			0.05
1,2,3,7,8-PeCDD			0.01
1,2,3,6,7,8-HxCDF			0.01
Negative direction	Sum of negative coefficients = -10		
2,3,7,8-TCDD			0.30
1,2,3,4,7,8-HxCDD			0.19
233'44'-PeCB(#105)			0.12
33'44'5-PeCB(#126)			0.06
1,2,3,4,7,8-HxCDF			0.06
23'44'55'-HxCB(#167)			0.05
233'44'5'-HxCB(#157)			0.05
OCDD			0.04
22'344'55'-HpCB(#180)			0.04
2344'5-PeCB(#114)			0.03
2,3,7,8-TCDF			0.03
1,2,3,6,7,8-HxCDD			0.02
1,2,3,4,6,7,8-HpCDF			0.01
1,2,3,4,6,7,8-HpCDD			0.01
22'33'44'5-HpCB(#170)			<0.01
1,2,3,7,8-PeCDF			<0.01
2,3,4,6,7,8-HxCDF			<0.01
OCDF			<0.01
<b>(b) Psychomotor Developmental Index (PDI)</b>			
	<b>Estimate (95% CI)<sup>a</sup></b>	<b>P</b>	<b>Weights</b>
	<b>-4.71 (-10.35, 0.94)</b>	<b>0.10</b>	
Positive direction	Sum of positive coefficients = 7.94		
2,3,4,7,8-PeCDF			0.19
233'44'-PeCB(#105)			0.18



1,2,3,7,8-PeCDD	0.16
1,2,3,6,7,8-HxCDF	0.16
2344'5-PeCB(#114)	0.10
22'344'55'-HpCB(#180)	0.09
OCDD	0.07
1,2,3,4,6,7,8-HpCDF	0.04
33'44'-TCB(#77)	0.02
Negative direction	Sum of negative coefficients = -12.6
2,3,7,8-TCDD	0.14
33'44'5-PeCB(#126)	0.13
1,2,3,4,7,8-HxCDF	0.12
233'44'5'-HxCB(#157)	0.08
1,2,3,4,6,7,8-HpCDD	0.08
22'33'44'5-HpCB(#170)	0.07
233'44'5-HxCB(#156)	0.07
1,2,3,4,7,8-HxCDD	0.06
23'44'5-PeCB(#118)	0.05
23'44'55'-HxCB(#167)	0.05
33'44'55'-HxCB(#169)	0.05
2,3,7,8-TCDF	0.04
1,2,3,7,8,9-HxCDD	0.02
2'344'5-PeCB(#123)	0.02
1,2,3,6,7,8-HxCDD	0.01
1,2,3,7,8-PeCDF	<0.01
2,3,4,6,7,8-HxCDF	<0.01
OCDF	<0.01

Models were adjusted for maternal age, education, household income, work status during pregnancy, smoking status during pregnancy, and fish intake during pregnancy (inshore and deep-sea fish).

<sup>a</sup> $\beta$  can be interpreted as the change in the BSID-II score for one quartile increase in all dioxins and DL-PCBs based on 200 bootstrap iterations.