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Effects of pre and postnatal exposure to low levels of polybromodiphenyl ethers on neurodevelopment and thyroid hormone levels at 4 years of age

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ABSTRACT

There are at present very few studies of the effects of polybromodiphenyl ethers (PBDEs), used as flame retardants in consumer products, on neurodevelopment or thyroid hormone levels in humans. The present study aims to examine the association between pre and postnatal PBDE concentrations and neurodevelopment and thyroid hormone levels in children at age 4 years and isolate the effects of PBDEs from those of PCBs, DDT, DDE and HCB. A prospective birth cohort in Menorca (Spain) enrolled 482 pregnant mothers between 1997 and 1998. At 4 years, children were assessed for motor and cognitive function (McCarthy Scales of Children's Abilities), attention-deficit, hyperactivity and impulsivity (ADHD-DSM-IV) and social competence (California Preschool Social Competence Scale). PBDE concentrations were measured in cord blood (N = 88) and in serum of 4 years olds (N = 244). Among all congeners analyzed only PBDE 47 was quantified in a reasonable number of samples (LOQ = 0.002 ng/ml). Exposure to PBDE 47 was analyzed as a dichotomous variable: concentrations above the LOQ (exposed) and concentrations below (referents).

Scores for cognitive and motor functions were always lower in children pre and postnatally exposed to PBDE47 than in referents, but none of these associations was statistically significant (β coefficient (95%CI) of the total cognition score: −2.7 (−7.0, 1.6) for postnatal exposure, and −1.4 (−9.2, 6.5) for prenatal exposure). Postnatal exposure to PBDE 47 was statistically significantly related to an increased risk of symptoms on the attention deficit subscale of ADHD symptoms (RR (95%CI) = 1.8 (1.0, 3.2)) but not to hyperactivity symptoms. A statistically significant higher risk of poor social competence symptoms was observed as a consequence of postnatal PBDE 47 exposure (RR (95%CI) = 2.6 (1.2, 5.9)). Adjustment for other organochlorine compounds did not influence the results. Levels of thyroid hormones were not associated to PBDE exposure.

This study highlights the importance of assessing the effects of PBDE exposure not just prenatally but also during the early years of life. In the light of current evidence a precautionary approach towards PBDE exposure of both mothers and children seems warranted.

1. Introduction

Flame retardants are a large family of compounds used in industrial products to inhibit or resist the spread of fire. Brominated flame retardants are the largest group on the market because of their low cost and high performance efficiency (Birnbaum and Staskal, 2004). Research on the toxicity of flame retardants has mainly focused on polybromodiphenyl ethers (PBDEs) due to their similarity to other persistent organic pollutants (POPs) with regard to high capacity to bioaccumulate and high toxicity (Hooper and McDonald, 2000; Thuresson et al., 2006). Further, levels of PBDEs in humans have increased over time (Meironyte et al., 1999; Noren and Meironyte, 2000; Betts, 2002; Fangstrom et al., 2008).

Concerns about the neurodevelopmental toxicity of PBDE compounds first emerged in studies with rodents, which have shown altered habituation capability, learning and memory functions and motor problems (Fonnum and Mariussen, 2009). These studies suggest that neurotoxic effects occur due to PBDE exposure in the period of rapid growth brain (also known as the brain growth spurt) (Darnerud et al., 2001; Schreiber et al., 2010). In humans this period spans from the 3rd trimester of pregnancy to at least 2 years after birth (Rice and Barone, 2000). These findings, and the fact that

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children are generally exposed to higher levels of PBDEs than their mothers through sources such as breastfeeding and contact with floor dust (Fischer et al., 2006; Toms et al., 2009; Lunder et al., 2010), raise the hypothesis that both prenatal and postnatal periods may be vulnerable to possible neurotoxic effects of PBDE exposures in humans. Thus far, only two epidemiological cohort studies, one in The Netherlands (Roze et al., 2009) and one in the US (Herbstman et al., 2010), have published results on neurodevelopmental effects of PBDEs in humans. Both find evidence that prenatal exposure to PBDEs may have an adverse effect on the neurodevelopment at age 4–6 years of the children, but associations are less consistent in the Dutch cohort where exposures were lower.

Neurotoxicity of PDBEs may act through an effect on the thyroid hormone levels, which are known to be essential for neonatal neurodevelopment (Williams, 2008), but only two studies have examined associations between PBDE exposure and thyroid hormone levels in children and these have reported both positive and negative associations (Herbstman et al., 2008; Roze et al., 2009).

There are at present very few studies in humans on the effects of PBDEs on neurodevelopment or thyroid hormone levels, and no studies on the effects of postnatal exposures, or on the influence of other organochlorine compounds (OCs). The present study aims to clarify these gaps by examining pre and postnatal PBDE concentrations in relation to neurodevelopment test scores and thyroid hormone levels of children at age 4 years in a Spanish birth cohort, and isolate the effects of PBDEs from those of other OCs (polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), dichlorodiphenyl dichloroethylene (DDE) or dichlorodiphenyl trichloroethane (DDT)).

2. Methods

This study is based on the Menorca birth cohort (INMA project, Spain), which focuses on environmental exposures and growth, development and health in children (Ribas-Fito et al., 2006a). All women presenting for antenatal care in a 12 months' period starting in mid-1997, were eligible and invited to participate. 482 mothers (94% of those eligible) were finally enrolled into the cohort. At age 4 years 470 mother–child pairs (98% of those enrolled) remained in the follow-up. All families signed a consent form to participate in the study.

2.1. Neurodevelopment assessment (cognitive function, ADHD symptoms and social competence)

At age 4 years, 422 children (88% of the original cohort) completed motor and cognitive capabilities tests. These were assessed with a standardized version of the McCarthy Scales of Children's Abilities (MSCA) adapted to the Spanish population (McCarthy, 1972). The Global Cognitive scale and five subscales (Verbal, Perceptive-Performance, Memory, Quantitative and Motor) were examined. In addition, a new summary measure was constructed to assess those cognitive tasks associated with executive functions (Julvez et al., 2007). A strict protocol was applied to avoid inter-observer variability, including inter-observer-trainings and three sets of quality controls (Julvez et al., 2007). The variability between the two neuropsychologists was lower than 5%. Further, children were assessed for social competence, attention deficit and hyperactivity problems. We used the California Preschool Social Competence Scale (CP-SCS) for evaluation of social competence (Levin et al., 1969). This scale was successfully adapted into a bilingual version (Spanish/Catalan) (Julvez et al., 2008). The Attention-Deficit Hyperactivity Disorder (ADHD) Criteria of the Diagnostic and Statistical Manual of Mental Disorders 4th Edition (ADHD-DSM-IV) were used to assess attention-deficit, hyperactivity and impulsivity. The CP-SCS and the ADHD-DSM-IV were completed by the children's teachers, since in Spain teachers who work with children between the ages of 3 and 6 years, spend approximately five hours per day with them. Continuous MSCA scales were standardized to a mean score of 100 with a standard deviation of 15 to homogenize all the scales. For CP-SCS a cut-off point corresponding to the 20th percentile was created to categorize those children with a 'normal' and a 'poor' social competence response. ADHD-DSM-IV scores above the 80th percentile were classified as "ADHD symptoms". This criterion was used because we were interested in subclinical symptoms as well as the clinical ADHD diagnosis: the 80th (or 20th) percentile is a criterion commonly used to distinguish between 'normal' and 'low' responses and has been used in previous studies (Jacobson and Jacobson, 2005; Julvez et al., 2007). Information for at least one of the neurodevelopmental outcomes was available for 422 children in the cohort.

2.2. Measurement of concentrations of PBDEs and other organochlorine compounds

PBDE concentrations were measured in 88 cord blood samples and 244 serum samples of 4 year old children. The 88 samples were the only remaining cord blood samples in the cohort (after analysis of other contaminants such as organochlorines). At the age of 4 years, analyses were limited to 244 children because of budget limitations; these were a random sample of all available blood samples. PBDE concentrations were measured in all available samples. Analyses were carried out in the Department of Environmental Chemistry of the Institute of Environmental Assessment and Water Research (IDAEA-CSIC) in Barcelona, Spain using gas chromatography (GC) with electron capture detection (Hewlett-Packard 6890N GC-ECD; Hewlett-Packard, Avondale, PA, USA) and GC coupled to chemical ionisation negative-ion mass spectrometry (Hewlett-Packard 5973 MSD), which has been previously described (Carrizo et al., 2006; 2007). Several congeners were analyzed (PBDE 12–13, 32, 17, 28–33, 47, 100, 119, 99, 116, 85, 126, 155, 153, 183, 66, 71, 154, 138, and 190), with a limit of detection (LOD) of 0.001 ng/ml and a limit of quantification (LOQ) of 0.002 ng/ml and recoveries between 88 and 118% (Vizcaino et al., 2009). The same method was used to measure concentrations of other OCs (PCB 28, 52, 101, 118, 153, 138 and 180, DDT, DDE and HCB) in cord blood and serum at age 4 (Carrizo et al., 2006). Among PBDEs, the most detected congener was PBDE 47 both in cord blood and serum samples at age 4 years (51.1% and 20.5% of the samples, respectively), followed by PBDE 99 (33.0% and 13.5%, respectively) and PBDE 100 (19.3% and 3.3%, respectively). The rest of congeners were detected in less than 17.4% in cord blood and 1.6% in serum samples at age 4 years (Carrizo et al., 2006).

2.3. Measurement of thyroid hormone levels

Levels of TSH (Thyroid-stimulating hormone), TT3 (Total Triiodothyronine) and free FT4 (Free Thyroxin) were measured from the serum samples obtained at age 4 by chemiluminescence assay (ARCHITECT system; Abbot Laboratories, Abbot Park, Illinois, USA) in the References Laboratory of Catalonia in 2004. Inter-assay coefficients of variation (CV) for the TSH, FT4 and TT3 measurements were under 5.2%, 7.8% and 5.3%, respectively, and intra-assay coefficients were 3.3%, 4.2% and 3.0%, respectively. The reference ranges proposed by the laboratory were 0.35–5 mU/l for TSH, 80–200 ng/dl for TT3 and 0.7–1.7 ng/dl for FT4. As all samples were collected in the morning, there should be no circadian variation effects. Samples were stored at −20 °C prior to analysis (Alvarez-Pedrerol et al., 2008). Numbers of children with information on thyroid hormones varied for: 294 (TSH), 307 (TT3) and 312 (FT4). All children had levels within the normal range (Alvarez-Pedrerol et al., 2008). Levels were normally distributed except for TSH, which were log-transformed before analysis.

2.4. Co-variables

Information on co-variables was obtained from mothers through questionnaires during pregnancy, birth and at age 1. Variables examined as co-variables in the current analysis include: sex and age of the child, evaluating psychologist, maternal age, social class (using the UK Registrar General's 1990 classification according to parental occupation by ISCO88 code) and education of the mother, smoking during pregnancy ("yes" if they smoke at the moment of the questionnaire), alcohol consumption (categorized by non-drinking (0 times a week) or drinking at least one time a week), fish consumption (times per week) and parity (first child or not). Type and duration of lactation was assessed by questionnaire at 6 months, 14 months and 2 years after delivery. Given the small size of the study populations and in order to keep models as simple as possible two categories were created for breastfeeding: children breastfeeding for less than 2 weeks, and children breastfeeding for 2 or more weeks (sensitivity analyses using three categories of lactation (non-breastfeeding, short-breastfeeding (1–16 weeks) and long-term breastfeeding (>16 weeks)) resulted in similar results). Pre-pregnancy body mass index (BMI) of the mother, gestational age and weight at birth were collected from clinical records.

2.5. Statistical methods

Given that PBDEs were quantified in a very small number of samples and since PBDE 47 was the congener mostly detected both in cord blood and serum at age 4 samples, we decided to analyze only PBDE 47 exposure in two categories: "referents", including children with samples <LOQ, and "exposed", including children with samples >LOQ.

Missing values in co-variables (gestational age, social class, education and pre-pregnancy BMI of the mother and duration of breastfeeding, 0.4% to 5.2% of missing variables within the original cohort) were imputed by multiple imputation (Royston, 2005) in order not to lose statistical power. This method is based on conditioning the missing variables density to given predictor variables, which in our case were sex, gestational age, age at delivery, weeks breastfeeding, parity, smoking, alcohol consumption, social class, maternal education and pre-pregnancy BMI. The same method was applied to impute concentrations of OCs when samples were missing (0% and 22% of cord blood and serum at age 4 samples, respectively) or when samples were below the LOQ or LOD (0.0–27.3% of prenatal and 1.1–3.1% of postnatal samples, except 59% of postnatal DDT samples).

Very few children (N = 49) had PBDE measurements in both cord blood and age 4 serum, and many of these were below LOQ, so simultaneous modeling of pre and postnatal exposure levels was not possible. All analyses (and imputations) were therefore conducted separately for children with cord blood levels and children with serum levels at age 4. β Coefficients for the association between the PBDE 47 exposure and the MSCA scores and thyroid hormone levels were calculated using linear regression models. Log-binomial regression models were used to analyze the binary outcomes (poor social competence and ADHD symptoms). Models always included sex, age and evaluating psychologist. Other potential confounders (co-variables listed above) were included in the initial models following a forward selection procedure. Variables that showed an association with the outcome ($p<0.2$) or that changed the β coefficient or the RR for PBDE exposure by more than 10% were included in adjusted models. Then, a backwards selection procedure identified covariates with $p<0.2$ or with a β coefficient or RR change >10% for inclusion in the final model. The same procedure was followed to create the models to study the association between postnatal PBDE 47 exposure and thyroid hormone levels at age 4. As thyroid hormone levels may be altered by postnatal PBDE 47 exposure and as they are related to neurodevelopment, they were also included as possible confounders in the models studying the association between postnatal PBDE 47 and neurodevelopment.

Since the distribution of the sum of PCBs, DDE, DDT and HCB was not normal we log-transformed concentrations before entering these compounds one by one and altogether in the models, in order to examine their influence on the relationship between PBDE 47 and neurodevelopment outcomes. OC concentrations measured at birth were included in the models of PBDE 47 levels at birth and those measured at age 4 were included in the models of PBDE 47 levels at age 4. All the analyses were done with STATA 10.

3. Results

Mothers of children with PBDE 47 measurements at birth (N = 88) had higher social class and education, and lower smoking levels than those from the original cohort (N = 482) (Table 1). Further, MSCA scores were higher and prevalence of ADHD symptoms and poor social competence substantially lower in these children. On the other hand, the population with PBDE 47 measurements at age 4 was very similar to the original cohort with respect to all maternal and child characteristics (Table 1).

The percentage of samples with quantifiable (>LOQ) PBDE concentrations was higher at birth than at age 4 (Table 2). PBDE 47 was the congener detected most frequently in both periods (51.1% and 20.5% of the samples, respectively), followed by PBDE 99 and PBDE 100. Despite this, maximum concentrations were higher at age 4 than at birth (PBDE 47 concentrations of 130.2 ng/gr lipid vs 16.8 ng/gr lipid). Concentrations of the 3 congeners are shown in Table 2, together with concentrations detected in the birth cohorts of the USA and The Netherlands.

Females were more likely than males to have a PBDE 47 concentration above the LOQ and therefore to be classified as PBDE 47 exposed. These differences were more accentuated at birth (Table 3). Breastfeeding determined age 4 PBDE 47 exposure, with a higher percentage of children breastfed for more than two weeks in the exposed group compared to the reference group (89.4% vs 74.1%, respectively). Exposure at age 4 years was further associated with higher social class and educational achievement (Table 3).

The MSCA total score for cognitive function and sub scores were all lower in the group of children with PBDE 47 concentrations above the LOQ (exposed) compared to those with concentrations below the LOQ (referents), but none of these differences was statistically significant (total score difference in exposed compared to referents: β coefficient = −1.4, 95%CI −9.2, 6.5 for prenatal and −2.7, 95%CI −7.0, 1.6 for postnatal exposure). Postnatal exposure to PBDE 47 was related to an increased risk of symptoms on the attention deficit subscale of ADHD (RR = 1.8, 95%CI 1.0, 3.2) but not to symptoms on the total ADHD scale (RR = 1.1, 95%CI 0.6, 2.1). Hyperactivity symptoms showed a reverse effect (RR = 0.4, 95%CI 0.2, 1.1). ADHD symptoms risk estimates were below 1 for prenatal exposure, except for hyperactivity, but the number of cases here was very small; for instance, there were only 2 cases of ADHD symptoms in the category of exposed children (n = 40). A higher risk of poor social competence symptoms was observed as a consequence of postnatal PBDE 47 exposure RR postnatal exposure: 2.6, 95%CI 1.2, 5.9.

Levels of TSH and FT4 did not seem to be affected by the postnatal exposure to PBDE 47. TT3 hormone levels were positively, but not statistically significantly, associated to postnatal PBDE 47 concentrations (postnatal β coefficient = 7.3, 95%CI −0.4, 14.9) (Table 4). This association may indicate a possible mechanism for the effect of PBDE 47 on neurodevelopment; we therefore also adjusted the analyses of PBDE 47 exposure and neurodevelopment outcomes for TT3 levels. These were not associated with neurodevelopment outcomes, nor did they influence the coefficients for PBDE 47 exposure (not shown).

Table 1
Characteristics of the original Menorca cohort (N = 482) and the populations with cord blood levels (N = 88) and serum levels at age 4 (N = 244).

	Original Menorca cohort (N = 482)	PBDE measured in cord blood (N = 88)	PBDE measured in cord blood at age 4 (N = 244)
Child's characteristics			
Sex (% males)	51.5	48.9	48.0
Pre-term (% <37 gestation weeks)	4.8	4.6	5.0
Breastfeeding (% >2 weeks)	77.2	82.1	77.2
Thyroid hormone			
TSH (mcIU/ml) (GM, (Min; Max))	1.6 (0.5; 5.0)	1.6 (0.5; 3.8)	1.6 (0.5; 5.0)
Total T3 (ng/dl) (GM, (Min; Max))	149.2 (25; 226)	148.5 (65; 219)	149.5 (25; 226)
Free T4 (ng/dl) (GM, (Min; Max))	1.0 (0.4; 1.4)	1.0 (0.8; 1.4)*	1.0 (0.4; 1.4)**
Organochlorines in cord blood (ng/ml), N = 405			
Σ PCBs (GM (Min; Max))	0.7 (0.2; 12.1)	0.9 (0.2; 12.1)	–
DDT (GM (Min; Max))	0.1 (0.0; 2.3)	0.1 (0.0; 1.1)**	–
DDE (GM (Min; Max))	1.1 (0.0; 19.6)	0.1 (0.1; 6.3)	–
HCB (GM (Min; Max))	0.7 (0.2; 9.8)	0.7 (0.2; 9.8)	–
Organochlorines in serum at age 4 years (ng/ml), N = 285			
Σ PCBs (GM (Min; Max))	0.9 (0.3; 41.2)	–	0.9 (0.3; 41.2)
DDT (GM (Min; Max))	0.1 (0.1; 0.7)	–	0.1 (0.1; 0.7)
DDE (GM (Min; Max))	0.9 (0.0; 43.9)	–	0.8 (0.0; 22.3)
HCB (GM (Min; Max))	0.3 (0.0; 157.0)	–	0.3 (0.0; 4.5)
Child's neurodevelopment scores			
Mc Carthy (mean, (SD))	100 (15.0)	102.2 (15.2)	99.0 (13.9)
ADHD (%)	22.4	11.7*	22.7
Attention deficit symptoms	21.0	14.3	22.7
Hyperactivity symptoms	21.4	15.6	20.5
CP-SCS (%)	18.8	9.1*	18.7
Mother's characteristics			
Age at delivery (years) (mean, (SD))	29.9 (4.6)	29.7 (4.3)	30.1 (4.3)
Pre-pregnancy BMI (kg/m2) (mean, (SD))	22.8 (3.7)	22.5 (3.4)	22.9 (3.8)
Social class (%)			
Professional, manager, technician	12.7	17.9	13.5
Skilled manual and non-manual	51.3	54.8	52.9
Partial skilled and unskilled 1	15.2	9.5	14.3
Unemployed	20.8	17.9	19.3
Completed secondary school or higher (%)	41.6	55.7**	42.0
Smoking during pregnancy (%)	21.2	17.1	20.1
Fish consumption (times/week) during pregnancy (mean, (SD))	1.6 (1.5)	1.6 (0.9)	1.8 (1.7)
First child (%)	49.2	54.6	46.7

(*$p<0.05$ and **$p<0.01$, compared to the original cohort).

Adjustment for other OCs did not change the associations between pre or postnatal PBDE 47 exposures and any of the neurodevelopment outcomes or thyroid hormone levels (results not shown).

4. Discussion

Our study suggests that postnatal exposure to PBDE 47 is associated with a higher risk of certain ADHD symptoms and social competence of children at age 4 years. Cognitive and motor function scores declined with higher postnatal and prenatal exposures to PBDE 47, but none of these associations was statistically significant and prenatal analyses were based on very few subjects. None of the findings was confounded by exposure to other OCs or by levels of thyroid hormones. We found little evidence for an association between postnatal PBDE 47 exposure and thyroid hormone levels at age 4 years.

Several studies have reported differences in PBDE levels between countries and regions, with higher levels (by at least one order of magnitude) in North-America compared to Europe, in part because of the higher standards for protection against flammability in the United States (Meironyte et al., 1999; Betts, 2002; Birnbaum and Staskal, 2004; Fangstrom et al., 2005; Ingelido et al., 2007; Antignac et al., 2008; Fangstrom et al., 2008; Polder et al., 2008). Correspondingly, we observed low levels of PBDE exposure in this Spanish study, which is in accordance with a recent published study measuring PBDE levels in maternal serum and cord blood samples from a birth cohort in Valencia, Spain (Vizcaino et al., 2010). These levels are also of similar magnitude to those used in a recent Dutch study (Roze et al., 2009), but much lower than those of a US birth cohort study (Herbstman et al., 2010). These differences in exposure levels might explain the different associations with the cognitive function between studies. In the American study (Herbstman et al., 2010) cord blood PBDE concentrations of 4 congeners were related to a worse mental and physical development among children at 1, 2, 3, 4 and 6 years, with strongest effects found at age 4. The Dutch cohort, with lower exposure levels, showed no correlations between prenatal exposure to 5 PBDEs congeners and cognitive function in children at age 5–6.

Although in both studies results were based on a small number of cases, nor the Dutch study (Roze et al., 2009) nor the present study, found associations between prenatal exposure to PBDEs and ADHD assessed by questionnaire. However, the Dutch study did observe a negative association between PBDE-47, 99 and 100 and sustained attention assessed by neuropsychological test. It is important to note that our analysis of postnatal PBDE exposure, with a larger number of cases, did find an increased risk of attention deficit symptoms. Our finding of a higher risk of poor social competence skills related to postnatal PBDE 47 exposure could not be compared directly to other studies since none evaluated this outcome. Our results for postnatal exposures taken together (for ADHD symptoms, poor social competence, and general cognitive functions) indicate that the postnatal period may be a vulnerable period for the effects of PBDEs. Proliferation, differentiation and synaptogenesis in cortical and subcortical structures continue into the postnatal period in humans. In fact, gross measures of the brain growth increase for at least 2–3 years after birth, whereas the hippocampus, an area related to behavior tasks and social and emotional function, maturates until the

Table 2
PBDE Levels (ng/gr lipid) and % of samples >LOD or LOQ of the present study and two other child cohort studies in the US and The Netherlands.

	Menorca cohort				US cohort (Herbstman et al., 2010)		Dutch cohort (Roze et al., 2009)	
	Cord blood levels (N = 88)		Serum levels age 4 (N = 244)		Cord blood levels (N = 152)[c]		Mother's blood, 35th pregnancy week (N = 62)	
	% >LOQ[a]	Median (Max)	% >LOQ[a]	Median (Max)	% >LOD[b]	Median (Max)	% >LOD[d]	Median (Max)
PBDE 47	51.1	2.10 (16.8)	20.5	0.12 (130.2)	81.4	11.2 (613.1)	96.8	0.9 (6.1)
PBDE 99	33.0	0.38 (11.6)	13.5	0.12 (72.1)	59.5	3.2 (202.8)	95.2	0.2 (2.1)
PBDE 100	19.3	0.38 (25.7)	3.3	0.12 (11.5)	63.6	1.4 (71.9)	95.2	0.2 (1.4)

[a] LOQ = 0.002 ng/ml (LOD = 0.001 ng/ml).
[b] LOD was not specified.
[c] Levels for those children with more than one neurodevelopment test included in the statistical analyses.
[d] LOD = 0.08·10–3–0.16·10–3 ng/ml.

child is 15 months old (Rice and Barone, 2000). A recent study found that hydroxilated metabolites of PBDE 47 cause disturbance of intracellular Ca^{2+}, very important in the correct neuronal function and development, in cells exposed to even lower levels to those detected in humans (Dingemans et al., 2010). This is especially important for synaptogenesis, which lasts until adolescence, in relation to postnatal exposure; several studies with rodents have found that the effects of many neurotoxic agents, among them PCBs (with similar structures to PBDEs), are more restricted to synaptogenesis when they are administered postnatally (Rice and Barone, 2000). Other neurotoxic mechanisms described for PBDEs are oxidative stress and apoptosis in hippocampal neurons (He et al., 2008), as well as detrimental effects on cytoeskeletal regulation and neuronal maduration in the developing cerebral cortex (Alm et al., 2008).

Table 3
Characteristics of the children with information for cord blood and serum concentrations at age 4 classified as "referents" (<LOQ) and as "exposed" (>LOQ). LOQ = 0.002 ng/ml. N missing = number of children without this information before imputation.

	Cord blood		Serum at age 4	
	Referents N = 43%	Exposed N = 45%	Referents N = 194%	Exposed N = 50%
Sex				
Males	65.1	33.3**	49.5	42.0
Females	34.9	66.7	50.5	58.0
Pre-term birth				
Yes	4.7	4.4	4.6	6.1
No	95.3	95.6	95.4	94.9
N missing	0		1	
Breastfeeding duration				
=<2 weeks	16.7	19.0	26.0	10.6*
>2 weeks	83.3	81.0	74.0	89.4
N missing	4		12	
Maternal age at delivery[a]				
18–28 years	46.5	22.2	30.9	44.0
28–31 years	25.6	40.0	35.1	26.0
31–42 years	27.9	37.8	34.0	30.0
Pre-pregnancy BMI[a]				
<20 kg/m^2	19.1	15.6	14.8	28.6
20–25 kg/m^2	69.0	62.2	61.4	57.1
>25 kg/m^2	11.9	22.2	23.8	14.3
N missing	2		6	
Social class				
Professional, manager, technician	15.4	20.0	11.2	22.0*
Skilled manual and non-manual	56.4	53.3	51.5	58.0
Partial skilled and unskilled	7.7	11.1	14.4	14.0
Unemployed	20.5	15.6	22.9	6.0
N missing	4		6	
Completed secondary school or higher				
Yes	48.8	62.2	39.7	50.0**
No	51.2	37.8	60.3	50.0
N missing	0		13	
Smoking during pregnancy				
Yes	18.6	15.6	19.6	22.0
No	81.4	84.4	80.4	78.0
Fish consumption during pregnancy[a]				
=<2 times/week	53.5	53.3	53.6	58.0
>2 times/week	46.5	46.7	46.4	42.0
Parity				
First child	55.8	53.3	43.8	58.0**
Second child or more	44.2	46.7	56.2	42.0

[a] Note: although shown as categorical here, these are entered as continuous variables in the statistical models.
* $p<0.05$.
** $p<0.01$.

One suspected mechanism through which PBDE may affect neurodevelopment is through interference with the thyroid hormone system. Influences of PBDE exposure on thyroid hormone levels have been described inconsistently in animal studies (Darnerud et al., 2001; Gee et al., 2008; Legler, 2008; Talsness et al., 2009) and very scarcely in humans (Boas et al., 2009). One child cohort study reported a negative association between BDE-100 and BDE-153 and levels of TSH and FT4 measured in cord blood, not for the whole study population but only among children with spontaneous unassisted vaginal delivery (Herbstman et al., 2008). In the Dutch birth cohort study cord blood levels of TT3 increased in relation to prenatal PBDE levels (Roze et al., 2009). In the present study we did not observe clear associations between postnatal PBDEs exposure and thyroid hormone levels at age 4. TT3 levels were elevated in the PBDE exposed group, but TT3 did not appear to explain the effect of PBDE exposure on neurodevelopment. Further studies are needed to confirm the role of thyroid hormones in the neurotoxic effects of PBDE exposure.

OCs, such as PCBs, HCB, DDT or DDE, may share exposure routes and effects with PBDEs, and due to structural similarities, toxicity studies have followed comparative approaches to those for PCBs to assess the effects of PBDEs on neurodevelopment (Fonnum and Mariussen, 2009). Prenatal exposure to OCs has been associated with deleterious effects on children's cognitive function and behavior (Wigle et al., 2007; Wigle et al., 2008) and also with thyroid hormone disruption (Boas et al., 2009). Earlier publications from the Menorca cohort also found that prenatal levels of DDT and HCB were associated to cognitive and behavioral dysfunction, respectively (Ribas-Fito et al., 2006b; Ribas-Fito et al., 2007). Thyroid hormone disruption was associated with prenatal DDT, HCB and PCBs exposure as well (Alvarez-Pedrerol et al., 2008). Thus, in the present study we considered it important to clarify whether the associations found between PBDEs and neurodevelopment and thyroid hormone disruption may actually be explained by other OCs exposures. This was not the case, not even for specific PCB congeners (153 and 180) assessed in sensitivity analyses. In fact, the correlation between prenatal levels of PBDE 47 and OCs was only moderate or low in this cohort (ΣPCBs = 0.25*, HCB = 0.17, DDE = 0.19, DDT = −0.17, *$p<0.05$). For postnatal levels these correlations diminished varying between 0.04 (HCB) and 0.13 (DDT), non significant, indicating that exposure sources may be quite different. It is well known that one important source of exposure to persistent pollutants is breastfeeding (Lakind et al., 2004). It is curious that in the present study, although a significantly higher percentage of children within the exposed group had been breastfed for more than two weeks, the correlation between duration of breastfeeding (weeks) and post-natal PBDE 47

Table 4
Regression (β) coefficients, relative risk (RR), and confidence intervals (95%CI) for neurodevelopment and thyroid outcomes in relation to PBDE 47 exposure; "exposed" group (>LOQ) compared to "referents" group (<LOQ). LOQ = 0.002 ng/ml.

	Cord blood			Serum at age 4		
	N Exposed	N Referents	β Coefficient (95%CI)[a]	N Exposed	N Referents	β coefficient (95%CI)[e]
MSCA total cognitive function score	41	37	−1.4 (−9.2; 6.5)	50	190	−2.7 (−7.0; 1.6)
Verbal	41	38	−0.4 (−7.6; 6.7)	50	190	−3.2 (−7.7; 1.3)
Performance	41	37	−1.4 (−9.7; 6.8)	50	191	−1.4 (−5.8; 3.0)
Quantitative	41	38	−2.5 (−10.6; 5.6)	50	190	−1.2 (−5.6; 3.3)
Memory	41	38	−3.1 (−10.1; 3.8)	50	190	−2.0 (−6.5; 2.6)
Motor	41	37	−2.8 (−11.4; 5.8)	50	190	−1.0 (−5.3; 3.3)
Executive function	41	38	−1.1 (−9.1; 6.8)	50	190	−2.8 (−7.1; 1.5)
	N cases/total	N cases/total	RR (95%CI)[b]	N cases/total	N cases/total	RR (95%CI)[f]
ADHD symptoms Total score	2/40	7/37	0.4 (0.1; 1.7)	9/44	41/176	1.1 (0.6; 2.1)
Attention deficit symptoms	2/40	9/37	0.3 (0.1; 1.3)	12/44	6/176	1.8 (1.0; 3.2)
Hyperactivity symptoms	6/40	6/37	1.4 (0.6; 3.8)	4/44	41/176	0.4 (0.2; 1.1)
	N cases/total	N cases/total	RR (95%CI)[c]	N cases/total	N cases/total	RR (95%CI)[g]
Poor social competence	2/35	4/31	1.8 (0.2; 18.0)	8/33	26/149	2.6 (1.2; 5.9)
				N Exposed	N Referents	β coefficient (95%CI)h
Thyroid hormones						
lnTSH				39	173	0.05 (−0.1; 0.2)
Total TT3				45	183	7.3 (−0.4; 14.9)
Free T4				42	182	0.01 (−0.04; 0.05)

Models adjusted for sex (a,b*,c*,e,f*,g*,h), age of the child (a,b,c,e,f,g), preterm (a*,b*,c,e*,h*), evaluating psychologist (a,e*), maternal age (a*,b*,f*), social class (a,c*,e*,f,g,h), education (a*,c*,e*,f,g*,h), parity (a,e*,h), smoking during pregnancy (a,c,e,f,g), alcohol consumption (a,e), pre-pregnancy BMI (b,c,e*,f,g), fish consumption (b,g*,h) and duration of breastfeeding (e*,f,g*,h). (Remained significant in the final model, *p<0.05).

levels was nonexistent (r = 0.07, p = 0.03). On the contrary, the correlation between duration of breastfeeding and post-natal levels of other OCs was higher (r between 0.38 and 0.60, p<0.01). These results altogether indicate that other sources of exposure to PBDEs in children from birth up to age 4 must play a role apart from breastfeeding, for instance house dust as it is suggested by other studies (Fischer et al., 2006; Frederiksen et al., 2009; Toms et al., 2009; Lunder et al., 2010). Further, in our cohort the correlation between prenatal and postnatal PBDE 47 levels was nonexistent (r = −0.06, not significant) in the 49 children for who we had this information, whereas these same correlations were much higher for some of the other OCs (r = 0.44**(PCBs), 0.26*(HCB), 0.62**(DDE), **p<0.01, *p<0.05). Despite our results, future studies should continue to examine the influence of other POPs in their analyses of PBDE effects. Additionally, some animal studies have suggested synergic adverse effects due to the combination of different compounds, specifically PCBs and mercury and PBDEs and mercury (Fischer et al., 2008; Fonnum and Mariussen, 2009). Such synergistic effects also need further elucidation in human studies. Finally, it has been shown in experimental studies that OH-PBDEs metabolites are more harmful than the parent PBDEs (Dingemans et al., 2008); therefore, it would be interesting for future birth cohort studies to measure these metabolites, instead of the parent compounds.

A limitation of this study is the small number of quantifiable samples due to the low levels of exposure; we were unable to analyze the continuous association between PBDEs concentration and neurodevelopment scores in the positive samples. However, a sensitivity analysis modeling continuous exposure (replacing below LOQ values with half the LOQ value) gave very similar results to our dichotomous analysis. The low exposure levels in this study also made it impossible to assess effects of different PBDEs congeners separately. The US birth cohort study did assess individual congeners and found very similar results for all of them, probably because all were highly correlated. We would expect similar findings in our Spanish population as correlations between PBDE 47, 99 and 100 were high (r = 0.35 to 0.77, p<0.01). Due to sample size problems, our study could not include prenatal and postnatal exposures simultaneously in statistical models to clarify the importance of each. Prenatal and postnatal exposures appeared to be largely unrelated in this population so it is unlikely that prenatal exposures would explain the results for postnatal exposures. It should be noted, however, that postnatal exposure levels were measured at the same time as the neurodevelopment outcomes and this may complicate the interpretation of any association. However, animal studies (Hooper and McDonald, 2000) have shown that tetra and penta PBDEs, groups in which PBDE47, 99 and 100 are included, have long half-lives (order of years); thus, the PBDE concentrations measured at age 4 represent exposure from the years preceding outcome assessment. Following the same reasoning, cord blood concentrations would represent exposure during pregnancy, which is why we refer to the cord blood levels as "prenatal exposure". Further, levels in maternal serum (weeks 10–13 of pregnancy) and cord blood have been shown to be similar after lipid normalisation (Vizcaino et al., 2010).

5. Conclusion

This study is the first to examine effects of both prenatal and postnatal exposures to PBDEs, and to explore the role of other persistent organic pollutants and thyroid hormones in this association. Findings of a potential adverse effect of postnatal exposure to PBDE 47 on ADHD symptoms and poor social competence of children at age 4 years require follow up in larger studies. This study highlights the importance of assessing the effects of PBDE exposure not just prenatally but also during the early years of life. In the light of current evidence a precautionary approach towards PBDE exposure of both mothers and children seems warranted.

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