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Childhood exposure to polybrominated diphenyl ethers and neurodevelopment at six years of age

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Highlights

Brominated flame retardants are ubiquitous in indoor environment.

We examined whether environmental exposures could affect children's neurodevelopment.

Prenatal concentrations of decaBDE were not associated with cognitive score at age 6.

Childhood PBDE concentrations were negatively associated with cognitive scores. □

This association is stronger for pentaBDE mixture than for decaBDE.

ABSTRACT

Mixtures of polybrominated diphenyl ethers (PBDEs) are present in indoor environments. Studies of the developmental effects of exposure to these chemicals in large prospective mother-child cohorts are required, with data on prenatal exposure and long-term follow-up of the children.

We aimed to investigate the relationship between prenatal and childhood exposure to PBDEs and neurodevelopment at the age of six years.

We determined the levels of PBDEs and other neurotoxicants in cord blood and dust collected from the homes of children for 246 families included in the PELAGIE mother-child cohort in France. We assessed two cognitive domains of the six-year-old children using the Wechsler Intelligence Scale for Children-IV.

Verbal comprehension scores were lower in children from homes with higher concentrations of BDE99 ($\beta_{\text{Detects}<\text{median_vs_NonDetects}}=-1.6$; 95% CI: -6.1, 2.9; $\beta_{\text{Detects}\geq\text{median_vs_NonDetects}}=-5.4$; -9.9, -1.0; $\text{ptrend} = 0.02$) and of BDE209 ($\beta_{\text{2nd_vs_1st_tertile}}=-1.8$; 95% CI: -6.1, 2.5; $\beta_{\text{3rd_vs_1st_tertile}}=-3.2$; -7.5, 1.2; $\text{ptrend} = 0.15$) in dust, particularly for boys ($\text{ptrend} = 0.02$ and 0.04 , respectively). Working memory scores seemed to be lower in children with higher BDE99 concentrations in dust ($\text{ptrend} = 0.10$). No association was observed with cord blood levels of BDE209.

Our findings are in agreement with those of four previous studies suggesting adverse cognitive outcomes among children associated with early-life exposure to penta-BDE mixtures, and provide new evidence for the potential neurotoxicity of BDE209. Several countries are in the process of banning the use of PBDE mixtures as flame-retardants. However, these compounds are likely to remain present in the environment for a long time to come.

Keywords: brominated flame retardant, neurodevelopment, childhood, pregnancy, exposure

1. INTRODUCTION

Polybrominated diphenyl ethers (PBDEs) have been widely used as flame retardants for several decades and are present in diverse industrial and household products, including electronic equipment, electrical kitchen appliances, plastic compounds, upholstery and textiles, and in building materials (Frederiksen *et al.* 2009; Vonderheide *et al.* 2008). They are not chemically bound to the materials concerned and are continuously released into the indoor environment.

Three mixtures of PBDEs have been commercially available: penta-(BDEs 47, 99, 100 and 153), octa-(mixtures of hexa- to deca-BDEs), and deca-(BDE209) BDEs. PentaBDEs and octaBDEs were withdrawn from sale in the European Union in 2004. DecaBDE is more stable and is thought to be less toxic. It has not been banned outright, but its use has been restricted in Canada, Sweden and some American states (Vonderheide *et al.* 2008). PBDEs have been found in sediment, air, soil, and fish, and remain a matter of concern because of their persistence in the environment and bioaccumulation (Frederiksen *et al.* 2009; Vonderheide *et al.* 2008). Food and house dust are the principal sources of exposure in the general population (Costa and Giodarno 2007). House dust is the predominant pathway of exposure to PBDEs in toddlers and children (up to 80% intake), due to their tendency to move around and play on or close to the floor and their hand-mouth behavior (Lorber 2008). An international survey of indoor dust contamination showed that North American house dust was contaminated with commercial formulations of both deca- and penta-BDEs, whereas house dust in the UK was contaminated principally with deca-BDE (Harrad *et al.* 2008). The concentrations of penta- and deca-BDE mixtures in indoor air and dust were found to be about an order of magnitude higher in North America than in Europe (Frederiksen *et al.* 2009).

PBDEs cross the placenta and have been found in cord blood and breast milk, suggesting that infants are exposed to these compounds perinatally (Mazdai *et al.* 2003). In humans, serum PBDE concentrations are highest in toddlers and infants, followed by children, and lowest in adults (Frederiksen *et al.* 2009). All forms appear to have similar toxicity profiles, but with decaBDE displaying lower potency than other congeners with lower bromination levels. It has been shown that the exposure of rodents to PBDEs, both pre- and postnatally, causes hyperactivity and lower levels of habituation, and disrupts task performance in learning and memory tests (Dingemans *et al.* 2011; Costa *et al.* 2014). Possible mechanisms of action of PBDEs on neurodevelopment have been proposed, including the disruption of thyroid hormones (Schreiber *et al.* 2010, Herbstman and Mall 2014), the increase of oxidative stress and apoptosis (Kodavanti *et al.* 2015), the disruption of Ca²⁺ intracellular signaling pathways (Kodavanti *et al.* 2015), and the induction of neurotransmitter changes (Bradner *et al.* 2013).

A number of epidemiological studies have reported subtle cognitive and behavioral changes in children (4-7 years old) prenatally exposed to PBDEs, as shown by the sum of concentrations of several congeners in maternal or cord blood (Eskenazi *et al.* 2013) or for specific congeners of pentaBDE mixtures (Chen *et al.* 2014; Gascon *et al.* 2011; Herbstman *et al.* 2010; Roze *et al.* 2009). These studies reported successively deficits in verbal memory (BDE153) and sustained attention (BDE47 and 99) using the NEPSY-II neuropsychological battery (Roze *et al.* 2009), low full-scale (BDE47, 99 and 100) and verbal (BDE47 and 100) IQ scores using the Wechsler Preschool and Primary Scale of Intelligence (WPPSI) (Herbstman *et al.* 2010), poor verbal comprehension using the Wechsler Intelligence Scale for Children-4th edition (WISC-IV) (sum of BDE47, 99, 100 and 153, or individually; Eskenazi *et al.* 2013), or a low full-scale IQ using the WPPSI (BDE47; Chen *et al.* 2014) in children between five and seven years of age that had been prenatally exposed to PBDEs. Gascon *et al.* (2011) reported lower verbal scores for the McCarthy Scales of Children's Abilities (MSCA) in four-year-old children prenatally exposed to BDE47, but the results were not statistically significant.

The potential impact of early-childhood exposure to PBDEs on children (12 to 36 months of age) has been investigated in three epidemiological studies using measurements in breast milk or colostrum. Two reported that

lower mental scores on the Bayley Scales of Infant Development (BSID) were associated with a higher BDE209 concentration in milk (Chao *et al.* 2011; Gascon *et al.* 2012), whereas the third (Adgent *et al.* 2014) suggested that cognitive skills, as measured using the Mullen Scales of Early Learning (MSEL), tended to be positively associated with PBDE concentrations in breast milk (sum of BDE28, 47, 99, 100 and 153).

Only two studies have examined PBDE exposure during childhood, based on the concentrations in the child's blood at the age of four (Gascon *et al.* 2011) or seven years (Eskenazi *et al.* 2013). Gascon *et al.* (2011) found no association between the BDE47 concentrations and MSCA evaluations of motor and cognitive functions, whereas Eskenazi *et al.* (2013) reported that higher concentrations were associated with lower scores for the processing speed subscale of the WISC-IV (sum of BDE47, 99, 100 and 153, and individually).

In this study, we investigated the association between prenatal and childhood exposure to PBDEs and neurodevelopment at the age of six years, focusing particularly on BDE209, which is still in use.

2. METHODS

2.1. Participants

The participants were mother and child pairs included in the French PELAGIE mother-child cohort, which was designed to document the impact of perinatal exposure to a range of environmental contaminants on intrauterine and childhood development (Chevrier *et al.* 2013; Petit *et al.* 2010). Between 2002 and 2006, 3421 women from diverse rural and urban areas in the Brittany region were invited at the beginning of their pregnancy (<19 weeks of gestation) by their gynecologists, obstetricians, or ultrasonographers, to participate in the PELAGIE cohort. Women completed a questionnaire at home, reporting sociodemographic and personal characteristics. They were asked to return the questionnaire in a prepaid envelope, together with a first-morning-void urine sample (>95%

of the cohort) which was stored at -20°C on receipt. Umbilical cord blood was then obtained from 62% of the cohort. Randomly chosen families with live-born singleton children ($n = 591$) were selected to participate in a neuropsychological follow-up study when the children reached six years of age, regardless of the availability of prenatal biological samples. The exclusion criteria were child < 6 years or > 6 years and 3 months old, gestational duration < 35 weeks, neonatal disorders (e.g. severe hypoglycemia, low Apgar score reflecting non-optimal health of the newborn a few minutes after birth), neonatal hospitalization or reanimation, genetic abnormalities, and child or mother death. In total, 570 families were considered to be eligible for this study.

Between 2009 and 2012, we were able to contact 446 of the eligible families by telephone (124 families had incorrect or unavailable phone numbers, or were not reachable by phone). Among them, 138 families refused to participate in the neuropsychological follow-up, 18 were excluded because of previous child neuropsychological or behavioral tests (to avoid bias due to the learning effect), and three did not complete the testing. In total, 287 families (64%) agreed to participate and underwent neuropsychological testing, which was conducted at home by two licensed psychologists blinded to the exposure status. One of the psychologists completed child neurodevelopmental assessments. The other was in charge of maternal interviews to document maternal verbal IQ using the Wechsler Adult Intelligence Scale-3rd edition (WAIS-III) (Wechsler 1997) and quality of the familial environment assessed with the HOME inventory (Home Observation for Measurement of the Environment) (Elardo and Bradley 1981). The morning of the home visit, mothers collected a first-morning-void urine sample of their child. During the home visit, they provided completed paper questionnaires about their sociodemographic background, and the environment and health of the child. We collected dust samples from vacuum cleaner bags for PBDE determinations in 246 homes (86%), and wipes were passed over the floors for lead determinations in all homes.

Written informed consent was obtained from the mother. The research was approved by the appropriate ethics committees (the French Consulting Committee for the Treatment of Information in Medical Research, no. 09.485, and the French National Commission for the Confidentiality of Computerized Data, no. 909347).

2.2. Neuropsychological assessment

The WISC-IV test battery was used to assess cognitive abilities in six-year-old children (Wechsler 2003). Six subtests were administered (similarities, vocabulary, comprehension, digit span, letter-number sequencing, and block design), leading to the calculation of two index scores: 1) the Verbal Comprehension Index (WISC-VCI; subtests similarities, vocabulary, comprehension) that assesses the formation of verbal concepts, a good predictor of readiness for school, and 2) the Working Memory Index (WISC-WMI; subtests digit span, letter-number sequencing,) that assesses the child's ability to memorize new information, to hold it in short-term memory, to concentrate, and to manipulate information. We did not administer all the scales of the WISC-IV due to time constraints.

2.3. Assessment of PBDE exposure

Concentrations of the main PBDE congeners present in the commercial mixtures, BDE28, 47, 99, 100, 153, 154, 183 and 209, were determined for 159 umbilical cord serum samples (55%) by the *Centre de Toxicologie du Québec* (CTQ, *Institut national de santé publique du Québec*, Québec, Canada), to document prenatal exposure. Two cross-validated methods were used to quantify the PBDEs. Serum samples (2 mL) were enriched with ^{13}C -labeled internal standards (BDE77- $^{13}\text{C}_{12}$, PCB194- $^{13}\text{C}_{12}$ and BDE209- $^{13}\text{C}_{12}$) and BDE101 and denatured with formic acid (series 1: 80% of the samples) and with reagent alcohol (series 2: 20% of the samples). The compounds were then automatically extracted with dichloromethane in a solid-phase extraction (series 1) and from the aqueous matrix with hexane, in a liquid-liquid extraction (series 2). The extracts were cleaned up on Florisil columns (series 1 and 2) and eluted in a mixture of hexane and dichloromethane (25:75). PBDEs were then analyzed using an Agilent GC-MS machine (Agilent Technologies; Mississauga, Ontario Canada), in split-

less mode, with a 15 m DB-XLB column (0.25 mm i.d., 0.1 µm film thickness) (see Chevrier *et al.* 2013 for more details about series 1 laboratory methods). Mass spectrometry was carried out using single-ion monitoring and negative chemical ionization (NCI), with methane as the gas source. The limit of detection (LOD) was 0.01 µg/L (series 1 and 2) for all PBDEs except BDE209, for which the LOD was 0.05 µg/L in series 1 and 0.02 µg/L in series 2. All congeners other than BDE209 were present in less than 1% of the samples.

Concentrations of total cholesterol (TC), free cholesterol (FC), triglycerides (TG) and phospholipids (PL) were also measured in these samples (in g/L), by an enzymatic method, making it possible to calculate the total lipid concentration as $1.677*(TC-FC) + FC + TG + PL$.

BDE 85, 99, 100, 119 and 209 were analyzed in vacuum cleaner bags collected from the 246 participants' homes by the LERES (Environment & Health Research Laboratory at EHESP, Rennes, France). The choice of these compounds was based on a health ranking of semivolatile organic compounds ingested through settled dust, on the basis of neurotoxicity and contamination data, as recently proposed (Bonvallot *et al.* 2010). The analytical methods are described in detail elsewhere (Blanchard *et al.* 2014; Mercier *et al.* 2014). Briefly, BDEs were extracted from 200 mg samples of sieved dust (100 µm) by pressurized liquid extraction (PLE) using dichloromethane. All organic extracts were then removed by solid-phase extraction (SPE) on aminopropyl columns before analysis by gas chromatography coupled with mass spectrometry (GC-MS) for BDE209 and gas chromatography coupled with tandem mass spectrometry (GC-MS/MS) for the other PBDEs. The detection rates were 15% for BDE85, 41% for BDE99, 12% for BDE100, 0% for BDE119, and 87% for BDE209. Detection of BDE85, 99, and 100 were highly correlated, but not with that of BDE209. Only the BDE99 (as a representative of the BDE85, 99, and 100 mixture) and BDE209 concentrations, expressed in ng/g, were retained as markers of childhood exposure .

2.4. Exposure to other chemicals

Exposures to other potential neurotoxicants (PCB, mercury, lead, organophosphate insecticides, tobacco smoke) were evaluated from measurements in environmental or biological samples.

PCB153 concentrations in umbilical cord plasma samples were determined simultaneously with those of the PBDE compounds, in the same laboratory. They were determined using a GC-MS system (Agilent Technologies; Mississauga, Ontario, Canada) with an Agilent Technologies 60 m DB-XLB column (0.25 mm i.d., 0.25 μm film thickness). The LOD was 0.01 $\mu\text{g/L}$.

We used the 2 cm of hair closest to the root from maternal hair samples collected at birth to assess total mercury exposure during the third trimester of pregnancy. The CTQ determined mercury levels by cold vapor atomic absorption spectrometry following acid digestion of the hair matrix. The LOD was 0.02 $\mu\text{g/g}$.

A single-surface sample of interior floor dust was collected from the living room of each home by wipe sampling ($\mu\text{g/m}^2$) according to a standard protocol (ASTM 2003). Samples were analyzed by the LERES laboratory, with a 7500ce inductively coupled plasma mass spectrometer (ICP-MS; Agilent Technologies, Massy, France) equipped with a quadrupole mass filter and an octopole reaction cell. The LOQ was 2 $\mu\text{g/m}^2$ for leachable lead and 1 $\mu\text{g/m}^2$ for total lead concentrations in dust. Both determinations were performed for each sample, as described by Le Bot *et al.* 2011, and the values obtained were strongly correlated with each other. Leachable lead concentration, a proxy for bioaccessible lead, was used to assess childhood exposure to lead at home (Le Bot *et al.* 2011, Etchevers *et al.* 2015).

Chemical analysis of six nonspecific organophosphate dialkylphosphate metabolites (DAPs) in the maternal urinary samples collected at the beginning of pregnancy was carried out by the LABOCEA Institute (Plouzané, France) on 10 mL urine samples, by solid-phase extraction followed by liquid chromatography and triple quadrupole mass spectrometry (LC-MS/MS, Quattro Ultima, Micromass/Waters, Saint-Quentin-en-Yvelines, France). One mL urine samples of urines from the 6-year-old children were subsequently analyzed by the same laboratory, using similar analytical methods with improved materials (UPLC-MSMS, Xevo TQ-S, Waters; Waters, Saint-Quentin-en-Yvelines, France) for dialkylphosphate metabolites and urinary cotinine. The LOQ values for the chemical analyses of maternal urine samples (values between the LOD and the LOQ not available) were 1.25 $\mu\text{g/L}$ for DEP, 1.7 $\mu\text{g/L}$ for DETP, 0.02 $\mu\text{g/L}$ for DEDTP, 0.2 $\mu\text{g/L}$ for DMP, 1 $\mu\text{g/L}$ for DMTP, and 0.45 $\mu\text{g/L}$ for DMDTP. The LOD values for the children's samples were 0.2 $\mu\text{g/L}$ for DEP, 0.1 $\mu\text{g/L}$ for DETP, 0.005 $\mu\text{g/L}$ for DEDTP, 0.06 $\mu\text{g/L}$ for DEDTP, 0.32 $\mu\text{g/L}$ for DMP and, 0.13 $\mu\text{g/L}$ for DMDTP. Censored values (i.e. <LOQ for maternal urine samples or <LOD for the children's samples) were not imputed. Metabolite

concentrations were converted from micrograms per liter to the corresponding molar concentrations (nanomoles per liter). Concentrations were summed to obtain overall concentrations of dialkylphosphate metabolites.

2.5. Covariates

The following potential confounding variables were considered, based on prior knowledge of the potential effects of environmental contaminants on neurodevelopment: 1) maternal and family characteristics: maternal age (years), education (< or \geq university level) and verbal IQ, presence of older siblings at home (no, yes), HOME score, duration of breastfeeding (none, ≤ 16 weeks, > 16 weeks), maternal smoking status at the beginning of pregnancy (no, yes), maternal fish intake (< or \geq twice a week), pre-pregnancy maternal body mass index (< or ≥ 25 kg/m²); 2) characteristics of the child: school attendance (preschool, primary school) and birth weight (grams); 3) testing characteristics: interviewer at testing (binary); and 4) co-exposure to other potential neurotoxicants: cord blood PCB153 concentration ($\mu\text{g/L}$; for the subsample $n = 159$), maternal hair mercury concentration (in tertiles; for the subsample $n = 159$), lead concentrations in the floor dust of the living room (in tertiles), environmental tobacco smoke (child's urinary cotinine concentration < or ≥ 6 $\mu\text{g/L}$), urinary DAP concentrations during pregnancy and during childhood (both in tertiles).

2.6. Statistical analysis

The association of WISC-VCI and WISC-VMI scores with childhood exposure variables (concentrations of BDE99 and 209 in household dust, simultaneously) was investigated using multiple linear regression analysis ($n = 246$). A specific adjustment set was chosen for each WISC cognitive domain. Covariates were included in the multivariate model if a p value < 0.20 was obtained for their association with the WISC score in bivariate analyses.

The associations of WISC scores with childhood exposures were assessed in the subsample of mother-child pairs for which cord blood samples were available ($n = 159$) with or without further adjustment for prenatal exposure (BDE209 concentration in cord blood). The same adjustment covariates were used and completed with cord

blood total lipid concentration as a forced covariate, and with cord blood PCB153 concentration and maternal hair mercury concentration if a p value <0.20 was obtained for their association with the WISC score in bivariate analyses.

Missing values for breastfeeding duration ($n = 1$), school attendance ($n = 1$) and maternal educational level ($n = 1$) were imputed from the modal value for participants, and the median value for prepregnancy maternal body mass index ($n = 1$), the urinary cotinine concentration of the child ($n = 1$) and maternal hair mercury concentration ($n = 12$). For each WISC index, missing values were replaced by regression analyses with highly correlated subtests (for working memory, Pearson's $\rho = 0.85$ and 0.89 for Letter-Number Sequencing and Digit Span, respectively; for verbal comprehension, Pearson's $\rho = 0.79$, 0.85 and 0.74 for similarities, vocabulary and comprehension, respectively; all $p < 0.0001$). This was performed for WISC-VCI ($n = 3$) and WISC-WMI ($n = 10$).

Due to the low detection rate, the cord blood BDE209 concentration was dichotomized (not detected/detected). Using equal-sized categories, the BDE209 concentration in dust was divided into tertiles and the BDE99 concentration was grouped into three classes: $<LOD$ (59.3%), and among detected values, $<median$ and $\geq median$. Ordinal variables coded using integer values (0, 1, 2) were used to perform trend tests.

We evaluated possible effect-modification by child's sex. We tested models without adjustment for birth weight as a sensitivity analysis, because birth weight may be considered as an intermediate variable.

The main effects were considered to be statistically significant if $p < 0.05$ in two-tailed tests. All analyses were performed with the SAS software (SAS/STAT version 9.3; SAS Institute Inc., Cary, NC).

3. RESULTS

The mean age of the mothers at the start of pregnancy was 30 years, and more than 70% had completed at least a university degree (Table 1). Approximately one quarter of the mothers smoked during pregnancy. Children were breastfed for a mean of 17 weeks and 25% attended primary school at age 6. PCB153 was detected in 100% and BDE209 in 27% of cord serum samples. Lead and BDE209 were detected in the dust of most of the homes at highly variable concentrations. BDE99 was detected in 41% of homes. The mean concentration of BDE209 in dust (1045 ng/g) was five times higher than that of BDE99 (200 ng/g) in the homes with detected concentrations in dust samples. The percentage of children considered to be exposed to environmental tobacco smoke (urinary cotinine concentration $\geq 6 \mu\text{g/L}$) was low (6%).

In the analysis restricted to the mothers for whom we had cord blood BDE209 determinations ($n = 159$), the sociodemographic characteristics were similar to the total sample, except that the percentage of smokers was slightly lower (19% vs 23%), and similar concentrations of BDE99 and BDE209 were observed in their homes. The means and standard deviations of the WISC-VCI and WISC-WMI scores did not differ between the total sample and the subsample for which cord blood samples were available (Supplemental Table 1).

For the total sample set, the WISC-VCI score was lower in the children with the highest concentrations of BDE99 ($\geq 54 \text{ ng/g}$) in house dust (p trend = 0.02 overall; p trend = 0.02 (boys) and 0.34 (girls); Table 2). Higher concentrations of BDE209 in house dust were simultaneously associated with lower WISC-VCI scores in boys (p trend = 0.04). Analyses of the three original subtests used to calculate the WISC-VCI showed that higher BDE99 concentrations in dust were associated with lower scores for two subtests: similarities (overall p trend = 0.005 overall; p trend = 0.02 (boys) and 0.07 (girls)) and vocabulary (p trend=0.02 overall; p trend=0.004 (boys) and 0.63 (girls)). The association with the BDE209 concentration in dust was statistically significant for the

comprehension subtest score (overall p trend = 0.007 overall; p trend = 0.01 (boys) and 0.27 (girls)) but not for the similarities and vocabulary subtest scores (Supplemental Table 2).

Dust BDE99 and BDE209 concentrations were not statistically significantly related to the WISC-WMI score (Table 2). Analyses of the two original subtests of the WISC-WMI showed that higher BDE99 concentrations in dust were associated with a lower score for Digit Span only (overall p trend = 0.02 overall; p trend = 0.11 (boys) and 0.05 (girls)) (Supplemental Table 3).

Very similar findings were obtained for the multiple models for both WISC indices whether birth weight was included or not (Supplemental Table 4), and for the crude models (Supplemental Table 5).

Associations of the WISC-VCI and WISC-WMI indices with PBDE concentrations in dust were slightly stronger in the subsample for which cord blood samples were available than those observed for the total sample (Table 3). The associations with BDE concentrations in dust were unaffected when cord blood BDE209 concentration was taken into account in the model, and no association was observed between cord blood BDE209 concentrations and WISC indices.

No association was observed between PBDE concentrations in cord blood or dust and block design subtest scores.

4. DISCUSSION

We found that children living in houses with higher concentrations of BDE99 or BDE209 in dust performed less well in WISC tests of verbal comprehension at the age of six years than children with little or no exposure. This association was more pronounced in boys than girls. The scores among boys exposed to the highest BDE99 house-dust concentrations (20%) were, on average, 8 points lower than for boys from homes with no detected BDE99 in the house dust (60%). There was also a 6 point-difference in the score between the boys exposed to

the highest one-third and lowest one-third of BDE209 dust concentrations. Higher concentrations of BDE99 in house dust were associated with poorer performance on both the similarities and vocabulary subscales, indicating a potential threat to learning abilities. In contrast, higher concentrations of BDE209 in house dust were associated with lower scores in the comprehension subtest, which assesses the ability of the child to understand social norms. A decrease of 3.6 points on the WISC working memory index was observed among the children living in homes with the highest concentrations of BDE99 in house dust (20%) relative to those living in homes with no detected BDE99 in dust (60%). The decrease was especially observed for the Digit Span subtest. We found no evidence of association between cord blood BDE209 concentrations and any of the WISC-IV scores.

Previous epidemiological studies have focused principally on the developmental neurotoxicity of PBDE congeners included in the technical penta- or octa-mixtures, likely due to analytical difficulties for higher brominated congeners. Most of the previous studies conducted in the Netherlands (Roze *et al.* 2009) and the US (Chen *et al.* 2014; Eskenazi *et al.* 2013; Herbstman *et al.* 2010) showed detrimental neurodevelopmental outcomes in association with prenatal exposure to PBDEs measured in maternal or cord blood (mostly for BDE47). However, no such associations were reported in a study carried out in Spain where exposure levels were much lower (Gascon *et al.* 2011). Studies in which the PBDE concentration was measured in breast milk (Adgent *et al.* 2014; Chao *et al.* 2011) or colostrum (Gascon *et al.* 2012), showed no adverse outcomes or better score for cognitive skills (Chao *et al.* 2011) in exposed children relative to children with little or no exposure. Our study did not address the potential effects of prenatal exposure to these PBDE mixtures because the analysis of cord blood was not sufficiently sensitive, making it impossible to document exposure to BDEs other than BDE209.

Our results for exposure to BDE99 in house dust during childhood replicate those of the CHAMACOS cohort study conducted in California (Eskenazi *et al.* 2013), where PBDE exposure levels are higher than in France. In

the CHAMACOS cohort, childhood exposure to PBDEs (mostly BDE47, 99, 100 and 153), measured in serum samples from seven-year-old children, was associated with a reduced global IQ score for the WISC-IV. By contrast, in the Spanish INMA-Menorca birth cohort (Gascon *et al.* 2011), no statistically significant trend was found for the relationship between serum BDE47 concentrations from four-year-old children and their cognitive function scores in the MCSA. However, the median serum BDE47 concentration in this cohort (0.12 ng/g) was lower than that in the CHAMACOS cohort, by a factor of almost 400 (47 ng/g) (note that the median BDE99 concentration was 100-fold lower: 0.12 ng/g in INMA *vs* 10.6 ng/g in CHAMACOS).

Far fewer studies have assessed exposure to BDE209. Two studies in Taiwan (Chao *et al.* 2011) and Spain (Gascon *et al.* 2012) reported a lower score for the BSID mental development index during infancy (< 18 months of age) specifically in association with BDE209 exposure, simultaneously assessed with several other PBDE congeners (7 congeners determined in colostrum in the study by Gascon *et al.* (2012); and 14 congeners determined in breast milk collected during the first month of life in the study by Chao *et al.* (2011)). These reports are consistent with our findings linking the concentrations of BDE209 in house dust during childhood with a lower verbal comprehension score at the age of six years.

Our findings support the potential contribution of exposure to residuals of the penta-PBDE mixture during childhood on child cognitive development, as previously reported by Eskenazi *et al.* (2013). They also provide new evidence to suggest that childhood exposure to residuals of the deca-BDE mixture (still in use in Europe) may also be detrimental, as also suggested by studies outside the US based on early-life exposure through breast milk (Chao *et al.* 2011; Gascon *et al.* 2012).

An association between early exposure to BDE99 and cognitive deficits in children is biological plausible as animal studies have shown altered spontaneous behavior in mice neonatally exposed to BDE99 (Hallgren *et al.* 2015). *In vitro* studies reported that BDE99 induces a decrease of neuronal cell differentiation via the disruption of cellular thyroid hormone signaling (Schreiber *et al.* 2010), changes in cytoskeletal regulation and expression of neuron-growth associated proteins (Viberg and Eriksson 2011), and modifications of dopamine and

acetylcholine neurotransmitter phenotypes (Slotkin *et al.* 2013). Possible mechanisms for the neurotoxicity of BDE209 might involve the cholinergic system and the induction of lipid peroxidation by reactive oxygen species (Liang *et al.* 2010). In addition, BDE209 induces changes in the expression of neuron-growth associated proteins, similarly to BDE99, in particular calmodulin-dependent protein kinase II and Gap43, involved in normal maturation of the brain (Viberg and Eriksson 2011).

Despite the modest statistical power of our study for testing potential effect-modification by sex, our findings suggest that boys are more susceptible than girls to the possible adverse impact of PBDE exposure on the verbal comprehension cognitive domain (5 statistically significant associations out of 8 for boys, and 0 out of 8 for girls). Sex-dependent effects have been reported in animal studies, with males appearing to be more affected in some, but not all studies (Costa & Giordano 2007). However, sex-specific effects have rarely been evaluated in epidemiological studies. Eskenazi *et al.* (2013) reported a second-order interaction (sex and age) in their assessment of motor function: poorer performance in the WRAVMA (Wide Range Assessment of Visual Motor Ability) pegboard test in association with pre- or postnatal exposure to PBDEs was observed principally in boys at the age of five years, and in girls at the age of seven years. Chen *et al.* (2014) also tested for interactions with sex but did not find any.

Our study has a number of strengths. It is based on a prospective cohort and one of the few to examine exposure during childhood. Exposure was assessed from the concentrations of the PBDEs found in house dust, with likely improved analytical sensitivity relative to serum concentrations. The PBDE measurement in house dust is relevant because household consumer products are thought to be the main source of exposure in children. However, unlike the use of serum PBDE concentrations as a marker of exposure, this approach does not take into account the individual behaviour of children, which may modify their uptake. Nevertheless, one Californian study has shown a correlation between blood and dust concentrations for several PBDE congeners, including the BDE99 (Bennett *et al.* 2014). We considered a large number of potential confounders, including the quality of the family environment (assessed through the HOME and maternal IQ), and we also took into account exposure

to known neurotoxicants, such as PCBs, lead, mercury, organophosphate pesticides, and environmental tobacco smoke, together with simultaneous exposure to several different PBDEs. We cannot rule out that other potential neurotoxicants with similar uses and/or sources of dust contamination than those of PBDEs, could explain our findings, such as perfluorinated compounds (D'Hollander *et al.* 2010).

The main limitation of this study concerns the measurement of prenatal exposure, as the method was insufficiently sensitive to detect PBDEs in cord blood. The study thus lacked sufficient power to examine the effects of prenatal exposure to BDE209, but it may also have introduced residual confounding into the study of postnatal exposure due to the failure to detect other important congeners in cord blood. Our assessment of exposure during childhood, based on concentrations in house dust, has some imprecision due to the use of domestic vacuum cleaner bags rather than the collection of standardized samples. However, this would have limited the likelihood of detecting statistically significant associations. The cross-sectional design is another limitation for interpreting the association between BDE dust concentrations and WISC scores, both measured at 6 years of age. However, a strong correlation between dust BDE99 concentrations from samples collected one year apart by vacuum cleaner was recently demonstrated, showing that dust may be an appropriate medium for assessing childhood exposure to BDE99 (Bennett *et al.* 2014). Another limitation is that the use of dust measurements prevents the comparison of our results with those of previous studies which are based on concentrations in breast milk or serum from children. Nevertheless, our results suggest that this domestic source of exposure to PBDEs may specifically contribute to cognitive deficits in childhood. Our ability to evaluate the magnitude of the potential impact of PBDEs on intellectual competence was limited, because all the scales of the WISC-IV were not administered. A complete assessment of potential neurodevelopmental toxicity would require the study of behavioural outcomes. We cannot rule out the possibility of reverse causation in our findings, although it is difficult to relate lower verbal comprehension or working memory performance to specific child behaviors such as increased crawling and/or hand-to-mouth contacts.

The women of the present study were slightly better educated (71.5% vs 63%) and smoked less during the beginning of their pregnancy (23.2% vs 28%) than the whole cohort (Petit et al. 2010, Chevrier et al. 2011) which was also more socially advantaged than the national population of pregnant women according to the results of a national perinatal survey in 2003 (Blondel et al. 2006). Despite these biases of enrolment and loss of follow-up, this study has confirmed well-established factors related to child neuropsychological outcomes, including enriching and cognitively stimulating environments, as assessed by maternal level of education, mothers' IQ, HOME score, or child elementary school (for working memory domain). Moreover, enrolment in the PELAGIE birth cohort took place throughout Brittany and did not represent one particular vulnerable area.

In conclusion, our findings reinforce knowledge on the adverse association between the indoor contaminants, in particular pentaBDE mixture congeners and BDE209, which has been much less studied, and the neurodevelopmental health of children. Several countries are in the process of banning the use of PBDE mixtures as flame retardants. However, these compounds are likely to persist in the environment for a long time to come.

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Conflict of interest

The authors have no conflicts of interest to declare.

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TABLE 1. Descriptive statistics for the study sample (PELAGIE live-birth subcohort, France)

Variables	Study sample (N=246)			
	N	Mean ± SD	Range	%
<i>Mother and family characteristics</i>				
Age at inclusion (years)	246	30.3 ± 4.1	21.5-44.4	
Education (% university)	246			71.5%
BMI before pregnancy	245	22.2 ± 3.5	14.7-39.1	
Number of children in the family	245	2.4 ± 0.7	1-5	
WAIS-III score (verbal IQ)	245	94 ± 11.2	62-127	
HOME	245	46.3 ± 4.0	31-56	
Smoking during pregnancy (% yes)	246			23.2%
<i>Child Characteristics</i>				
Sex (% girls)	246			52.4%
Birth weight (grams)	246	3415 ± 435	2340-4660	
Breastfeeding duration (weeks)	246	17.1 ± 26.3	0-208	
School attendance (% primary)	246			24.8%
<i>Contaminants and biological measures^b</i>				
Cord blood BDE209 (µg/L) (% detected; out of 159)	43	0.19 ± 0.24	0.02-1.2	27.0%
Cord blood PCB153 (µg/L) (% detected; out of 159)	159	0.13 ± 0.07	0.03-0.48	100%
Cord blood lipids ^a (mg/L)	159	3509 ± 1649	980-11000	
Dust BDE99 (ng/g) (% detected; out of 246)	100	200 ± 584	27-4239	40.7%
Dust BDE209 (ng/g) (% detected; out of 246)	213	1045 ± 2368	112-26776	86.6%
Lead in living room dust (µg/m ²) (% detected; out of 246)	230	6.8 ± 19.4	1-233	93.5%
Hair mercury levels (nmol/g) ^c	159	4.0 ± 2.5	0-13	
Urinary cotinine concentration in the 6-year-old child ≥ 6 µg/L (% out of 246)	15			6.1%

^a Lipids: cholesterol, triglycerides and phospholipids

^b For contaminants and biological determinations, statistics were calculated on samples with a detected value

^c 12 values were imputed by the complete-data median value (3.4)

TABLE 2. Association between concentrations of BDE99 and BDE209 measured in house dust and WISC-IV scores at the age of 6 years (PELAGIE cohort, France)

	WISC-VCI						WISC-WMI					
	All (n=246)		Males (n=117)		Females (n=129)		All (n=243)		Males (n=115)		Females (n=128)	
	β^a	(95% CI)	β^a	(95% CI)	β^a	(95% CI)	β^b	(95% CI)	β^b	(95% CI)	β^b	(95% CI)
Dust BDE99												
Not detected	ref		ref		ref		ref		ref		ref	
<54 ng/g	-1.57	(-6.07, 2.94)	0.37	(-6.26, 7.00)	-3.38	(-9.99, 3.22)	-1.43	(-5.74, 2.87)	-5.16	(-12.41, 2.09)	-1.13	(-7.02, 4.77)
\geq 54 ng/g	-5.45	(-9.95, -0.96)	-8.24	(-14.31, -2.16)	-2.72	(-9.93, 4.49)	-3.61	(-7.92, 0.70)	-4.16	(-10.84, 2.52)	-3.02	(-9.42, 3.37)
<i>p trend</i>	0.02		0.02 ^c		0.34 ^c		0.10		0.13		0.35	
Dust BDE209												
<257 ng/g	ref		ref		ref		ref		ref		ref	
257-627 ng/g	-1.82	(-6.15, 2.51)	-2.62	(-8.78, 3.55)	-0.96	(-7.61, 5.69)	3.83	(-0.40, 8.06)	4.50	(-2.22, 11.21)	2.96	(-3.06, 8.99)
>627 ng/g	-3.16	(-7.52, 1.19)	-6.36	(-12.53, -0.19)	-1.70	(-8.36, 4.96)	3.23	(-0.94, 7.39)	4.18	(-2.47, 10.83)	3.05	(-2.91, 3.01)
<i>p trend</i>	0.15		0.04		0.78		0.13		0.23		0.31	

Abbreviations: WISC-VCI, WISC verbal comprehension index; WISC-WMI, WISC working memory index.

Concentrations of BDE99 and 209 in household dust were included simultaneously in the models.

^a Adjustment for maternal education, WAIS-III score, HOME score and prepregnancy fish intake, breastfeeding duration, lead concentrations in house dust, and birth weight.

^b Adjustment for maternal age, education, WAIS-III score and smoking status at the beginning of pregnancy, HOME score, breastfeeding duration, number of older siblings, child school attendance, lead concentrations in house dust, birth weight, and interviewer.

^c Test for potential modification of the association between dust BDE99 and WISC-VCI score by child's sex: $p=0.15$. Other potential effect-modifications by sex (for BDE209 or for WISC-WMI) were $p>0.20$.

TABLE 3. Association between concentrations of BDE99 and BDE209 measured in house dust and WISC-IV scores at the age of 6 years, taking cord blood BDE209 levels into account (PELAGIE cohort, France)

	WISC-VCI (<i>n</i> =159) ^a				WISC-WMI (<i>n</i> =157) ^b			
	β	(95% CI)	β^c	(95% CI)	β	(95% CI)	β^c	(95% CI)
Dust BDE99								
Not detected	ref		ref		ref		ref	
<54 ng/g	-5.85	(-11.86, 0.16)	-5.91	(-11.93, 0.10)	-4.02	(-9.83, 1.79)	-4.02	(-9.86, 1.82)
\geq 54 ng/g	-5.70	(-11.59, 0.18)	-6.01	(-11.94, -0.08)	-2.56	(-8.36, 3.23)	-2.56	(-8.42, 3.29)
<i>p trend</i>		0.03		0.02		0.27		0.27
Dust BDE209								
<257 ng/g	ref		ref		ref		ref	
257-627 ng/g	-2.78	(-8.24, 2.69)	-2.93	(-8.41, 2.55)	5.69	(0.23, 11.14)	5.69	(0.20, 11.18)
>627 ng/g	-4.68	(-10.39, 1.02)	-4.83	(-10.55, 0.89)	2.65	(-2.94, 8.24)	2.65	(-2.97, 8.28)
<i>p trend</i>		0.11		0.10		0.27		0.27
Cord blood BDE209								
Not detected	-		ref		-		ref	
Detected	-		-2.38	(-7.74, 2.98)	-		-0.006	(-5.44, 5.43)
<i>p</i>				0.38				0.99

Abbreviations: WISC-VCI, WISC verbal comprehension index; WISC-WMI, WISC working memory index.

Concentrations of BDE99 and 209 in household dust were included simultaneously in the models.

^a Same adjustment sets as proposed in the Table 2 for WISC-VCI (maternal education, WAIS-III score, prepregnancy fish intake, HOME score, breastfeeding duration, lead concentrations in house dust, birth weight), plus cord plasma PCB153 and lipid levels, and maternal hair mercury levels.

^b Same adjustment sets as proposed in the Table 2 for WISC-WMI (maternal age, education, WAIS-III score, smoking status at the beginning of pregnancy, HOME score, breastfeeding duration, number of older siblings, child school attendance, lead concentrations in house dust, birth weight, interviewer), plus cord plasma PCB153 and lipid levels, and maternal hair mercury levels.

^c Also adjusted for cord blood BDE209 detection