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Title

Association between prenatal exposure to organochlorine pesticides and the mental and psychomotor development of infants at ages 6 and 18 months: The Hokkaido Study on Environment and Children's Health

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Abstract

Organochlorine pesticides (OCPs) are environmental contaminants that persist in the environment and bioaccumulate through the food chain in humans and animals. Although previous studies have shown an association between prenatal OCP exposure and subsequent neurodevelopment, the levels of OCPs included in these studies were inconsistent. A hospital-based prospective birth cohort study was conducted to examine the associations between prenatal exposure to relatively low levels of OCPs and neurodevelopment in infants at 6 (n = 164) and 18 (n = 115) months of age. Blood samples were analyzed using gas chromatography/mass spectrometry techniques to quantify 29 OCPs. The Bayley Scales of Infant Development 2nd edition (BSID-II) was used to assess the Mental and Psychomotor Developmental Index. After controlling for confounders, we found an inverse association between prenatal exposure to *cis*-heptachlor epoxide and the Mental Developmental Index at 18 months of age. Furthermore, infants born to mothers with prenatal concentrations of *cis*-heptachlor epoxide in the highest quartile had Mental Developmental Index scores –9.8 (95% confidence interval: –16.4, –3.1) lower than that recorded for infants born to mothers with concentrations of *cis*-heptachlor epoxide in the first quartile (p for trend < 0.01). These results support the hypothesis that prenatal exposure to OCPs, especially *cis*-heptachlor epoxide, may have an adverse effect on the neurodevelopment of infants at specific ages, even at low levels.

Organochlorine pesticide

Prenatal exposure

Birth cohort

Highlights
- We studied the association between prenatal exposure to low levels of OCP and infant neurodevelopment through a prospective birth cohort study
- Infants prenatally exposed to *cis*-HCE showed a decreased Mental Developmental Index at 18 months
- Prenatal exposure to OCPs affects infant neurodevelopment

1. Introduction

Organochlorine pesticides (OCPs) are environmental contaminants, the exposure to which has potential effects such as alterations in basic cellular signaling processes and endocrine function (Saeedi Saravi and Dehpour, 2016). OCPs are categorized as persistent organic pollutants (POPs), meaning they persist in the environment and bioaccumulate through the food chain in the fatty tissue of humans and animals. These compounds have been banned or restricted under the Stockholm Convention since 2004, however, they can still be found in the environment.

In Japan, the use of most OCPs for agricultural purposes was prohibited during the 1970s. The pesticides heptachlor, dieldrin, and chlordane were used for termite control until the 1980s, despite being banned from agricultural use. Although never registered for agricultural use, hexachlorobenzene (HCB) was used for industrial purposes until the 1980s. In Japan, mirex and toxaphene have never been produced or used for industrial or agricultural purposes (Ministry of the Environment Government of Japan, 2017). However, these compounds were detected in the air, soil, and water, as well as in animals and humans (Ministry of the Environment Government of Japan, 2014). For example, in our previous study, we detected 21 OCPs in the serum of pregnant Japanese women (Kanazawa et al., 2012).

Although prenatal exposure to OCPs is known to be associated with impaired neurodevelopment in children, previous studies have yielded inconsistent results. Two previous studies conducted in New York City and Oswego, New York, showed no association between dichlorodiphenyldichloroethylene (DDE) concentration and health conditions in newborns (Engel et al., 2007; Stewart et al., 2000). However, a study in Massachusetts reported that polychlorinated biphenyl (PCB) and DDE negatively

affected attention-related ability in infants, within 5 days after birth (Sagiv et al., 2008).

Many studies that have evaluated neurodevelopment in order to examine the effects of prenatal exposure to OCPs used the Bayley Scales of Infant Development (BSID) or the BSID second edition (BSID-II). These are suitable for infants up to 4 years and consist of the Mental Development Index (MDI) and Psychomotor Development Index (PDI).

In a Mexican study, high concentrations of *p,p'*-DDE (1436.9 ng/g lipid) in maternal serum during the first trimester of pregnancy were found to be associated with a significant reduction in the resulting child's PDI at 6 months, MDI at 24 months, and both PDI and MDI at 12 months (Eskenazi et al., 2006). Two other studies reported that prenatal exposure to moderate concentrations of *p,p'*-DDE and DDE (119.06 ng/g lipid and 132.47 ng/g lipid, respectively) was not associated with a reduction in BSID scores among 14-month-old infants (Forns et al., 2012; Gascon et al., 2013). However, in a Spanish study Ribas-Fito et al. (2003) reported that cord serum *p,p'*-DDE concentrations (2.0 ng/mL) were associated with a decrease in MDI and PDI among children at 13 months old. Although it is difficult to compare OCP concentrations across studies because the *p,p'*-DDE values were achieved using cord serum samples in Ribas-Fito et al., the concentrations reported by them were lower than those observed in many other studies. Therefore, concentration level of OCP might not be the only determinant of impaired neurodevelopment caused by prenatal OCP exposure.

Torres-Sanchez et al. (2007) detected DDE levels in maternal serum during the first trimester of pregnancy (6.8 ng/mL), and found that its concentration was related to a reduction in PDI at 1, 3, 6 and 12 months, even at an exposure level lower than those reported in the previous two studies. However, the same cohort demonstrated no significant association between BSID score at 12–30 months and exposure to

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dichlorodiphenyltrichloroethane (DDT) and DDE (Torres-Sanchez et al., 2009). The findings of these studies suggest that the association between DDE and infant neurodevelopment might be reversible. Thus, we hypothesized that the key determinant of the relationship between OCP exposure and neurodevelopment might be the timing of the administration of developmental assessments, as well as the levels of OCPs.

The aim of the present study was to clarify the association between prenatal exposure to relatively low levels of OCPs and neurodevelopment in infants at ages 6 and 18 months. In Japan, Kanazawa et al. (2012) reported low serum OCP concentrations, especially *p,p'*-DDE (0.61 ng/g wet weight in maternal serum), which were less than one-tenth of those reported by Eskenazi et al. (2006) in Mexico. If there is a significant association between prenatal OCP exposure and neurodevelopment at either 6 or 18 months, despite relatively low OCP concentrations, the result might indicate that OCP levels are not the only determinants of neurodevelopment in the setting of OCP exposure. Additionally, implementation of BSID-II at two different time points may allow investigation as to whether the timing of neurodevelopmental assessment is an important factor in examining the association between prenatal OCP exposure and neurodevelopment.

2. Methods

2.1. Study population

This prospective birth cohort study was based on the Sapporo Cohort, the Hokkaido Study on Environment and Children's Health (Kishi et al., 2013; Kishi et al., 2011). A total of 514 pregnant women were recruited for the study between July 2002 and July 2005 from the Sapporo Toho Hospital in Hokkaido, Japan. All participants were native

Japanese women residing in Sapporo and surrounding areas. The participants completed a self-administered questionnaire after the second trimester of their pregnancy. The questionnaire obtained baseline information such as education level, family income, working during pregnancy, tobacco/smoking history, and alcohol consumption. The perinatal information for the mothers and infants was collected from their medical records. We also screened for postpartum depression at 1 and 6 months after delivery using the Japanese version of the Edinburgh Postnatal Depression Scale (EPDS) (Okano et al., 1996).

The inclusion criteria for the analyses at 6 and 18 months were as follows: no serious illnesses or complications during pregnancy and delivery, singleton babies born at term (37–42 weeks gestation), Apgar score >7 at 1 minute, and infants without congenital anomalies or diseases. According to these criteria, 475 were selected among all 514 participants of the Sapporo Cohort. Of these 475 women, 362 had OCP data. Among them, 290 mothers did not agree with the BSID-II assessment, or did not participate in the BSID-II assessment at the appropriate age. Finally, 164 and 115 participants had available BSID-II scores at 6 and 18 months, respectively. A total of 185 participants, who had BSID-II data for either 6 or 18 months were analyzed and included in the study.

Written informed consent was obtained from all participants. The protocol used in this study was approved by the Institutional Ethics Board for epidemiological studies at the Hokkaido University Graduate School of Medicine, Hokkaido University Center for Environmental and Health Sciences.

2.2. *Exposure measures*

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The detailed methodology for the measurement of OCPs has been described in our previous report (Kanazawa et al., 2012). The 29 OCPs evaluated in this study were 6 DDTs [*o,p'*-DDT, *p,p'*-DDT, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-dichlorodiphenyldichloroethane (DDD), *p,p'*-DDD], 5 chlordanes (*cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, oxychlordane), 3 drins (aldrin, dieldrin, and endrin), 3 heptachlors [heptachlor, *cis*-heptachlor epoxide (HCE), *trans*-HCE], HCB, 4 hexachlorocyclohexane isomers [α- hexachlorocyclohexane (HCH), β-HCH, γ-HCH, δ-HCH], mirex, and 6 toxaphenes (Parlar-26, Parlar-41, Parlar-40, Parlar-44, Parlar-50, and Parlar-62). The internal standards (for the clean-up and syringe spikes) were ^{13}C-labeled isomers or d-isomers obtained from Cambridge Isotope Laboratory, Inc. (Andover, MA, USA). The concentrations of OCPs were measured according to the methods recommended by the Ministry of the Environment, Japan, using gas chromatography/negative-ion chemical-ionization mass spectrometry (GC/NCIMS) and gas chromatography/hi-resolution mass spectrometry (GC/HRMS). All procedures were performed by IDEA Consultants, Inc. (Shizuoka, Japan).

2.3. Developmental measures

We used the BSID-II (Bayley, 1993) to assess infants' mental and psychomotor development at 6 and 18 months of age. The BSID-II is a developmental screening tool that has been standardized for use as an infant assessment instrument in the United States and is widely used in both clinical and research settings. The BSID-II mental scale assesses the age-appropriate level of cognitive, language, and personal/social development in children. The motor scale assesses fine and gross motor development. The mental and motor scores are based on a calibrated scale constructed using raw

scores and are represented as index scores. The mean (±SD) values of the MDI and the PDI were 100 (±15). All infants were examined by one examiner in a quiet, private room in the presence of the parent/s. The indicators of neurodevelopment were evaluated by three occupational therapists with clinical experience in the field of developmental disabilities. First, the scoring was performed by the examiner who assessed all of the infants. Two other examiners then verified and confirmed the scores by reviewing videotaped evaluations. The examiners were unaware of the participants' OCP levels. In addition, we used the Index of Child Care Environment (ICCE) to assess the child care environment of the participants at 6 and 18 months old (Anme et al., 1997; Anme et al., 2013).

2.4.Data analysis

We analyzed the correlations between BSID-II scores and the characteristics of the mothers and infants using the Pearson's correlation coefficient test and Student's t-test. We performed multiple regression analysis to examine the association between BSID-II scores and the OCP concentrations in maternal serum samples. The values were log10 transformed and potential confounders were selected according to the findings from previous studies. Subsequently, we used directed acyclic graphs (DAGs) to finally determine adjustment factors. The set of variables selected for adjustment were age at delivery, annual income, first-born status, smoking during pregnancy, and education level. For model 2, EPDS and ICCE were added to the list, because these were the most influential factors for BSID after birth in our previous study (Otake et al., 2014). Infant sex and maternal blood sampling period for OCP measurements were included as a priori in both models.

To facilitate interpretation of study results, post hoc power analysis was conducted using G* Power3 software (Faul et al., 2007). Multi-regression analysis was used with 8 predictors (model 2), detectable effect size f^2 was 0.096 at 6-months (n = 164), and 0.140 at 18-months (α = 0.05, β = 0.2). According to Cohen's guidelines (1988), $f^2 \geq$ 0.02, $f^2 \geq$ 0.15, and $f^2 \geq$ 0.35 represent small, medium and large effect sizes respectively. Therefore, our analysis can detect medium to small size effect both at 6 and 18 months.

Previously, our group reported that prenatal exposure to dioxin congeners is negatively associated with MDI and PDI of infants at 6 months old (Nakajima et al., 2006) and that an inverse association exists between prenatal exposure to perfluorooctanoate (PFOA) and MDI only among female infants at 6 months old (Goudarzi et al., 2016). Therefore, prenatal dioxin and PFOA levels were added to the aforementioned confounders in an additional adjustment model, which is shown in Supplemental Tables 1 and 2.

We also evaluated whether the relationship between BSID score and OCP exposure differs based on sex (Boucher et al., 2013). A multivariate linear regression model was constructed with the interaction terms for BSID and OCP for the 6 and 18-month-old participants. However, owing to a lack of such an association (the smallest $p_{interaction}$ = 0.073 for mirex for PDI at 6 months, and $p_{interaction}$ > 0.1 for other OCPs for both 6 and 18-month-old participants), we performed a subsequent analysis stratified for sex which showed no significant association between mirex and PDI (β=1.29, CI: -8.45 11.03 for male, β=-10.2, CI: -21.80 1.40) or between any other OCPs and BSID. We therefore proceeded with our analyses with both male and female infants grouped together. For the assessment of the dose–response relationship between OCP exposure and BSID score, prenatal OCP concentrations in maternal blood samples were divided into quartiles and the least square means (LSMs) and 95% confidence intervals (CIs) were

calculated. The p for trend was estimated using the linear contrast coefficients –3, –1, +1, +3 assigned to quartiles 1, 2, 3, and 4, respectively. The LSM of the BSID-II scores for each quartile was compared using the Hsu–Dunnett method to accommodate for multiple comparisons. Results were considered significant if p < 0.05. We performed all the statistical analyses using SPSS (Version 22.0; SPSS, Chicago, IL, USA) and JMP pro 12 (SAS Institute Inc., NC, USA).

3. Results

The BSID scores and basic characteristics of the study population are presented in Table 1. Maternal age and educational level, gestational age, birth weight, and birth length were significantly higher among those who were included in the analysis compared to those who were excluded. Among the included group, the mean (±SD) MDI was 90.7 (±5.6) and PDI was 89.7 (±10.4) for infants at 6 months old, whereas the mean (±SD) MDI and PDI at 18 months old were 82.9 (±11.3) and 86.2 (±11.4), respectively. The ICCE was 22.6 (±2.5) at 6 months and 28.4 (±3.4) at 18 months old. The proportion of participants with an annual household income of more than 5 million yen was higher in the 18 month analysis than in the 6 month analysis. Moreover, the proportion of participants who smoked during pregnancy and those with male and first-born infants were lower at the 18 month analysis (Table 1).

Maternal serum OCP concentrations are presented in Table 2. Of 29 OCPs, 15 compounds that were detected among >80% of participants are reported. There was no difference in OCP concentrations across participant groups.

Table 3 shows the BSID scores according to characteristics of the mothers and infants at 6 and 18 months old. At 6 months, mother who did not work during pregnancy showed

lower MDIs (p < 0.05). For infants, gestational age was positively correlated with MDI (r = 0.188, p < 0.05) and PDI (r = 0.256, p < .01). At 18 months old, mothers with an annual income above 5 million demonstrated significantly higher PDIs (p < 0.05); a similar trend was also observed for the MDI (p = 0.06). Female infants showed both higher PDI and MDI scores than male infants (p < 0.05). The ICCE demonstrated a significant correlation with MDI (r = 0.190, p < 0.05), and a marginally significant correlation with PDI (r = 0.174, p = 0.06).

Tables 4 and 5 show the association between prenatal exposure to OCPs and BSID-II scores (MDI, PDI) at 6 and 18 months. At 6 months, no significant association between OCPs and the BSID score was observed. However, at 18 months, we found a negative association between *cis*-HCE exposure and the MDI in the crude model (β = –8.99; 95% confidence interval [CI]: –17.66, –0.32), model 1 (β = –9.72; 95% CI: –18.60, –0.85) and model 2 (β = –10.07; 95% CI: –18.79, –1.76). Therefore, children in the cohort who had the highest concentrations of *cis*-HCE showed a low score in the MDI after adjusting for appropriate confounders.

Additionally, we included dioxin and PFOA levels in the models for the 6 month and 18 month analyses (Supplemental Tables 1 and 2) considering the possibility of co-exposures. Dioxin adjusted model did not change the results both at 6 months and 18 months results. The PFOA adjusted model did not change the results at 6 months, while the association between cis-HCE and MDI changed to being marginally significant at 18 months.

We also examined the association between the *cis*-HCE quartiles and MDI (Figure 1 and Supplemental Table 3). Figure 1 shows the dose–response relationship between the quartiles of *cis*-HCE and decreased MDI at 18 months old. In the adjusted model, p for

trend was significant (p < 0.05). Furthermore, infants born to mothers with prenatal concentrations of *cis*-HCE within the fourth quartile had MDI scores that were –9.14 (95% confidence interval: –15.96, –2.31) lower than those recorded for infants born to mothers with concentrations of *cis*-HCE in the first quartile (p for trend < 0.01).

4. Discussion

This study is one of the few to have examined the association between prenatal exposure to OCPs and neurodevelopment in early life. As we have already reported, the maternal OCP concentrations observed in Japan are relatively low when compared to other countries (Kanazawa et al., 2012). In this study, we examined the association between prenatal exposure to low levels of OCPs and the subsequent neurodevelopment of infants at 6 and 18 months old using the BSID-II. We found an inverse association between prenatal exposure to *cis*-HCE and the MDI at 18 months old. Furthermore, the *cis*-HCE dose–response trend analysis showed a significant decrease in MDI at 18 months old.

The concentrations of the OCPs in this study were similar to those reported in two Japanese studies (Fukata et al., 2005; Sugiura-Ogasawara et al., 2003), which involved participants exposed to OCPs during approximately the same period as in the present study. According to Govarts et al. (2012), the level of *p,p'*-DDE exposure in the previous Japanese cohorts and in the present study were close to the mean of European cohorts. Precise comparisons between the concentrations of OCPs in the present study and those in previous studies are limited, because most previous studies investigated lipid-based OCP concentrations in serum or plasma samples. We divided the wet-weight-based OCP concentrations by median fat content among participants in the

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same cohort (n = 426) to calculate the approximate lipid-based OCP concentration. We found that the median concentration of *p,p'*-DDE was 183.8 ng/g lipid, much lower than that reported by Eskenazi et al. (2006) among people in Salinas Valley, CA, USA (1436.9 ng/g lipid; geometric mean in maternal serum), and similar to that reported by Gascon et al. (2013), among people in Spain (132.47 ng/g lipid; GM in maternal serum). Furthermore, mirex, Parlar-26, and Parlar-50 were also detected in this study, despite the fact that they have never been used in Japan.

Although we examined comparably low levels of exposure to OCPs, we found inverse associations between prenatal exposure to OCPs and BSID score at 18 months old. This result suggests that OCP level is not necessarily a critical determinant of neurodevelopment. Furthermore, we found an association between *cis*-HCE concentration and the MDI at 18 months but not at 6 months, even though fewer infants were examined at 18 months (n = 115 vs n = 164).

The impact of OCPs, especially *cis*-HCE, on neurodevelopment in infancy and early childhood are not well understood. In an animal studies, the neurotoxicological outcomes of perinatal HCE exposure in rats suggested developmental delays, alterations in gamma-aminobutyric acidergic neurotransmission, and neurobehavioral changes, including cognitive deficits, even at low doses (Moser et al., 2001). For humans, the Concise International Chemical Assessment Document (CICAD) describes a study conducted in Oahu, Hawaii, USA, in which gestational HCE exposure was associated with a slower acquisition of behaviors at 4 and 8 months, but not at 18 and 36 months (http://apps.who.int/iris/bitstream/10665/43441/1/9241530707_eng.pdf). On the other hand, the CICAD also reported that any association between HCE and cognitive deficits among the 399 children aged $\geq$ 5 years was probably due to other co-exposures, to

either polychlorinated biphenyls or unidentified confounders, in a study conducted in San Francisco Bay, California, USA (World Health Organization, 2006). In the present study, regression models adjusted for dioxin TEQs still indicated a significant adverse association between *cis*-HCE and the MDI. These results indicate that prenatal exposure to *cis*-HCE could affect MDI independent of dioxin. For the PFOA adjusted model, the association between cis-HCE and MDI did not reach significance. In this model, the analyzed participants were smaller than the original analysis because of the lack of PFOA data. This may have caused a loss of statistical power. However, these results might show a latent association between PFOA and MDI. It is necessary to determine the extent of simultaneous exposure to both cis-HCE and PFOA in future analyses. In addition to the simultaneous exposure to multiple POPs, we must consider the most suitable time-point at which to assess neurodevelopment. *cis*-HCE had inverse associations with the BSID score at 18 months old, but not at 6 months old, which indicates that the effect of OCP on neurodevelopment might be observed during a specific time-window. However, there are other possible explanations for these results. One possibility is that a different subset of participants contributed data at 6 and 18 months old. To test this possibility, we performed multiple regression analysis for those for whom neurodevelopmental assessment data was available for both 6 and 18 months (N = 94). As in model 2, in the adjusted model, the (Supplemental Table 4), associations between *cis*-HCE and MDI were still significant (β=-10.07, CI: -18.79 -1.36) at 18 months. However, there were no significant or marginally significant associations seen for the analysis of 6 month old infants. Total tendency at 18 months remained the same for both groups of participants. Furthermore, to ascertain follow-up bias, we compared characteristics among those who had 2 neurodevelopment assessments (both at 6 and 18

months, N = 94), those who had only the 6-month assessment (N = 70), and those who had only the 18-month assessment (N = 21). As a result, we found significant differences among only those who smoked during pregnancy (Supplemental Table 5). Although the number of mothers who smoked during pregnancy was smaller in the assessment group than the other two groups, there was no difference between the 6 month only and the 18 month only assessment groups. In addition, the results of the regression model were the same in both the assessment group and the 18 month only group. We considered that the loss to follow-up does not explain the different results between the 6 month and the 18 month analysis. Another possibility is that OCP might affect one specific aspect of brain development. In our results, annual income, infant sex, and ICCE had a strong relationship with the PDI and MDI at 18 months old (Table 3). Annual income and infant sex, in particular, had significant positive associations with PDI more than other confounders in model 1 (data not shown). These variables might be important environmental factors for the development of an infant's ability at 18 months old but not at 6 months old. At 6 months old, infants obtain basic cognitive functions that are affected by biological factors such as maternal age. We hypothesize that environmental factors, such as *cis*-HCE, might affect only higher level functions of brain development. Overall, associations were larger for PDI compared to MDI in this study. These trends were in line with the previous study (Torres-Sanchez et al., 2007). One possibility for this is that thyroid hormone might mediate the adverse effect by OCP. One animal study showed that POPs significantly suppressed thyroid hormone-induced Purkinje cell dendrite arborization in the cerebellar culture (Ibhazehiebo et al., 2011); this is significant since the cerebellum is known as the locus of motor control (Fine et al., 2002). Our results may also be explained by the time-course of human ability

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developments. Development of motor function is salient in early childhood while mental functions develop gradually from early childhood to school age according to brain development. In early childhood, there might be ceiling effect for measuring adverse effects on mental development, but not on motor development. Continuous neurodevelopmental examinations would have to be conducted to determine these possibilities. The major strength of our study was that the outcomes of infant neurodevelopment were measured by a well-trained examiner to minimize inter-examiner bias, and twice at different time points, which allowed for investigation of the association between prenatal OCP exposure and infant neurodevelopment over time. In particular, we were able to assess the time-course of neurodevelopment on the basis of the 6 and 18 month old BSID scores for the same groups of infants in the prospective birth cohort. Moreover, OCP exposure was quantified by GC/NCIMS and GC/HRMS with high accuracy and precision.

One limitation of this study is that only the BSID-II, which was not standardized in Japan, was used for the neurodevelopmental evaluation of infants. The mean ($\pm$SD) of MDIs and PDIs were 90.7 ($\pm$5.6) and 89.7 ($\pm$10.4) at 6 months and 82.9 ($\pm$11.3) and 86.2 ($\pm$11.4) at 18 months, respectively. These scores were lower than the standard scores, likely owing to the cultural and language differences between Japan and the United States. Although we observed lower mean BSID-II scores among our study population, Oka et al. (2005) reported a high correlation between the BSID-II and the Kyoto Developmental Test that has been standardized in Japan. Thus, the BSID-II scores of our study population were considered to be validated and suitable for analyses.

Another limitation was that a limited number of participants agreed to participate in the BSID follow-up. Comparison of characteristics showed an older maternal age,

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higher educational levels, longer gestational period, heavier birth weight, and taller birth length for those with BSID scores (shown as included group) than those who did not have BSID data (shown as the not included group) (Table 1). The number of mothers smoking during pregnancy tended to be smaller in the included group than in the not included group. These results might indicate that the group analyzed for BSID-II in 6M and 18M were healthier than those not included. However, these characteristics did not directly correlate with the BSID-II score at 18 months (Table 3). Therefore, we considered that the effects were minimum towards the 18-month BSID results. However, at 6 months, gestational age was positively correlated with both MDI ($r = 0.19$, $p < 0.05$) and PDI ($r = 0.26$, $p < 0.01$). Therefore, because of the relatively longer gestational days for the group with BSID data, the effect of OCP may be underestimated. Although, in several epidemiological studies, sex-specific association between prenatal chemical exposure and childhood neurodevelopment were observed (Boucher et al., 2013; Goudarzi et al., 2016), we could not compare the results between sex directly, because the number of participants was very small at 18 months old (male, 51; female, 64). However, an interaction between sex and OCP exposure was not found, and a subsequent analysis stratified for sex showed no significant association between any OCP and BSID (data is not shown); therefore, we did not conduct any further analyses according to infant sex. It is also noted that these results should be carefully considered. Statistically, there remains the possibility that the significant association between BSID-II score and *cis*-HCE or mirex were observed by chance, because there is a 5% chance of incorrectly rejecting the null hypothesis in repetitive analyses (Hubbard, 2011). Moreover, we cannot exclude the possible influence of chemical compounds other than dioxin and PFOA, which were not measured in the present study. As

concentrations of OCPs were strongly correlated with each other (Supplemental Table 5), we cannot rule out the possibility that other OCPs affected our results; therefore, further studies are needed to confirm the results in this study, as well as results of those investigating simultaneous exposure to multiple compounds via statistical analyses (using structural equation modeling or weighted quantile sums, for example). In conclusion, we found that prenatal exposure to low levels of OCPs adversely affected neurodevelopment in infants at 18 months old, but not at 6 months old. In particular, the association between *cis*-HCE and MDI was significant when dioxin exposure was used as an adjustment factor, and was marginally significant when PFOS was used. Our results indicate that prenatal exposure to *cis*-HCE affects infant neurodevelopment. OCPs persist in the environment around the world at higher concentrations than those seen in Japan. Further studies are needed to clarify the time-course of neurodevelopment in the presence of prenatal exposure to these OCP compounds.

Conflict of interest statement

None of the authors have any conflicts of interest to report.

Acknowledgments

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Table 1. Characteristics of mothers and infants.

Characteristic		Included (N = 185) Mean± SD or No.(%)	6M BSID-II (N = 164) Mean± SD or No.(%)	18M BSID-II (N = 115) Mean± SD or No.(%)	n	Excluded (N =290) Mean± SD or No.(%)	p-value
Mother							
Age at delivery (years)		31.4 ± 4.9	31.3 ± 4.7	31.4 ± 4.9	290	30.29 ± 4.9	0.02
Education Level (years)	<13	69 (37.3)	61 (37.2)	42 (36.5)		146 (50.3)	0.01
	≥13	116 (62.7)	103 (62.8)	73 (63.5)		144 (49.7)	
Annual income (million yen)	<5	120 (64.9)	103 (62.8)	78 (67.8)		207 (71.4)	0.10
	≥5	65 (35.1)	61 (37.2)	37 (32.2)		80 (27.6)	
Worked during pregnancy		22 (11.9)	19 (11.6)	11 (9.6)		27 (9.3)	0.44
Smoked during pregnancy		25 (13.5)	21 (12.8)	9 (7.8)		58 (20.0)	0.08
Alcohol intake during pregnancy		54 (29.2)	47 (28.7)	32 (27.8)		96 (33.1)	0.42
Blood sampling period	During pregnancy	123 (66.5)	110 (67.1)	75 (65.2)		111 (38.3)	0.51
	After delivery	62 (33.5)	54 (32.9)	40 (34.8)		66 (22.8)	
EPDS 1M		2.68 ± 3.9	4.05 ± 4.5	3.56 ± 3.5	237	3 ± 3.8	0.90
EPDS 6M		4.07 ± 4.4	2.71 ± 3.8	2.39 ± 3.2	211	4.13 ± 4.1	0.42
Infant							
Sex	Male	90 (48.6)	79 (48.2)	51 (44.3)		138 (47.6)	0.85
	Female	95 (51.4)	85 (51.8)	64 (55.7)		152 (52.4)	
Gestational age (days)		277 ± 8.0	277 ± 8.1	277 ± 8.0	290	275 ± 9.9	0.03
Birth weight (g)		3121 ± 334.0	3129 ± 330.4	3109 ± 307.9	290	3037 ± 411.2	0.02
Birth length (cm)		48.3 ± 1.6	48.4 ± 1.5	48.3 ± 1.5	290	47.9 ± 2.2	0.04
First-born		93 (50.3)	84 (51.2)	54 (47.0)		133 (45.9)	0.40
Duration of breast-feeding	< 3Months	19 (10.3)	17 (10.4)	11 (9.6)		25 (8.6)	0.63
	≥ 3Months	150 (81.1)	131 (79.9)	102 (88.7)		165 (56.9)	
BSID-II mental index score: MDI			90.7 ± 5.6	82.94 ± 11.3			

BSID-II motor index score: PDI	89.7	± 10.4	86.23	± 11.4
ICCE	22.6	± 2.5	28.44	± 3.4

BSID-II: Bayley Scales of Infant Development second edition, EPDS:Edinburgh Postnatal Depression Scale, ICCE: Index of Child Care Environment

Total of 185 participants who had BSID-II data for either 6 or 18 months were included.

6M BSID-II and 18M BSID-II indicates participants with available OCP data who were followed up until 18 months and completed the BSID assessment at 6 and 18 months, respectively.

Table 2 Concentrations of OCPs (pg/g wet weight) in maternal serum.

	Detection Limit	Detection Rate	Median (range)					
			ALL (N = 185)		6M BSID-II (N = 164)		18M BSID-II (N = 115)	
Oxychlordane	0.90	100	37.7	(8.5–250.9)	38.1	(8.5–250.9)	37.0	(9.2–250.9)
cis-nonachlor	0.40	100	9.8	(1.6–38.1)	9.9	(1.6–38.1)	9.2	(1.6–38.1)
trans-nonachlor	0.50	100	67.0	(13.1–513.5)	67.1	(13.1–513.5)	66.9	(13.1–513.5)
p,p'-DDD	0.40	90	1.4	(0.2–6.3)	1.4	(0.2–6.3)	1.7	(0.2–6.2)
o,p'-DDE	0.40	85	1.3	(0.2–6.2)	1.4	(0.2–6.2)	1.4	(0.2–6.2)
p,p'-DDE	0.60	100	634.0	(99.5–4575.7)	656.2	(99.5–4575.7)	650.5	(103.3–4575.7)
o,p'-DDT	0.60	98	3.5	(0.3–13.3)	3.7	(0.3–13.3)	3.8	(0.3–13.2)
p,p'-DDT	0.40	100	24.3	(2.4–121.5)	26.0	(2.4–121.5)	26.3	(6.2–121.5)
Dieldrin	0.80	100	16.7	(4.1–53.5)	17.2	(4.1–53.5)	16.8	(5.1–47.9)
cis-HCE	0.40	100	25.3	(6.2–200.5)	25.7	(6.2–200.5)	26.0	(7.6–200.5)
HCB	0.90	100	101.1	(36.8–239.8)	102.7	(36.8–239.8)	100.4	(36.8–239.8)
βHCH	0.60	100	154.1	(23.7–1667.1)	158.5	(23.7–1667.1)	152.8	(23.7–1667.1)
Mirex	0.50	100	5.9	(0.9–35.0)	6.1	(0.9–30.1)	5.7	(1.9–35.0)
Parlar-26	1.00	97	4.7	(0.5–18.9)	4.9	(0.5–18.9)	4.6	(0.5–17.3)
Parlar-50	2.00	96	7.1	(1.0–27.2)	7.6	(1.0–27.2)	6.6	(1.0–23.3)

DDD, dichlorodiphenyldichloroethane; DDE, dichlorodiphenyldichloroethylene; DDT,cis-heptachlor epoxide; cis-HCE

ALL indicates participants with available OCP data who have either BSID-II data at 6 or 18 months.

6M BSID-II and 18M BSID-II indicates participants with available OCP data who were followed up until 18 months and completed

Table3 BSID−II MDI and PDI scores at 6 months (n = 164) and 18 months (n = 115) according to characteristics of the mothers and infants

Characteristic		6M No.	MDI Mean±SD	MDI p−value	PDI Mean±SD	PDI p−value	18M No.	MDI Mean±SD	MDI p−value	PDI Mean±SD	PDI p−value
Mother											
Age (years)		164	r = 0.03	0.73	r = −0.11	0.17	115	r = −0.03	0.77	r = 0.08	0.37
Education level (years)	<13	61	91.5 ± 4.4	0.15	90.9 ± 10.3	0.29	42	83.8 ± 12.8	0.53	85.8 ± 12.1	0.78
	≥13	103	90.2 ± 6.2		89.1 ± 10.4		73	82.4 ± 10.4		86.5 ± 11.0	
Annual income (million yen)	<5	103	90.6 ± 5.7	0.86	90.2 ± 10.5	0.46	78	81.6 ± 11.2	0.06	84.6 ± 11.3	0.03
	≥5	61	90.8 ± 5.6		89.0 ± 10.2		37	85.8 ± 10.9		89.6 ± 10.8	
Worked during pregnancy	No	145	90.3 ± 5.4	0.04	89.7 ± 10.1	0.89	104	82.8 ± 11.6	0.68	86.5 ± 11.8	0.50
	Yes	19	93.1 ± 6.8		90.1 ± 12.4		11	84.3 ± 8.3		84.0 ± 6.4	
Smoked during pregnancy	No	143	90.7 ± 5.6	0.66	89.9 ± 10.4	0.54	106	83.2 ± 11.3	0.49	86.3 ± 11.4	0.95
	Yes	21	90.1 ± 6.2		88.4 ± 10.0		9	80.4 ± 11.3		86.0 ± 11.0	
Caffeine intake during pregnancy (mg/day)		164	r = −0.04	0.65	r = −0.07	0.39	115	r = −0.10	0.30	r = −0.07	0.44
Alcohol consumption during pregnancy		164	r = 0.16	0.045	r = 0.04	0.59	115	r = 0.03	0.77	r = 0.01	0.91
Blood sampling period	During	110	90.5 ± 5.1	0.50	88.8 ± 9.7	0.08	75	83.5 ± 11.4	0.49	86.0 ± 11.5	0.76
	After delivery	54	91.1 ± 6.6		91.8 ± 11.3		40	82.0 ± 11.0		86.7 ± 11.1	
EPDS 1M		164	r = −0.14	0.08	r = −0.11	0.17	110	r = −0.14	0.15	r = −0.16	0.10
EPDS 6M		155	r = −0.09	0.25	r = 0.02	0.77	115	r = −0.13	0.18	r = −0.09	0.37
Infant											
Sex	Male	79	90.8 ± 5.3	0.77	89.1 ± 10.0	0.42	51	80.2 ± 9.7	0.02	82.8 ± 10.6	<.01
	Female	85	90.5 ± 6.0		90.4 ± 10.7		64	85.1 ± 12.0		89.0 ± 11.3	
Gestational age (days)		164	r = 0.19	0.02	r = 0.26	<.01	115	r = 0.17	0.07	r = 0.15	0.11
Birth weight (g)		164	r = 0.03	0.73	r = 0.05	0.52	115	r = 0.13	0.16	r = 0.08	0.41
Birth length (cm)		164	r = 0.03	0.73	r = 0.10	0.20	115	r = 0.15	0.12	r = 0.12	0.20
Head circumference (cm)		164	r = −0.03	0.76	r = −0.06	0.43	115	r = −0.11	0.25	r = −0.08	0.43
First born	Yes	80	89.9 ± 6.3	0.08	89.2 ± 9.9	0.51	61	84.1 ± 10.8	0.23	87.8 ± 11.9	0.12
	No	84	91.4 ± 4.9		90.3 ± 10.9		54	81.6 ± 11.7		84.5 ± 10.6	

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		n	Mean	SD	p	Mean	SD	p	n	Mean	SD	p	Mean	SD	p
Duration of breast−feeding	< 3Months	17	90.3	± 5.4	0.80	91.2	± 12.1	0.55	11	86.3	± 17.1	0.26	88.0	± 11.1	0.61
	≥ 3Months	131	90.7	± 5.8		89.6	± 9.8		102	82.3	± 10.4		86.1	± 11.5	
ICCE		164	r = 0.01		0.88	r = −0.07		0.39	115	r = 0.19		0.04	r = 0.17		0.06

Student's t−test, Pearson's correlation coefficient test

BSID−II: Bayley Scales of Infant Development second edition, EPDS:Edinburgh Postnatal Depression Scale, ICCE: Index of Child Care Environment

Table4 Association between prenatal exposure to OCPs and MDI and PDI at 6 months (n = 164).

	MDI						PDI					
	Crude		Model 1		Model 2		Crude		Model 1		Model 2	
	β[a]	95% CI	β[a]	95% CI	β[a]	95% CI	β[a]	95% CI	β[a]	95% CI	β[a]	95% CI
Oxychlordane	2.62	(−0.86, 6.09)	1.22	(−2.71, 5.14)	1.36	(−2.54, 5.26)	1.03	(−5.40, 7.47)	1.58	(−5.62, 8.78)	1.85	(−5.36, 9.05)
cis−Nonachlor	1.67	(−1.68, 5.02)	0.34	(−3.32, 3.99)	0.76	(−2.89, 4.41)	−2.57	(−8.74, 3.60)	−2.62	(−9.31, 4.07)	−2.15	(−8.89, 4.58)
trans−Nonachlor	2.17	(−1.05, 5.39)	0.89	(−2.69, 4.46)	1.17	(−2.38, 4.72)	0.37	(−5.59, 6.34)	0.65	(−5.9, 7.21)	0.96	(−5.62, 7.53)
p,p'−DDD	0.49	(−1.95, 2.93)	0.23	(−2.24, 2.7)	0.31	(−2.17, 2.78)	0.81	(−3.68, 5.29)	0.61	(−3.91, 5.14)	0.44	(−4.13, 5.01)
o,p'−DDE	0.36	(−2.18, 2.90)	0.24	(−2.33, 2.81)	0.52	(−2.04, 3.09)	−0.36	(−5.03, 4.32)	−0.1	(−4.81, 4.61)	0.13	(−4.61, 4.87)
p,p'−DDE	−0.49	(−3.40, 2.42)	−0.84	(−3.79, 2.12)	−0.79	(−3.72, 2.14)	−1.93	(−7.28, 3.42)	−1.26	(−6.67, 4.16)	−1.21	(−6.63, 4.21)
o,p'−DDT	−0.39	(−3.33, 2.54)	−0.14	(−3.11, 2.83)	0.21	(−2.76, 3.17)	−1.20	(−6.60, 4.20)	−0.44	(−5.88, 5)	−0.04	(−5.52, 5.43)
p,p'−DDT	−0.21	(−3.62, 3.21)	−0.59	(−4.02, 2.84)	−0.02	(−3.47, 3.43)	−2.86	(−9.13, 3.42)	−2.76	(−9.03, 3.52)	−2.32	(−8.68, 4.05)
Dieldrin	−1.34	(−5.89, 3.21)	−2.15	(−6.8, 2.5)	−1.5	(−6.17, 3.16)	−4.03	(−12.39, 4.33)	−4.01	(−12.53, 4.51)	−3.35	(−11.96, 5.26)
cis−HCE	−0.54	(−4.38, 3.30)	−1.42	(−5.34, 2.49)	−0.92	(−4.84, 2.99)	0.22	(−6.84, 7.28)	0.45	(−6.73, 7.64)	0.97	(−6.27, 8.21)
HCB	2.72	(−2.72, 8.16)	0.79	(−4.99, 6.58)	1.35	(−4.43, 7.14)	−2.86	(−12.89, 7.17)	−3.19	(−13.78, 7.41)	−2.27	(−12.96, 8.42)
βHCH	0.56	(−2.39, 3.50)	−0.75	(−3.97, 2.47)	−0.52	(−3.73, 2.69)	−2.76	(−8.17, 2.64)	−2.33	(−8.22, 3.56)	−1.94	(−7.87, 3.98)
Mirex	0.70	(−2.71, 4.11)	−0.8	(−4.7, 3.1)	−0.44	(−4.33, 3.45)	−4.61	(−10.85, 1.63)	−4.67	(−11.79, 2.45)	−4.2	(−11.36, 2.96)
Parlar−26	0.38	(−2.57, 3.34)	−0.29	(−3.37, 2.79)	0.07	(−3, 3.15)	−3.62	(−9.03, 1.79)	−3.38	(−9, 2.23)	−2.99	(−8.65, 2.67)
Parlar−50	0.35	(−2.68, 3.38)	−0.33	(−3.48, 2.82)	0.02	(−3.12, 3.17)	−3.43	(−8.99, 2.12)	−2.99	(−8.74, 2.77)	−2.6	(−8.4, 3.19)

Model 1: Adjusted for annual income, first-born status, smoking during pregnancy, education level, blood sampling period, and infant sex.

Model 2: Model 1 + postpartum depression at 1 month and child care environment. [a] β is the point increase in developmental score per tenfold increase of OCP level.

DDD, dichlorodiphenyldichloroethane; DDE, dichlorodiphenyldichloroethylene; DDT,cis−heptachlor epoxide; cis−HCE dichlorodiphenyltrichloroethane;

HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; OCPs, organochlorine pesticides, CI; confidence interval MDI, mental development indices; PDI, psychomotor development indices

* p < 0 .05

Table5 Association between prenatal exposure to OCPs and MDI and PDI at 18 months (n = 115).

	MDI						PDI					
	Crude		Model 1		Model 2		Crude		Model 1		Model 2	
	β^a	95% CI	β^a	95% CI	β^a	95% CI	β^a	95% CI	β^a	95% CI	β^a	95% CI
Oxychlordane	−1.88	(−10.07, 6.31)	0.29	(−8.81, 9.39)	−0.51	(−9.48, 8.46)	−1.92	(−10.18, 6.34)	−2.09	(−11.19, 7.02)	−2.77	(−11.81, 6.27)
cis−Nonachlor	0.50	(−7.34, 8.34)	1.22	(−7.24, 9.68)	0.45	(−7.9, 8.8)	−1.05	(−8.95, 6.86)	−2.56	(−11.02, 5.91)	−3.2	(−11.61, 5.21)
trans−Nonachlor	−2.42	(−10.00, 5.16)	0.05	(−8.31, 8.41)	−0.27	(−8.5, 7.96)	−3.06	(−10.69, 4.58)	−3.05	(−11.4, 5.31)	−3.32	(−11.6, 4.96)
p,p'−DDD	−2.43	(−8.77, 3.92)	−1.93	(−8.27, 4.4)	−1.32	(−7.6, 4.96)	−2.32	(−8.72, 4.08)	−2.58	(−8.92, 3.76)	−2.01	(−8.35, 4.32)
o,p'−DDE	1.32	(−4.79, 7.42)	0.33	(−5.74, 6.39)	−0.3	(−6.29, 5.7)	0.42	(−5.75, 6.58)	−1.49	(−7.56, 4.59)	−2	(−8.04, 4.04)
p,p'−DDE	−2.73	(−9.82, 4.35)	−1.78	(−8.82, 5.26)	−1.55	(−8.49, 5.38)	−3.71	(−10.84, 3.42)	−4.02	(−11.04, 3)	−3.8	(−10.77, 3.17)
o,p'−DDT	2.01	(−5.22, 9.24)	1.04	(−6.14, 8.22)	1.04	(−6.05, 8.12)	−1.04	(−8.34, 6.25)	−2.74	(−9.91, 4.44)	−2.69	(−9.83, 4.45)
p,p'−DDT	−5.41	(−14.02, 3.20)	−4.32	(−12.81, 4.16)	−4.24	(−12.6, 4.12)	−6.26	(−14.93, 2.40)	−5.84	(−14.31, 2.64)	−5.82	(−14.23, 2.58)
Dieldrin	−4.16	(−14.86, 6.55)	−6.6	(−17.51, 4.32)	−6.37	(−17.12, 4.38)	−1.07	(−11.89, 9.75)	−4.07	(−15.06, 6.91)	−3.94	(−14.84, 6.96)
cis−HCE	−8.99	(−17.66,	−9.72	(−18.6,	−10.07	(−18.79, −1.36)*	−3.16	(−12.04, 5.73)	−4.02	(−13.08, 5.04)	−4.33	(−13.31, 4.65)
HCB	1.41	(−11.49, 14.30)	3.31	(−9.93, 16.54)	2.57	(−10.47,	−5.15	(−18.12, 7.82)	−5.4	(−18.64, 7.84)	−6.09	(−19.22, 7.04)
βHCH	−2.32	(−9.38, 4.73)	0.35	(−7.21, 7.91)	0.38	(−7.08, 7.83)	−3.91	(−11.00, 3.18)	−3.19	(−10.74, 4.36)	−3.26	(−10.76, 4.24)
Mirex	−1.19	(−9.18, 6.80)	−0.55	(−9.69, 8.59)	−1.17	(−10.19, 7.86)	−2.91	(−10.95, 5.14)	−7.1	(−16.16, 1.96)	−7.59	(−16.59, 1.4)
Parlar−26	1.00	(−6.09, 8.08)	0.10	(−7.18, 7.37)	−0.82	(−8.01, 6.37)	−0.71	(−7.85, 6.44)	−2.9	(−10.16, 4.37)	−3.72	(−10.95, 3.5)
Parlar−50	2.30	(−5.03, 9.64)	1.20	(−6.31, 8.71)	0.21	(−7.23, 7.65)	0.09	(−7.32, 7.49)	−2.46	(−9.98, 5.06)	−3.33	(−10.81, 4.16)

Model 1: Adjusted for annual income, first-born status, smoking during pregnancy, education level, blood sampling period, and infant sex.

Model 2: Model 1 + postpartum depression at 6 months and child care environment.

[a] β is the point increase in developmental score per tenfold increase of OCP level. DDD, dichlorodiphenyldichloroethane; DDE, dichlorodiphenyldichloroethylene; DDT,cis−heptachlor epoxide; cis−HCE

HCB, hexachlorobenzene; HCH, hexhexachlorocyclohexane; OCPs, organochlorine pesticides, CI; confidence interval; MDI, mental development indices; PDI, psychomotor development indices
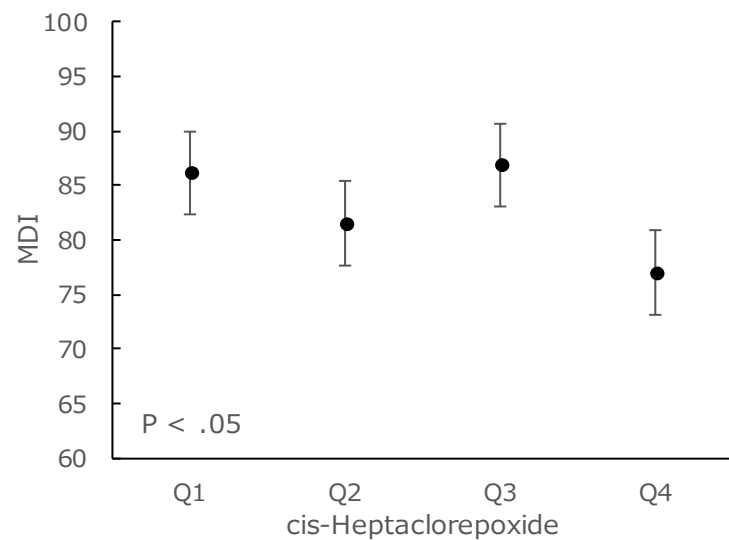
* p < 0 .05

Fig. 1. The dose-response relationship between the quartiles of cis-heptachlor epoxide and decreased MDI among infants at 18 months of age.

LSMs were adjusted for annual income, first-born, smoking during pregnancy, education level, blood sampling period, and infant sex, presence of postpartum depression at 6 months, and child care environment. LSMs are indicated in black circles and the error bars depict the upper and lower 95% CI. Q: quartile.