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List of abbreviations:

BDE	Brominated diphenyl ether(s)
BMI	Body mass index (kg/m^2)
CI	95% Confidence Interval
CV	Coefficient of variation
EU	European Commission
FSH	Follicle-Stimulating Hormone
LH	Luteinizing Hormone
LOAEL	Lowest observed adverse effect level
LOD	Limit of Detection
LOQ	Limit of Quantitation
PBDE(s)	Polybrominated Diphenyl Ether(s)
SGA	Small for gestational age
SHBG	Sex-Hormone Binding Globulin
TDS	Testicular Dysgenesis Syndrome
WGA	Weight for gestational age

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Article descriptor

Exposure assessment

Outline of section headers

Abstract

Background: Polybrominated diphenyl ethers (PBDEs) are widely used in Western countries.

Objectives: As the prevalence of cryptorchidism appears to be increasing, we investigated whether exposure to PBDEs was associated with testicular maldescent.

Methods: In a prospective Danish-Finnish study 1997-2001 all boys were examined for cryptorchidism. Whole placentas (95 cryptorchid / 185 healthy boys) and individual breast milk samples (62 / 68) were analysed for 14 PBDEs and infant serum samples for gonadotropins, sex-hormone binding globulin (SHBG), testosterone and inhibin B.

Results

Placentas: In 86 placenta-milk pairs, placenta PBDE concentrations in fat were lower than in breast milk, and a larger proportion of congeners were non-detectable. There was no significant difference between boys with and without cryptorchidism for individual congeners, the sum of 5 most prevalent or all 14 congeners. *Breast milk:* The concentration of PBDEs in breast milk was significantly higher in boys with cryptorchidism than controls (sum of BDE 47, 153, 99, 100, 28, 66 and 154: median 4.16 vs. 3.16 ng/g fat, $p<0.007$). There was a positive correlation between the sum of PBDEs and serum LH ($p<0.033$). The sum of PBDEs in breast milk did not differ between Denmark and Finland (median 3.52 vs. 3.44 ng/g fat), but significant differences in some individual congeners were found.

Conclusions: Two different proxies were used for prenatal PBDE exposure, and levels in breast milk, but not in placenta, showed an association with congenital cryptorchidism. Other environmental factors may contribute to cryptorchidism. Our observations are of concern as human exposure to PBDEs is high in some geographical areas.

Introduction

Polybrominated diphenyl ethers (PBDEs) are widely used as flame-retardants and the general population is exposed through products such as upholstery, building materials, insulation, electronic equipment and contaminated food. PBDEs are added to polymers without being chemically bound and can leach into the environment, where they settle with air particles and sludge. They are persistent and some, i.e. BDE-47, BDE-99 and BDE-153, can accumulate in lipid-rich tissues (Agency for Toxic Substances and Disease Registry 2004; Sjödin et al. 2003).

Concentrations of PBDE in human European breast milk samples are generally low as compared to the US, and considered to be well below the estimated LOAEL of 1 mg/kg/day (Darnerud et al. 2001). Two technical mixtures, penta- and octa-mixtures of PBDEs, have been banned from use in Europe since 2003 (Darnerud et al. 2001), and Swedish studies indicated a decrease in breast milk levels since the middle of the 1990's (Meironyte et al.1999; Sjödin et al. 2003). However, annual production rates of some PBDEs are still considerable in some areas (Alaee et al. 2006; Betts 2002; Law et al. 2006). Animal studies show that some PBDEs exhibit endocrine disrupting activity, which has predominantly been studied for thyroid hormone transport and metabolism (Legler and Brouwer 2003), but data on adverse effects on reproductive outcome after gestational exposure are also emerging (Lilienthal et al. 2006).

The prevalence of cryptorchidism in newborn boys appears to have increased in some areas such as Great Britain and Denmark over the past decades, and its current prevalence is considerably higher in Denmark than in Finland (Anonymous 1986; Boisen et al. 2004). Although the reason for this is as yet unknown, the rapid increase in prevalence suggests that environmental factors are involved (Sharpe 2006; Skakkebæk et al. 2001). Adverse effects of fetal exposure to environmental chemicals on testicular descent and hormonal function may be detectable during the short physiological activation of the pituitary-gonadal axis at approximately three months of age (Andersson et al. 1998; Main et al. 2000; Main et al. 2006a; Suomi et al. 2006).

This study aimed at evaluating the association between exposure to 14 PBDEs (BDE-28, 47, 66, 71, 75, 77, 85, 99, 100,119, 138, 153, 154, 183) in newborn boys and the position and function of the testes.

Materials and Methods

The study was conducted according to the Helsinki II declaration after informed oral and written consent of the parents. The ethical committees (Finland: 7/1996, Denmark: KF01-030/97) and the Danish Data Protection Agency (1997-1200-074) approved the study.

Study population

Breast milk samples and placentas were obtained from a joint prospective, longitudinal cohort study performed 1997 – 2001 at Turku University Hospital, Turku, Finland, and the National University Hospital (Rigshospitalet, Hvidovre Hospital) Copenhagen, Denmark. This bi-national study aimed at establishing contemporary prevalence rates for cryptorchidism and hypospadias, and at evaluating risk factors by means of questionnaires and biological samples (blood, placentas, breast milk). Exposure measurements were prospectively planned to include persistent and non-persistent chemicals (EU grant QLK4-CT-2001-00269), some of which have been previously reported (Damgaard et al. 2006; Main et al. 2006b; Shen et al. 2005; Shen et al. 2006; Shen et al. 2007). Recruitment strategy, inclusion criteria and clinical examination of the children, i.e. the identification of cryptorchidism, have been previously described (Boisen et al. 2004; Main et al 2006a; Suomi et al 2006) and were strictly standardized. Boys with normally descended testis, including retractile testes, were used as controls in this study under the terms controls or healthy boys. Boys with undescended testes (non-palpable, inguinal, suprascrotal, high scrotal), either uni-or bilaterally at birth, were included in the group of cryptorchid boys.

All boys were examined at birth and at three months of age before the results of chemical analyses were known. Birth weight and length was obtained from hospital records. The supine length of the children was measured with infantometers (Denmark: Kidimeter, Raven Equipment Ltd., United Kingdom; Finland: Pedihealth Ky, Oulu, Finland). Weight was measured on a digital scale (Solotop Oy, Baby scale model,

Helsinki, Finland). Weight for gestational age was calculated using national standards as % deviation from the expected mean (Marsál et al. 1996; Pihkala et al. 1989), -22% being equivalent to –2 standard deviations.

Biological samples

In Denmark biological samples were collected from all participants (case-cohort design). In Finland, due to lack of storage space, biological samples were collected from boys with cryptorchidism at birth and matched controls (matching criteria: parity, maternal smoking (yes/no), diabetes (yes/no), gestational age (± 7 days) and date of birth (± 14 days)) as a nested case-control design.

From this bio-bank a total of 280 placentas (168 Danish/ 112 Finnish) and 130 breast milk samples (65 Danish/ 65 Finnish) were selected for PBDE measurements, this number was determined by funding. Birth examination data were used for classification of cryptorchid and healthy boys.

In Finland, placentas were selected from 56 case-control pairs, in which both placentas were available. In Denmark, all available placentas from cryptorchid boys were chosen (n=39). Control placentas were selected randomly from the total Danish cohort (n=129).

Hereafter, 65 breast milk samples were selected for each country, aiming, if possible, at equal numbers of samples from boys with and without cryptorchidism and at including the same mothers as for placenta analyses. Only samples with a volume > 125 ml were included to ensure that all chemicals could be analysed. Milk from mothers of 29 cryptorchid boys and 36 randomly chosen control boys was included in Denmark. In Finland, milk samples were chosen from mothers of cryptorchid boys (n=33), matched controls (n=18) or random controls (n=14).

In 86 boys milk and placentas could be selected from the same mother-child pair (10 Danish boys with cryptorchidism, 33 Danish controls, 20 Finnish boys with cryptorchidism, 23 Finnish controls).

Collection of samples

Whole placentas were collected by the midwives and frozen immediately in two layers of polyethylene bags (–20^{o}C). Placentas were not bled prior to storage.

Each mother collected one breast milk sample. We wished to assess the average concentration of PBDEs during the period preceding the endogenous hormone surge in infants. Thus, each sample consisted of several small aliquots collected over successive feedings over several weeks and frozen in household freezers in 250 ml Pyrex glass bottles (1515/06D, Bibby Sterilin, Staffordshire, UK) with Teflon coated caps. The mothers were instructed orally and in writing to feed the baby, then to sample aliquots (hind milk), starting from 1 month after birth. This start point was chosen after discussion with the ethics committee for human subject studies to ensure that breastfeeding had been well established. Mothers were instructed to collect samples into a clean household glass or porcelain container avoiding, if possible, the use of breast pumps, and to freeze every portion immediately. Breast milk was delivered frozen to the hospital at the 3 months' examination and stored at -20^{o} C. In 57 of the 65 Danish mothers, but no Finnish mothers, information on breast pump use was obtained at sample delivery, 26 (46%) had used a pump on one or more occasions. No information was obtained on the type of breast pump (glass or plastic).

Blood samples

Venous non-fasting blood samples (4 ml) were drawn from the infants at the 3 months' examination (median age: 3.0 months, range: 2.4-4.1), the overall success rate of obtaining a sample in the study being 74%. After clotting, the blood samples were centrifuged and the sera were separated and stored at -20 °C. All samples were analysed as duplicates and blinded for the technician at one laboratory (Rigshospitalet, Denmark) for reproductive hormones. Each run contained samples of both cryptorchid and healthy boys from both countries (up to 160 samples per analysis) to minimize any effect of inter-assay variation.

Hormone analyses

Serum Follicle-Stimulating Hormone (FSH), Luteinizing Hormone (LH) and sex-hormone binding globulin (SHBG) were analysed by time-resolved immunofluorometric assays (Delfia®, Wallac Inc., Turku, Finland). Detection limits were 0.06 and 0.05 IU/l for FSH and LH, respectively, and 0.23 nmol/l for SHBG. The intra- and interassay coefficients of variation (CV) were <5% in both gonadotropin assays and <6%

for SHBG. Serum testosterone was measured by radioimmunoassay (Coat-a-Count®, Diagnostic Products Corp., Los Angeles, CA), with a detection limit of 0.23 nmol/l and intra- and interassay CVs <10%. Free testosterone index was calculated: ((testosterone x 100)/SHBG). Serum inhibin B was analysed by a double antibody enzyme-immunometric assay (Main et al. 2006a). The detection limit was 20 pg/ml, and intra- and interassay CVs were <15 % and <18 %, respectively. Ratios between hormones were calculated: LH/testosterone, LH/free testosterone, FSH/inhibin B.

Analysis of PBDE

All PBDE analyses for both milk and placenta were performed at the laboratory at the Department of Environmental Health in Kuopio, Finland. Placentas were defrosted, the umbilical cord and all readily removable membranes were discarded. Whole placentas were homogenized in a mixer (Büchi Mixer B-400, Büchi Laboratories AG, Flawil, Switzerland) and 75 g of the homogenate was lyophilized. Dried homogenate was pulverized in a mortar and slurry was made by adding dichloromethane and cyclohexane (1:1 v/v) and concentrated sulphuric acid. This slurry was spiked with six ^{13}C-labelled PBDE internal standards (BDE 28, 47, 77, 99, 153, and 183) (Wellington Laboratories Inc., Guelph, Canada). Fat was not determined, and fat based results were relying on the gravimetrically measured fat contents obtained from a German partner in this EU project (Shen et al. 2005; Shen et al. 2006; Shen et al. 2007).

Breast milk samples (average volume: 70 ml) were thawn in sample bottles in a water bath (+ 40 ºC) for one hour and homogenized. Fat was extracted with a mixture of diethyl ether and hexane (1:1.4 v/v) after addition of sodium oxalate solution and ethanol (1:5 v/v). Fat content was determined gravimetrically after exchange of the solvent to hexane. An average 1.5 g of fat was spiked with the same set of internal standards used with placenta.

The procedure for decomposition of fat and sample clean up has been described previously (Kiviranta et al. 2004). The quantification of 14 PBDE analytes (BDE 28, 75, 71, 47, 66, 77, 100, 119, 99, 85, 154, 153, 138, and 183) was performed by selective-ion recording using a high resolution mass- spectrometer, Autospec Ultima (Micromass Inc., Manchester, UK) at resolution 10 000. Gas chromatographic separation of the PBDEs was

performed with a HP 6890 gas chromatograph with fused silica capillary column (DB5-MS, 60 m, 0.25 mm, 0.25 μm; J&W Scientific, Folsom, CA, USA). As a recovery standard for internal PBDE standards polychlorinated biphenyl 159 (PCB 159) was used.

In the analysis of PBDEs at the Department of Environmental Health (National Public Health Institute, Kuopio, Finland) the technicians and chemists were blinded. Laboratory and cross-sample contamination was monitored by analyzing procedural blank samples. The concentrations of these blank samples were much lower than the concentrations in placenta and breast milk, on average 3.6 and 2.6% of the average sum of PBDEs in placenta and milk, respectively.

Recoveries of individual internal PBDE standards were >60%. Median limit of quantification (LOQ) for placentas corresponding to a signal to noise ratio of 3:1, was 0.006 ng/g fat (range: 0.004 - 0.14 ng/g fat). Corresponding LOQs for breast milk were 0.004 ng/g fat (range: 0.0003 - 0.12 ng/g fat). In placenta, CVs for individual congeners were 10-20%, and >20% at concentration levels 0.1-1 ng/g fat and <0.1 ng/g fat, respectively. In breast milk samples corresponding values were <10%, 10-20%, and >20% at concentrations levels >1 ng/g fat, 0.1-1 ng/g fat, and <0.1 ng/g fat, respectively. Concentrations < LOQ were considered to be equal to nil (lower bound results). The laboratory has successfully participated in interlaboratory comparison studies of PBDEs in different biological matrices including breast milk (Becher et al. 2001, Småstuen and Becher 2004, Småstuen and Becher 2005). The Finnish Accreditation Service, FINAS, has verified the competence of the laboratory (testing laboratory T077) in performing PBDE analyses in biological samples according to the EN ISO/IEC 17025 standard.

Statistics

Population characteristics are given as medians and percentiles (2.5^{th}, 97.5^{th}). Differences between boys with / without cryptorchidism and between Danish / Finnish populations were analysed by Mann-Whitney U-test. Note, that 86 boys participated with both breast milk and placenta samples.

Country differences for (log-transformed) PBDE concentrations in breast milk and placenta were tested by multiple linear regression including maternal age, parity (1, 2 and ≥3) and pre-pregnancy body mass index (BMI kg/m^2) in the model.

Correlations between individual PBDE congener concentrations, and between PBDE concentrations and date of childbirth within the Danish and Finnish cohort, respectively, were tested by Spearman correlation on non-transformed data. For each country the date of birth for the first child was set at zero, the date of birth for all consecutive children was then calculated as numbers of days elapsed since the first child of the same country-specific cohort. This variable was applied to control for any time trends in flame-retardant concentration in the study period from 1997-2001.

Differences in PBDE concentrations between boys with and without cryptorchidism were tested in a multiple regression model including as covariates: maternal age, parity, maternal pre-pregnancy BMI and childbirth within the cohort (days) to control for factors that could affect PBDE concentrations in the sample. Prematurity and being small for gestational age are well-known risk factors for cryptorchidism. As the number of premature (5 Danish/3 Finnish) and SGA children (3 Danish/1 Finnish) was small in this study, analyses were carried out both with and without inclusion of these parameters. Analyses were only carried out for the most abundant seven congeners in breast milk (and their sum) and for the most abundant 5 congeners in placenta.

Multiple linear regression analysis was used to assess the correlation between serum levels of reproductive hormones (log-transformed LH, FSH, SHBG, inhibin B), square root transformed serum testosterone or free testosterone index and log-transformed PBDE concentrations in breast milk. Covariates included in these analyses were: country of origin, testicular position (cryptorchidism/control) and age at blood sampling (months).

Results

There were no significant differences in maternal age, reported smoking and parity between cryptorchid and control boys (Table 1, 2). Gestational age and birth weight were slightly lower in cryptorchid Danish (but not Finnish) boys than controls. Diabetes was more prevalent in Finnish (but not Danish) mothers of cryptorchid boys than their controls, as we could not always find controls matched by this criterion. The date of childbirth within the study period did not differ significantly between cryptorchid boys and controls ($p=0.327$ Denmark/ $p=0.949$ Finland).

Danish mothers were slightly older than Finnish (30.6 years (23.6-38.8) versus 28.7 years (21.4-39.7), $p<0.011$), had a lower parity ($p<0.033$) and smoked more frequently ($p<0.04$). The prevalence of diabetes mellitus was higher among Finnish women ($p<0.004$), but BMI before pregnancy ($p=0.678$) did not differ significantly between the countries ($p=0.225$).

Breast milk

Seven PBDEs were measurable in all breast milk samples (Table 3). Median concentrations were higher in Danish but not Finnish cryptorchid boys than in controls, reaching statistical significance for BDE 47, 100, 28, 66 and 154. The sum of all seven congeners was significantly higher in cryptorchid boys than controls if both countries were analysed together (Figure 1, $p<0.007$), also if prematurity and being small for gestational age were included in the model ($p<0.035$). Similar results were obtained for PBDE expressed as ng/l (data only shown for the sum of all 14 congeners in Table 3). Table 4 shows the remaining seven congeners, which were below detection limit in a substantial number of samples, and the sum of all 14 congeners.

The concentrations of PBDE congeners in milk samples showed large variations between congeners and individuals (Figure 2). Individual congener concentrations were positively correlated with each other ($r= 0.178$-0.955, $p<0.0001$).

There were no significant country differences in the sum of all congeners (Denmark 3.52 ng/g (1.26-14.2) versus Finland 3.44 (1.25-13.94), $p=0.754$) or the sum of the most prevalent seven congeners BDE 47, 153, 99, 100, 28, 66 and 154 (Denmark 3.24 ng/g (1.26-51.1) versus Finland 3.23 (1.26-51.0), $p=0.629$).

Similar results were obtained if only milk samples from mothers of healthy boys were analysed (data not shown). The estimated daily intake of PBDEs (sum of all) for an infant at three months of age (median 6.58 kg, consuming 120 ml breast milk/kg) was 16 ng/kg/d (median, range: 6-121 ng/kg/d). Breast milk lipid (% w/w) differed significantly between the countries (mean ± SD: 2.99 ± 1.38 in Denmark, 4.52 ± 1.56 in Finland, $p<0.0001$). The lipid content was not significantly correlated with the sum of PBDE congeners (data not shown).

The pattern of congener distribution differed between the countries. BDE 28 was significantly higher in Finland than in Denmark ($p<0.021$), with a similar tendency for BDE 47 ($p<0.077$), which was the most prevalent congener. BDE 153 ($p<0.0001$), BDE 66 ($p<0.026$) and BDE 183 ($p<0.022$) were significantly higher in Denmark than in Finland with a similar tendency for BDE 99 ($p<0.073$). For congeners at very low concentrations the percentage of measurable samples was higher in Denmark than in Finland (Table 4). There was no significant effect of breast pump use during collection of samples on the concentration of any BDE congener in the Danish breast milk samples.

In Danish, but not the Finnish samples, the date of childbirth within the cohort was significantly correlated with the concentration of BDE 154 ($r=-0.346$, $p<0.005$), BDE 85 ($r=-0.434$, $p<0.0001$) and BDE-75 ($r=+0.376$, $p<0.002$) with similar tendencies for BDE 66 ($r=-0.211$, $p=0.092$) and BDE 77 ($r=-0.214$, $p=0.087$).

Serum LH levels correlated positively with the sum of seven PBDEs in breast milk ($r= 0.218$, $p<0.033$) as well as with individual congeners BDE 47 ($r=0.227$, $p<0.027$), BDE 100 ($r=0.293$, $p<0.004$) and BDE 154 ($r=0.203$, $p<0.048$). Country-specific analyses showed significant associations between serum LH and the above listed PBDEs and their sum for Finnish milk samples, but not Danish (data not shown). No other reproductive hormones or their ratios were significantly correlated with the concentration of PBDEs.

Placentas

The average placental levels of 14 PBDE congeners per gram fat were lower than in breast milk (Table 5), and more samples were non-detectable. Therefore, the sum of 5 congeners was used instead of 7 congeners used for milk. This had a minor influence on the total, as the sum of 5 and 14 were very close.

The distribution of congeners resembled the distribution in breast milk, with BDE-47 and BDE–153 constituting the main fraction of PBDEs. There was no significant country difference for the sum of all 14 congeners (p=0.198) or the sum of five (BDE-47, BDE-153, BDE-99, BDE-100 and BDE-28, p=0.192). This was also true when only placentas from healthy boys were analysed (data not shown). The concentrations of the five most prevalent congeners were positively correlated with each other (r= 0.171-0.827, p<0.0001). Placenta lipid content (% w/w) differed significantly between the countries (mean ±SD: 1,09 ± 0.17 Denmark, 1.21 ± 0.13 in Finland, p<0.0001). Placenta lipid content was not significantly correlated with the sum of PBDE congeners (data not shown).

In Danish placentas the date of childbirth within the cohort was significantly correlated with the concentration of BDE 66 (r=-0.246, p<0.001), in the Finnish placentas with BDE 85 (r=+0.239, p<0.01) and BDE 153 (r=+0.275, p<0.003).

There was no significant difference in the placenta concentration of the five most prevalent PBDEs between cryptorchid boys and healthy boys in Denmark (p=0.10-0.976) or Finland (p=0.09-0.835) or for their sum (p=0.312 and p=0.128, respectively). The results remained non significant if prematurity and being small for gestational age were included in the model. There were no correlations between placental PBDEs and serum reproductive hormones in three months old infants.

Paired samples

The median concentrations (ng/g fat) of the 14 congeners in placenta were lower than the concentration found in the paired breast milk samples (n=86), Table 6, but there were significant correlations between the measurements, except for BDE-85 and 138.

The sum of all congeners in milk was 3.39 (1.43-48.2) for boys with cryptorchidism and 3.15 (1.07-24.9) for controls (p=0.228), in placenta 1.22 (0.64-9.32) for boys with cryptorchidism and 1.17 (0.49-5.46) for controls (p=0.871). Infant reproductive hormones at 3 months of age (27 Danish, 35 Finnish boys) were not correlated to PBDE concentrations in placenta or milk in this data subset.

Discussion

This is to our knowledge the first study describing an association between congenital cryptorchidism in humans and exposure to PBDEs. An association was found for the sum of seven PBDEs (BDE-47, 153, 99, 100, 28, 66, 154) in breast milk as well as for the individual congeners BDE-47, 100, 28, 66 and 154. Concentrations of BDE-47, 100 and 154 were also positively correlated with increasing serum LH values. This suggested that a higher gonadotropin drive was necessary to ensure normal testosterone production by the Leydig cells (Suomi et al. 2006), and thus a subtle primary testicular dysfunction.

This study assessed infant exposure by measuring the concentration of PBDEs in breast milk, which reflects the accumulated body burden of the mother (Inoue et al. 2006; Jensen and Slorach 1991; Waliszewski et al. 2001). It also is a proxy for prenatal fetal exposure as PBDEs, especially the lower brominated compounds, can pass the placenta (Bi et al. 2006; Mazdai et al 2003) and are transferred to breast milk during lactation (Darnerud and Risberg 2006). However, when exposure was assessed by measurement of PBDE in placenta, the results did not support the above findings, despite the fact that 86 mother-child pairs were represented with both milk and placenta samples. At present time it is not clear why this is the case.

The concentration of all PBDE congeners per gram fat in placenta was considerably lower than in milk, and more congeners were non-detectable. These differences did not have any remarkable influence on analytical errors, because the amount of fat used for analysis was similar for placentas and milk samples. In theory, placenta should be a better proxy for fetal exposure than breast milk, as it represents the direct route of chemical transfer during pregnancy. The number of placentas analysed in this study was larger than the number of milk samples, and should thus better represent the pool of cases and controls. We could not establish any obvious selection bias of milk donors. On the other hand, due to higher concentrations milk analyses were somewhat more reliable towards the lower end of concentration. This should not be important, as the main group differences were seen at the higher end of PBDE concentrations. It is currently unknown, whether PBDEs accumulate in placenta, but our paired samples indicated that this may not be the case. We found positive correlations between measurements in placenta and breast milk, but 3-4 times lower absolute

concentrations in placenta. Placenta concentrations may resemble measurements in single blood samples and thus reflect the situation at delivery, but not the long-term exposure.

There is some controversy as to whether the placental transport of PBDEs may differ between lower and higher brominated PBDEs. An American study reported a strong correlation of lipid-adjusted BDE concentrations in maternal serum at term and cord blood (Mazdai et al. 2003), whereas studies from countries with a generally lower exposure level found weaker correlations and higher values in maternal than in fetal samples (Bi et al. 2006). Segregation from serum into breast milk samples did not always follow a 1:1 pattern. The congener distribution appeared to be similar for lower brominated BDEs (Bi et al. 2006; Mazdai et al. 2003), whereas higher brominated BDEs such as BDE-209 were ten times higher in serum than in breast milk (Inoue et al. 2006). Thus, the relative distribution of congeners between placenta and breast milk may also depend on the fat composition of these two matrices causing different solubility. We found significantly higher fat concentrations in the Finnish than the Danish samples, which may reflect dietary habits in the two countries, as both long-term and short-term diet as well as the nutritional status may influence content and composition of breast milk lipids (Ortiz-Olaya et al.1996).

Current knowledge about human reproductive health consequences after exposure to PBDEs is very limited (Agency for Toxic Substances and Disease Registry 2004). A study from Taiwan showed an association between the sum of 12 PBDE congeners in breast milk, 10 of which were the same as in our study, and lower birth weight and length (Chao et al. 2007). Recently, a Swedish case-control study found higher values of PBDEs (sum of BDE-47, 153 and 99) in blood samples from mothers of young men with testicular cancer than in age-matched controls (Hardell et al. 2006). However, maternal PBDE exposure was assessed 15-25 years after the critical time period, i.e. at the time of the cancer diagnosis, which considerably weakens the possibility to establish a causal link between exposure and outcome. Also in this study, exposures to other persistent chemicals occurred simultaneously, and it cannot be determined how these substances interact in their effect on reproductive development. Testicular cancer is the most severe clinical symptom of the testicular dysgenesis syndrome (TDS), which also encompasses impaired semen quality, congenital cryptorchidism and hypospadias (Skakkebæk et al. 2001). TDS may be caused by genetic, hormonal or environmental factors

(Sharpe 2006; Skakkebæk et al.2001). Prenatal exposure to PBDEs may have an adverse effect on testicular growth and differentiation in utero.

In animal studies, penta-brominated PBDEs showed anti-androgenic activity (Stoker et al. 2005). A peripubertal single-dose exposure to a commercial mixture of penta-BDEs delayed the onset of puberty in male and female rats (Stoker et al. 2004) and suppressed the growth of the seminal vesicles and ventral prostate. In adult rats, a single dose exposure significantly increased LH concentration in serum (Stoker et al. 2005). Our observation of an association between PBDE levels in breast milk and serum LH in infants at three months of age were in line with these animal data. Gestational exposure of rats to BDE-99 caused a shortening of the anogenital distance in male and female rats, reduction in primordial and secondary ovarian follicles, a lower sperm count and lower oestradiol and testosterone levels in adulthood (Kuriyama et al. 2005; Lilienthal et al. 2006). In vitro assays showed a competitive androgen receptor binding for BDE-47, 99 and 100. BDE- 47, 71 and 100 inhibited dihydrotestosterone-induced transcriptional activity (Stoker et al. 2005). As testicular descent is highly androgen-dependent (Toppari 2003), the adverse effect of PBDE on testicular descent could be caused by their anti-androgenic properties.

In addition, BDE-47, 100, 75 and 51, in particular their hydroxylated metabolites, are weakly estrogenic and BDE-153, 166 and 190 anti-estrogenic in vitro (Legler and Brouwer 2003; Meerts et al. 2001). Exposure of female rats to BDE-99 lead to the formation of abundant vesicles and vacuolization of the ovary (Talsness et al. 2005), which showed signs of compromised steroidogenesis. Prenatal female exposure to PBDE-99 in rats affected estrogen target genes in the uterus (Ceccatelli et al. 2006). Thus, the delicate balance between androgens and estrogens in the fetus may become altered by PBDE exposure. In mice, the metabolism of BDE-47 was highly dependent on the developmental stage of the animal, being slowest in pups (Staskal et al. 2006). Whether this also plays a role for human fetal development is currently unknown. Most exposure doses used in animal studies are several orders of magnitude higher than the levels of PBDEs found in breast milk in our study. However, there is emerging evidence that also low-dose exposure to e.g. BDE-99 close to levels found in human adipose tissue may have an adverse effect on the reproductive health of the offspring (Anonymous 2005; Kuriyama et al. 2005).

The distribution pattern of BDE congeners in breast milk corresponded to the distribution of BDE congeners in commercially available mixtures of PBDEs (Alaee et al. 2006; Darnerud et al. 2001; Law et al. 2006; Lilienthal et al. 2006). The absolute concentrations found in our study are within the same order of magnitude as reported from other Nordic and European countries (Jaraczewska et al. 2006; Kalantzi et al. 2004; Lind et al. 2003; Strandman et al. 2000) as well as China (Bi et al 2006) and Japan (Eslami et al 2006; Inoue et al 2006). There are, however, significant geographical differences between and within these countries, which point towards differences in general contamination levels. In our study, the total amount of PBDEs did not differ between Finnish and Danish milk or placenta samples, but the pattern of congener distribution varied, which indicated different sources and timing of exposure. PBDE levels reported from American studies of breast milk appear to be considerably higher (Betts 2002). In contrast, the previous exponential increase in penta-PBDEs in Swedish breast milk samples since the 1970's has reversed since the late 1990's (Law et al. 2006; Meironyte et al. 1999; Sjödin et al 2003), when penta-BDEs were gradually phased out. The collection of breast milk samples in our study covered a 5-year period, and we found a negative correlation between the level of PBDEs in breast milk and the infant date of birth. This is in line with the expected decline in the use of penta-PBDEs in the two regions. The high variability in PBDE concentrations between individual mothers has been described also for other human matrices (McDonald 2002), and may reflect variability in both exposure and metabolism. Deca-BDE can through sunlight exposure be converted into lower brominated BDEs, which are more readily absorbed from the intestine and bioaccumulate due to their longer half-life than deca-BDE (Watanabe and Tatsukawa 1987). It is, however, as yet unknown how much this process contributes to human environmental exposure to lower brominated PBDEs.

Exposure to PBDEs cannot explain the observed geographical difference in the prevalence of cryptorchidism between Denmark and Finland. Breast milk contains significant levels of other persistent and non-persistent chemical compounds (Damgaard et al. 2006; Main et al. 2006b), which can affect perinatal testicular development. Mothers with high levels of PBDE exposure may be exposed to high levels of other persistent chemicals. Thus, the combined exposure to multiple environmental factors may cause the association between congenital cryptorchidism and PBDE concentration in breast milk (Koppe et al. 2006). In addition,

other lifestyle factors and genetic susceptibility may play a role (Damgaard et al. 2007; Sharpe 2006; Virtanen et al. 2006). In our total study population, the geographical difference in the prevalence of cryptorchidism was mainly observed for mild forms of undescended testes, which had a high degree of spontaneous postnatal descent (Boisen et al. 2004). This pattern was also seen in this subpopulation, in which PBDEs was analyzed. However, also mild and transient forms of cryptorchidism are associated with a subtle impairment of primary testicular function (Suomi et al.2006).

In conclusion, two different biological matrices were used in this study for the assessment of infant perinatal exposure. Breast milk, but not placenta, showed an association with congenital cryptorchidism. There are valid arguments for either matrix, and risk assessment will require more scrutiny. The association between PBDE contamination levels in breast milk and congenital cryptorchidism is still of concern, as exposure to PBDEs is considerable in some areas.

References

Agency for Toxic Substances and Disease Registry. 2004. Toxicological profile for polybrominated biphenyls and polybrominated diphenyl ethers (PBBs and PBDEs). Available: www.atsdr.cdc.gov/toxprofiles/tp68.html [accessed 30 March 2007]

Alaee M, Arias P, Sjödin A, Bergman A. 2006. An overview of commercially used brominated flame-retardants, their application, their use in different countries/regions and possible modes of release. Environ Int 29:683-689

Andersson A-M, Toppari J, Haavisto A-M, Petersen JH, Simell T, Simell O, et al. 1998. Longitudinal reproductive hormone profiles in infants: peak of inhibin B levels in infant boys exceeds levels in adult men. J Clin Endocrinol Metab 83:675-681.

[Anonymous]. 1986. Cryptorchidism: an apparent substantial increase since 1960. John Radcliffe Hospital Cryptorchidism Study Group. Br Med J 293:1401-1404.

[Anonymous]. 2005. Polybrominated diphenyl ether levels among United States residents: daily intake and risk of harm to the developing brain and reproductive organs. Integrated Environmental Assessment and Management 1:343-354.

Betts K. 2002. Rapidly rising PBDE levels in North America. Environ Sci Technol 36:50A-52A.

Becher G, Nicolaysen T, Thomsen C. 2001. Interlaboratory comparison on dioxins in food 2001. Final Report 4. Oslo, Norway:Folkehelsa.

Bi X, Qy W, Sheng G, Zhang W, Mau B, Chen D, et al. 2006. Polybrominated diphenyl ethers in South China, maternal and fetal blood and breast milk. Environ Pollut 144:1024-1030.

Boisen KA, Kaleva M, Main KM, Virtanen HE, Haavisto A-M, Schmidt IM et al. 2004. Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. Lancet 363:1264-1269.

Chao HR, Wang SL, Lee WJ, Wang YF, Papke O. 2007. Levels of polybrominated diphenyl ethers (PBDEs) in breast milk from central Taiwan and their relation to infant birth outcome and maternal menstruation effects. Environ Int 33:239-245.

Ceccatelli R, Faass O, Schlumpf M, Lichtensteiger W. 2006. Gene expression and estrogen sensitivity in rat uterus after developmental exposure to the polybrominated diphenyl ether PBDE-99 and PCB. Toxicol 220:104-116.

Damgaard IN, Skakkebaek NE, Toppari J, Virtanen HE, Shen H, Schramm KW, et al. 2006. Persistent pesticides in human breast milk and cryptorchidism. Environ Health Perspect 114:1133-1138.

Damgaard IN, Jensen TK, Petersen JH, Skakkebæk NE, Toppari T, Main KM. 2007. Cryptorchidism and maternal alcohol consumption during pregnancy. Environ Health Perspect 115:272-277.

Darnerud PO, Eriksen GS, Johannesson T, Larsen PB, Viluksela M. 2001. Polybrominated diphenyl ethers: occurrence, dietary exposure and toxicology. Environ Health Perspect 109 (Suppl 1):49-68.

Darnerud PO, Risberg S. 2006. Tissue localization of tetra- and pentabromodiphenyl ether congeners (BDE-47, 85 and -99) in perinatal and adult C57BL mice. Chemosphere 62:485-493.

Eslami B, Koizumi A, Ohta S, Inoue K, Aozasa O, Harad K, et al. 2006. Large-scale evaluation of the current level of polybrominated diphenyl ethers (PBDEs) in breast milk from 13 regions of Japan. Chemosphere 63:554-561.

Hardell L, van Bavel B, Lindstrom G, Eriksson M, Carlberg M. 2006. In utero exposure to persistent organic pollutants in relation to testicular cancer risk. Int J Androl 29:228-234.

Inoue K, Harada K, Takenaka K, Uehara S, Kono M, Shimizu T, et al. 2006. Levels and concentration of polychlorinated biphenyls and polybrominated diphenyl ethers in serum and breast milk in Japanese mothers. Environ Health Perspect 114:1179-1185.

Jaraczewska K, Lulek J, Cobaci A, Voorspoels S, Kaluba-Skotarczak A, Drews K, et al. 2006. Distribution of polychlorinated biphenyls, organochlorine pesticides and polybrominated diphenyl ethers in human umbilical cord serum, maternal serum and milk from Wielkopolska region, Poland. Sci Total Environ 372:20-31.

Jensen AA, Slorach SA. 1991. Chemical contaminants in human milk. Boston, MA:CRC Press.

Kalantzi OL, Martin FL, Thomas GO, Alcock RE, Tang HR, Drury SC, et al. 2004. Different levels of polybrominated diphenyl ethers (PBDEs) and chlorinated compounds in breast milk from two U.K. regions. Environ Health Perspect 112:1085-1091.

Kiviranta H, Ovaskainen ML, Vartiainen T. 2004. Market basket study on dietary intake of OCDD/Fs, PCBs and PBDEs in Finland. Environ Int 30:923-932.

Koppe JG, Bartonova A, Bolte G, Bistrup ML, Busby C, Butter M, et al. 2006. Exposure to multiple environmental agents and their effect. Acta Paediatr (Suppl) 95:106-113.

Kuriyama SN, Talsness CE, Grote K, Chahoud I. 2005. Developmental exposure to low dose PBDE-99: effects on male fertility and neurobehaviour in rat offspring. Environ Health Perspect 113:149-154.

Law RJ, Allchin CR, de Boer J, Covaci A, Herzke D, Lepom P, et al. 2006. Levels and trends of brominated flame-retardants in the European environment. Chemosphere 64:187-208.

Legler J, Brouwer A. 2003. Are brominated flame-retardants endocrine disrupters? Environ Int 29:879-885.

Lilienthal H, Hack A, Roth-Härer A, Wichert Grande S, Talsness CE. 2006. Effects of developmental exposure to 2,2'4,4',5-pentabromodiphenyl ether (PBDE-99) on sex steroids, sexual development, and sexually dimorphic behaviour in rats. Environ Health Perspect 114:194-201.

Lind Y, Darnerud PO, Atuma S, Aune M, Becker W, Bjerselius R, et al. 2003. Polybrominated diphenyl esters in breast milk from Uppsala County, Sweden. Environ Res 93:186-194.

Main KM, Schmith IM, Skakkebæk NE. 2000. A Possible Role for Reproductive Hormones in Newborn Boys: Progressive Hypogonadism without the postnatal Testosterone Peak. J Clin Endocrinol Metab 85:4905-4907.

Main KM, Toppari J, Suomi AM, Kaleva M, Chellakooty M, Schmidt IM, et al. 2006a. Larger testes and higher inhibin B levels in Finnish than in Danish newborn boys. J Clin Endocrinol Metab 91:2732-2737.

Main KM, Mortensen GK, Kaleva M, Boisen K, Damgaard I, Chellakooty M, et al. 2006b. Human Breast Milk Contamination with Phthalates and Alterations of Endogenous Reproductive Hormones in Three Months Old Infants. Environ Health Perspect 114:270-276.

Marsál K, Persson PH, Larsen T, Lilja H, Selbing A, Sultan B. 1996. Intrauterine growth curves based on ultrasonically estimated fetal weights. Acta Paediatr 85:843-848.

Mazdai A, Dodder NG, Anernathy MP, Hites RA, Bigsby RM. 2003. Polybrominated diphenyl ethers in maternal and fetal blood samples. Environ Health Perspect 111:1249-1252.

McDonald TA. 2002. A perspective on the potential health risks of PBDEs. Chemosphere 46:745-755.

Meerts IA, Letcher RJ, Hoving S, Marsh G, Bergman A, Lemmen JG, et al. 2001. In vitro estrogenicity of polybrominated diphenyl ethers, hydroxylated PBDEs and polybrominated bisphenol A compounds. Environ Health Perspect 109:399-407.

Meironyte D, Noren K, Bergman A. 1999. Analysis of polybrominated diphenyl ethers in Swedish human milk. A time-related trend study, 1972-1997. J Toxicol Environ Health A, 58:329-341.

Ortiz-Olaya N, Flores ME, De Santiago S. 1996 Significance of lipid consumption during lactation. Rev Invest Clin 48:473-478.

Pihkala J, Hakala T, Voutilainen P, Raivio K. 1989. Characteristic of recent fetal growth curves in Finland. Duodecim 105:1540-1546.

Sharpe RM. 2006. Pathways of endocrine disruption during male sexual differentiation and masculinization. Best Pract Res Clin Endocrinol Metab 20:91-110.

Shen H, Main KM, Kaleva M, Virtanen H, Haavisto AM, Skakkebæk NE. et al 2005. Prenatal organochlorine pesticides in placentas from Finland: exposure of male infants born during 1997-2001. Placenta 26: 512-514.

Shen H, Virtanen HE, Main KM, Kaleva M, Andersson AM, Skakkebæk NE. et al. 2006. Enantiomeric rations as an indicator of exposure processes for persistent pollutants in human placentas. Chemosphere 62:390-395.

Shen H, Main KM, Virtanen HE, Damgaard IN, Haavisto AM, Kaleva M. et al. 2007. From mother to child: Investigation of prenatal and postnatal exposure to persistent bioaccumulating toxicants using breast milk and placenta biomonitoring. Chemosphere 67:S256-S262.

Sjödin A, Patterson DGJr, Bergman A. 2003. A review on human exposure to brominated flame-retardants - particularly polybrominated diphenyl ethers. Environ Int 29:829-839.

Skakkebæk NE, Rajpert-De Meyts E, Main KM. 2001.Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod 16:972-978.

Småstuen Haug L, Becher G. 2004. Interlaboratory comparison on dioxins in food 2004. Fifth round of an international study. 2004:6. Oslo, Norway:Norwegian Institute of Public Health.

Småstuen Haug L, Becher G. 2005. Interlaboratory comparison on dioxins in food 2005. Sixth round of an international study. 2005:7. Oslo, Norway:Norwegian Institute of Public Health.

Strandman T, Koistinen J, Variainen T. 2000. Levels of some polybrominated diphenyl ethers (PBDEs) in placenta and human milk. Organohalogen Compounds 47:61-64.

Staskal DF, Diliberto JJ, Birnbaum LS. 2006. Disposition of BDE 47 in developing mice. Toxicol Sci 90:309-316.

Stoker TE, Laws SC, Crofton KM, Hedge JM, Ferrell JM, Cooper RL. 2004. Assessment of DE-71, a commercial polybrominated diphenyl ether (PBDE) mixture in the EDSP male and female pubertal protocols. Toxicol Sci 78:144-155.

Stoker TE, Cooper RL, Lambright VS, Wilson F, Gray LE. 2005. In vivo and in vitro anti-androgenic effects of DE-71, a commercial polybrominated diphenyl ether (PBDE) mixture. Toxicol Appl Pharmacol 207:78-88.

Suomi A-M, Main KM, Kaleva M, Schmidt IM, Chellakooty M, Virtanen HE, et al. 2006. Hormonal changes in 3-month-old cryptorchid boys. J Clin Endocrinol Metab 91:953-958.

Talsness CE, Shakibaei N, Kuriyama SN, Grande SW, Sterner-Kock A, Schnitker P, et al. 2005. Ultra structural changes observed in rat ovaries following in utero and lactational exposure to low doses of a polybrominated flame retardant. Toxicol Lett 157:189-202.

Toppari J. 2003. Physiology and disorders of testicular descent. Endocr Dev 5:104-109.

Virtanen HE, Tapanainen AE, Kaleva MM, Suomi AM, Main KM, Skakkebæk NE, et al. 2006. Mild gestational diabetes as a risk factor for congenital cryptorchidism. J Clin Endocrinol Metab 91:4862-4865.

Waliszewski SM, Aquirre AA, Infanzon RM, Silva CS, Siliceo J. 2001. Organochlorine pesticide levels in maternal adipose tissue, maternal blood serum, umbilical blood serum, and milk from inhabitants of Veracruz, Mexico. Arch Environ Contam Toxicol 40:432-438.

Watanabe I, Tatsukawa R. 1987. Formation of brominated dibenzofurans from the photolysis of flame-retardantdeca-bromodiphenyl ether in hexane solution by UV and sunlight. Bull Environ Contam Toxicol 39:953-959.

Table 1: Population characteristics (medians, 2.5^{th} – 97.5^{th} percentiles) for breast milk samples

	Denmark			Finland		
	Breast Milk samples					
	Controls	Cryptorchidism	P*	Controls	Cryptorchidism	P*
N	36	29		32	33	
Maternal age (years)	29.8	29.8	0.979	27.7	29.7	0.306
	(23.0-42.6)	(25.8-39.2)		(19.9-38.5)	(21.5-39.9)	
BMI (kg/m^2)[a]	22.9	23.0	0.997	22.8	22.0	0.106
	(19.1-31.6)	(17.9-30.5)		(18.4-35.6)	(18.2-27.9)	
Diabetes Yes/No	1/35	1/28	0.877	0/32	4/29	0.044
Smoking Yes/No	9/27	7/22	0.937	4/28	6/27	0.529
Parity 1	28	20	0.451	19	17	0.405
2	4	5		9	9	
≥3	4	4		4	7	
Gestational age (days)	284	278	0.039	280	281	0.768
	(253-297)	(219-296)		(160-296)	(249-296)	
WGA[b] %	1.89	0.62	0.687	-1.59	1.17	0.451
	(-23.5/35.6)	(-29.7/30.5)		(-21.9/20.0)	(-30.8/27.3)	
Birth weight (kg)	3.73	3.52	0.139	3.46	3.83	0.397
	(2.65-5.15)	(1.31-4.85)		(2.86-4.46)	(2.51-4.66)	
Birth Length (cm)	53	52	0.264	51	52	0.826
	(48-57)	(35-59)		(47-55)	(46-55)	

Cryptorchidism at 3 months (n)	-	4	-	-	25	-
Prematurity (n)[c]	1	4	-	2	1	-
SGA (n)[d]	1	2	-	0	1	-
Infant blood samples (n)	21	24		25	26	

[a]BMI: maternal pre-pregnancy BMI, [b]WGA=weight for gestational age, [c] prematurity: < 259 days of gestation,

[d]SGA (small for gestational age), *p: difference between cryptorchid boys and controls

Table 2: Population characteristics (medians, 2.5th – 97.5th percentiles) for placentas

	Denmark			Finland		
	Placentas					
	Controls	Cryptorchidism	P*	Controls	Cryptorchidism	P*
N	129	39		56	56	
Maternal age (years)	31.0	29.5	0.723	28.1	29.1	0.349
	(22.7-38.5)	(25.7-45.7)		(20.6-38.1)	(20.1-42.2)	
BMI (kg/m^2)[a]	22.1	21.5	0.199	22.3	23.1	0.062
	(18.2-35.5)	(17.8-36.1)		(17.8-31.7)	(17.8-37.5)	
Diabetes Yes/No	2/127	0/39	0.435	0/55	10/46	0.001
Smoking Yes/No	32/91	11/28	0.788	9/47	4/48	0.793
Parity 1	81	26	0.628	31	31	0.997
2	36	10		19	19	
≥3	12	3		6	6	
Gestational age (days)	283	276	0.001	280	280	0.940
	(254-298)	(195-294)		(255-293)	(258-303)	
WGA[b] %	0.29	-0.83	0.921	-0.32	-1.12	0.895
	(-23.3/34.9)	(-39.5/44.4)		(-21.7/25.7)	(-27.9/25.0)	
Birth weight (kg)	3.63	3.45	0.038	3.54	3.63	0.807
	(2.49-5.06)	(0.75-4.75)		(2.85-4.61)	(2.55-4.58)	
Birth Length (cm)	53	52	0.230	51	51	0.955
	(47-57)	(32-60)		(47-55)	(48-55)	

Cryptorchidism at 3 months (n)	-	8	-	-	33	-
Prematurity (n)[c]	6	6	-	3	1	-
SGA (n)[d]	5	2	-	0	3	-
Infant blood samples (n)	88	25		45	35	

[a]BMI: maternal pre-pregnancy BMI, [b]WGA=weight for gestational age, [c] prematurity: < 259 days of gestation,

[d]SGA (small for gestational age), *p: difference between cryptorchid boys and controls

Table 3: Seven polybrominated diphenyl ethers (PBDEs), detectable in all breast milk samples from Danish and Finnish boys with and without cryptorchidism

PBDE	Denmark			Finland			Both countries		
ng/g fat									
	Controls	Cryptorchid	P*	Controls	Cryptorchid	P*	Controls	Cryptorchid	P*
N	36	29		32	33		68	62	
Sum of 7	3.21	4.12	0.017	3.08	4.27	0.111	3.16	4.16	0.007
congeners	(1.09-9.07)	(1.34-18.78)		(1.04-29.17)	(1.43-56.30)		(1.08-21.47)	(1.39-51.62)	
47	1.05	1.53	0.018	1.24	1.82	0.062	1.12	1.56	0.003
	(0.45-3.63)	(0.34-11.7)		(0.4-15.20)	(0.65-38.90)		(0.42-12.87)	(0.45-33.13)	
153	1.0	1.18	0.226	0.67	0.68	0.394	0.81	0.94	0.156
	(0.31-3.35)	(0.64-3.14)		(0.22-2.97)	(0.27-3.63)		(0.28-3.08)	(0.33-3.35)	
99	0.44	0.64	0.132	0.39	0.48	0.366	0.42	0.53	0.091
	(0.07-1.58)	(0.07-2.31)		(0.13-5.94)	(0.09-13.10)		(0.10-3.19)	(0.09-10.48)	
100	0.26	0.37	0.019	0.30	0.37	0.128	0.27	0.37	0.008
	(0.10-0.81)	(0.10-1.98)		(0.12-1.42)	(0.11-5.18)		(0.10-1.37)	(0.11-4.65)	
28	0.10	0.13	0.030	0.12	0.17	0.099	0.10	0.15	0.005

	(0.03-0.29)	(0.04-0.74)		(0.03-2.44)	(0.05-0.68)		(0.03-2.19)	(0.05-0.71)	
66	0.04	0.08	0.0001	0.03	0.032	0.451	0.03	0.05	0.002
	(0.01-0.25)	(0.01-0.26)		(0.01-1.38)	(0.01-0.19)		(0.01-0.57)	(0.01-0.25)	
154	0.04	0.09[a]	0.0001	0.04	0.04	0.536	0.04	0.05	0.001
	(0.01-0.13)	(0.00-0.18)		(0.02-0.18)	(0.02-0.54)		(0.01-0.17)	(0.01-0.50)	
Sum of all 14	83.4 (29.2-	119.3 (34.6-	0.009	163.2 (17.7-	119.2 (64.3-	0.581	104.2 (26.1-	119.3 (36.4-	0.046
ng/l	396.9)	757)		1308.5)	2657.4)		1188.4)	1785.1)	

Concentrations are given as median (2.5^{th}-97^{th} percentiles), *p-values are adjusted for maternal age, BMI, parity, and date of childbirth, for the combined data also for country of origin, [a] one unmeasurable sample

Table 4: Seven less prevalent polybrominated diphenyl ethers (PBDEs) in breast milk samples from Danish and Finnish boys with and without cryptorchidism

PBDE	Denmark				Finland			
ng/g fat								
	Controls		Cryptorchid		Controls		Cryptorchid	
N	36		29		32		33	
		%		%		%		%
Sum of 14 congeners	3.27 (1.11-9.12)	-	4.27 (1.34-19.10)	-	3.11 (1.04-29.5)	-	4.27 (1.43-56.44)	-
183	0.05 (0.0-0.58)	78	0.05 (0.0-0.23)	62	0.0 (0.0-0.17)	44	0.0 (0.0-0.49)	27
85	0.005 (0.0-0.05)	67	0.07 (0.0-0.22)	97	0.0 (0.0-0.12)	6	0.0 (0.0-0.08)	36
75	0.001 (0.0-0.01)	61	0.0 (0.0-0.01)	28	0.0 (0.0-0.06)	34	0.0 (0.0-0.03)	36
77	0.001 (0.0-0.03)	53	0.01 (0.0-0.04)	65	0.0 (0.0-0.12)	19	0.0 (0.0-0.02)	15
119	0.0 (0.0-0.01)	25	0.0 (0.0-0.01)	48	0.0 (0.0-0.02)	12	0.0 (0.0-0.01)	15
138	0.0 (0.0-0.02)	28	0.0 (0.0-0.03)	28	0.0 (0.0-0.01)	3	-	0

Concentrations are given as median (2.5^{th}-97.5^{th} percentiles), %: percentage of detectable samples, BDE-71 was not detected in any sample

Table 5: Polybrominated diphenyl ethers (PBDEs) in placentas from Danish and Finnish boys with and without cryptorchidism. Congeners BDE-153, 99, 100 and 28 were detected in all samples.

PBDE	Denmark				Finland			
ng/g fat								
	Controls n=129		Cryptorchid n=39		Controls n=56		Cryptorchid n=56	
		%		%		%		%
47	0.39	100	0.40	100	0.60	100	0.52	100
	(0.19-1.64)		(0.20-2.17)		(0.12-6.19)		(0.12-4.07)	
153	0.44	100	0.41	100	0.20	100	0.20	100
	(0.20-1.21)		(0.21-0.98)		(0.08-0.92)		(0.10-0.53)	
99	0.23	100	0.18	100	0.19	100	0.14	100
	(0.10-0.99)		(0.13-0.68)		(0.04-1.57)		(0.04-1.20)	
100	0.11	100	0.10	100	0.11	100	0.10	100
	(0.05-0.40)		(0.06-0.56)		(0.03-0.79)		(0.04-0.68)	
28	0.03	100	0.03	100	0.04	100	0.04	100
	(0.01-0.06)		(0.01-0.40)		(0.01-0.52)		(0.01-0.12)	
Sum of 5 congeners	1.28	100	1.13	100	1.16	100	1.05	100
	(0.59-3.26)		(0.79-4.45)		(0.35-9.65)		(0.35-6.51)	
66	0.0 (0.0-0.03)	29	0.0 (0.0-0.04)	38	0.01 (0.0-0.20)	64	0.01 (0.0-0.04)	62
154	0.0 (0.0-0.03)	39	0.0 (0.0-0.02)	5	0.01 (0.0-0.10)	79	0.0 (0.0-0.09)	34
183	0.0 (0.0-0.14)	26	0.0 (0.0-0.09)	10	-	0	0.0 (0.0-0.07)	5
85	0.0 (0.0-0.01)	2	-	0	0.0 (0.0-0.08)	25	0.0 (0.0-0.04)	34
75	(0.003)	1	-	0	-	0	-	0
77	-	0	-	0	0.0 (0.0-0.02)	4	-	0
119	0.0 (0.0-0.002)	3	-	0	-	0	(0.01)	2

138	-	0	-	0	0.0 (0.0-0.02)	2	-	0
Sum of 14 congeners	1.31		1.13		1.18		1.06	
	(0.61-3.31)		(0.79-4.48)		(0.35-9.89)		(0.36-6.67)	

Concentrations are given as medians (2.5^{th} -97.5^{th} percentiles), %: percentage of detectable samples, congener BDE-71 was not detected in any sample.

Table 6: Correlations between PBDE measurements in paired placenta and breast milk samples of 43 Danish and 43 Finnish boys. Due to non-detectable levels, correlations could not be computed for BDE-71 and BDE-119. Median concentrations (ng/g fat) are given for milk and placenta (95% interval in parenthesis).

Congener	Median Placenta	Median Milk	r	p
28	0.03 (0.02-0.39)	0.12 (0.03-1.85)	0.73	0.0001
47	0.42 (0.19-2.84)	1.27 (0.45-14.7)	0.64	0.0001
66	0.0 (0.0-0.06)	0.04 (0.01-0.27)	0.31	0.004
71	0.0 (0.0-0.0)	0.0 (0.0-0.0)	-	-
75	0.0 (0.0-0.0)	0.0 (0.0-0.02)	0.21	0.057
77	0.0 (0.0-0.004)	0.0 (0.0-0.03)	0.30	0.006
85	0.0 (0.0-0.06)	0.0 (0.0-0.12)	-0.02	0.823
99	0.19 (0.06-1.30)	0.42 (0.09-5.27)	0.55	0.0001
100	0.11 (0.05-0.43)	0.29 (0.10-1.41)	0.74	0.0001
119	0.0 (0.0-0.0)	0.0 (0.0-0.01)	-	-
138	0.0 (0.0-0.0)	0.0 (0.0-0.02)	-0.05	0.664
153	0.32 (0.11-0.93)	0.85 (0.27-3.11)	0.81	0.0001
154	0.0 (0.0-0.09)	0.04 (0.01-0.18)	0.26	0.015
183	0.0 (0.0-0.18)	0.02 (0.0-0.38)	0.26	0.015
Sum	1.19 (0.59-6.11)	3.23 (1.16-27.6)	0.66	0.0001

Figure Legends

Figure 1: Sum of the 7 most prevalent PBDEs in breast milk samples (BDE 47, 153, 99, 100, 28, 66, 154, log transformed values) from Denmark and Finland in boys with cryptorchidism (n=62, shaded) and healthy boys (n=68, open). The box plot shows medians and interquartiles.

Figure 2: Concentration (percentiles) of the sum of all PBDEs, BDE 47 and 100 (ng/g fat) in human breast milk samples from Denmark (red, n=65) and Finland (blue, n=65) 1997-2001 (solid line: boys with cryptorchidism, dotted line: healthy boys). Note the differences in absolute values in the Y-axis.

Figure 1

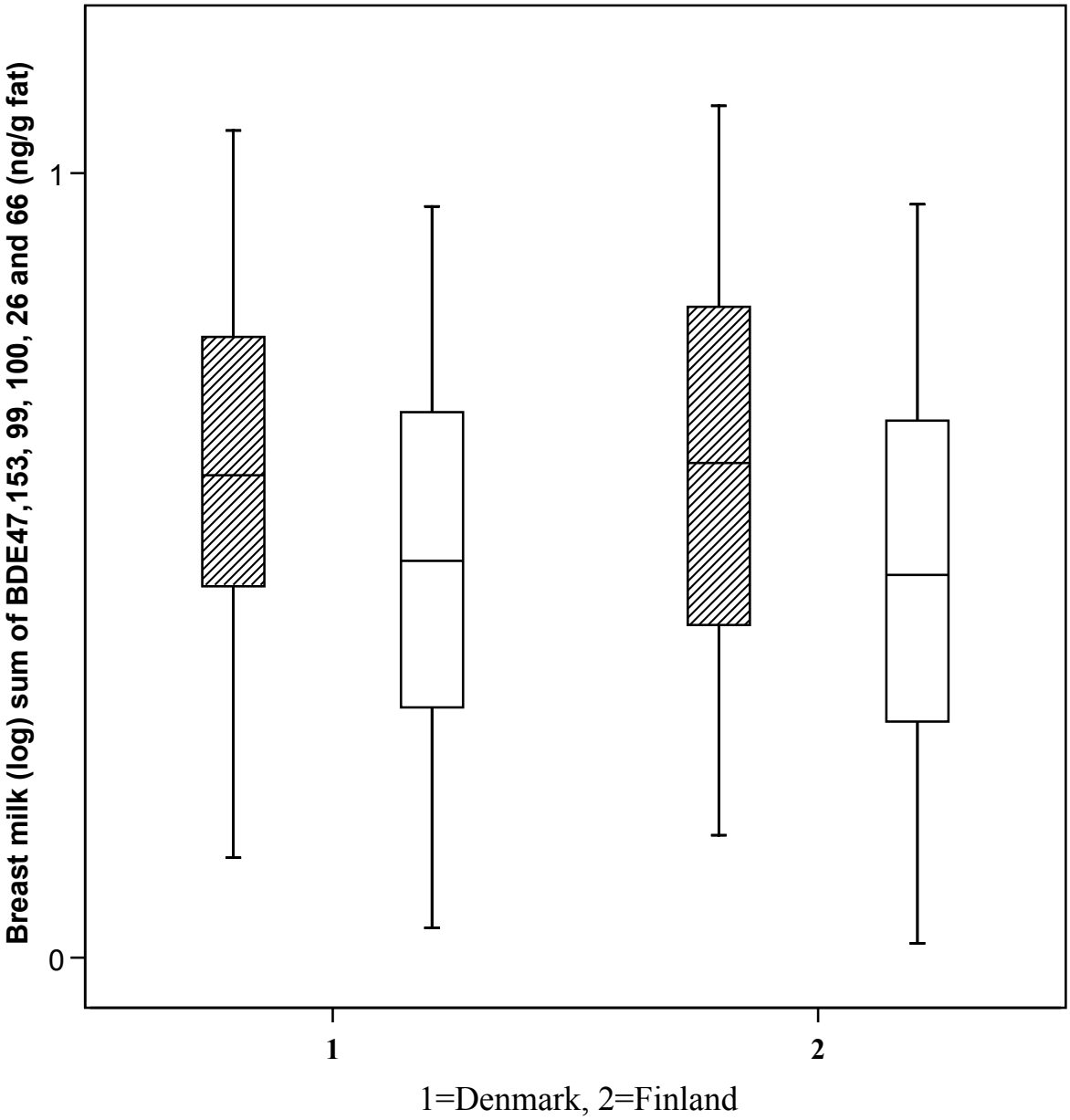
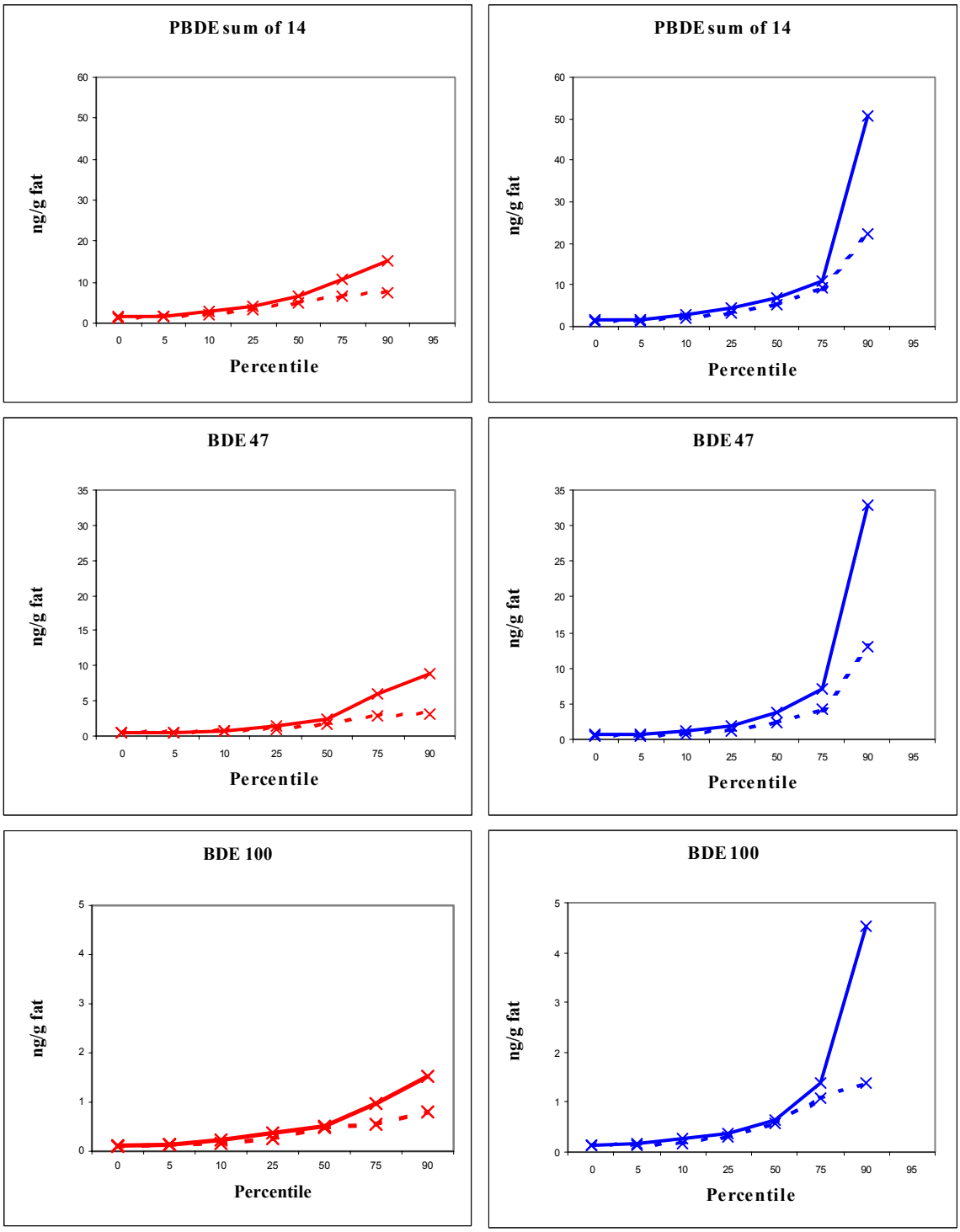

Breast milk (log) sum of BDE47,153, 99, 100, 26 and 66 (ng/g fat)
1
0
1
2
1=Denmark, 2=Finland

Figure 2


PBDE sum of 14
ng/g fat
Percentile
PBDE sum of 14
ng/g fat
Percentile
BDE 47
ng/g fat
Percentile
BDE 47
ng/g fat
Percentile
BDE 100
ng/g fat
Percentile
BDE 100
ng/g fat
Percentile