Concentrations of persistent organochlorine compounds in human milk and placenta are higher in Denmark than in Finland

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BACKGROUND: A significantly reduced male reproductive health status, including a higher prevalence of cryptorchidism and hypospadias, has been documented in Danish men compared with Finnish men. Exposure to environmental pollutants with endocrine disrupting activities has been suggested as a possible contributing factor. In this study, we investigated whether there was a difference in milk and placental concentrations of persistent organohalogen compounds, between the two countries.

METHODS: Organohalogenes were analysed by high-resolution gas chromatography–high-resolution mass spectrometry in human milk samples from Finland (n = 65) and Denmark (n = 65) and in placentas from Finland (n = 112) and Denmark (n = 168). RESULTS: 1,1-Dichloro-2,2-bis(4-chlorophenyl)ethane (p,p'-DDE) was the dominant pollutant. β-Hexa-chloro-cyclohexane (β-HCH), hexachlorobenzene (HCB), endosulfan-I, dieldrin, oxychlordane (OXC), cis-heptachloroepoxide (c-HE) and 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (p,p'-DDT) were the other main organochlorines detected. Danish samples had significantly higher concentrations of p,p'-DDE, p,p'-DDT, β-HCH, HCB, dieldrin, c-HE and OXC than did the Finnish samples. Levels of organobrominated compounds were very low and most were undetectable in the majority of samples. BB-153 and BB-155 were the most abundant polybromobiphenyl congeners. BB-153 was more abundant in Danish milk samples compared with Finnish samples, whereas BB-155 was more abundant in the Finnish milk.

CONCLUSIONS: The organochlorine levels were higher in Danish, than in Finnish, samples, suggesting a higher exposure for Danish infants.

Keywords: placenta; breast milk; organochlorine pesticides; organobromine compounds; infants

Introduction

Danish men have a higher prevalence of testicular cancer and a reduced semen quality compared with Finnish men (Adami et al., 1994; Jørgensen et al., 2002). Additionally, there is a higher prevalence of cryptorchidism and hypospadias among Danish compared with Finnish newborn boys (Virtanen et al., 2001; Boisen et al., 2004, 2005). It has been speculated that these differences between Denmark and Finland may be due to differences in exposures to environmental endocrine disrupting chemicals, especially during sensitive periods of development.

Several persistent organohalogen compounds have been shown to have endocrine disrupting activities (Toppari et al., 1996). Persistent organohalogen compounds bioaccumulate along the food chain in lipid-rich tissues. Humans are exposed to these compounds almost exclusively through the diet. The compounds can be transferred to the fetus across the placenta during pregnancy (Jacobson et al., 1984; Foster et al., 2000; Waliszewski et al., 2000) and to the newborn baby by breastfeeding (Nair et al., 1996; Anderson and Wolff, 2000). In this study, we investigated the exposure of Danish and Finnish infants to the organochlorine compounds pentachlorbenzene (PeCB), hexachlorobenzene (HCB), hexachloro-cyclohexane (HCH) (α-, β-, γ-, δ- and ε-), di-chlorodi-phenyl-trichloro-ethane (DDT)-related compounds [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (p,p'-DDT), 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenylethane(o,p'-DDT),...
1,1-dichloro-2,2-bis(4-chlorophenyl)ethane (p,p′-DDD), 1,1-
dichloro-2-(2-chlorphenyl)-2,2-(4-chlorophenyl)ethane (o,p′-
DDD), 1,1-dichloro-2,2-bis(4-chlorophe nyl)ethane (p,p′-DEE)
and 1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane
(o,p′-DEE), octachlorostyrene (OCS), pentachloroanisole (PCA),
aldrin, dieldrin, cis-chlordane (c-CHL) and trans-CHL
(t-CHL), heptachlor, oxychlordane (OXC), cis-heptachloroepoxide
(c-HE), trans-HE (t-HE), methoxychlor (MOC), mirex, endosulfan
(END)-I and organobromine compounds [polybromobiphenyl
(PBB) congeners, pentabromobenzene (PeBB) and hexabro-
mobenzene (HeBB)], by analysing placenta and human milk
samples from a prospective birth cohort study. The levels of
these compounds in the Finnish placentas have been discussed
elsewhere (Shen et al., 2005). In addition to looking for possi-
ble geographical differences in the concentrations of these
contaminants, we also explored some maternal factors, which
have been suggested to affect the prenatal (placental levels)
and post-natal (breast milk levels) exposure of children.

Some of the investigated compounds are the so-called chiral
compounds existing in two enantiomeric forms. For chiral
compounds, the biological activity may be enantiomer selec-
tive, e.g. different thalidomide enantiomers contain, respecti-
vively, the sedative and the teratogenic activity (Von Blaschke
et al., 1979). Likewise, the interaction of o,p′-DDT (Hoekstra
et al., 2001) and MOC metabolites (Hu and Kupfer, 2002)
with estrogen receptors has been shown to be enantiomer
specific (Bocchinfuso and Korach, 1997). In addition, as
the enantiomers of chiral compounds may be differently prone
to biodegradation, the ratio between the enantiomers (enanti-
meric ratio, ER) may offer information on the exposure
history. Thus, comparing the ERs of persistent compounds
in human samples with the ERs in the environment may tell if
the exposure is recent or historical. Therefore, we also
measured the concentration of the enantiomers of some of the
chiral compounds and compared ERs between Danish and
Finnish samples.

Materials and Methods

Placentas and breast milk samples were derived from a joint prospec-
tive, longitudinal birth cohort study in an urban setting in Finland
(Turku University Hospital) and Denmark (the University Hospital
of Copenhagen) from 1997 to 2001. This study aimed to describe
regional prevalence rates and risk factors (lifestyle and exposure)
for cryptorchidism by means of questionnaires and biological
samples. Recruitment, participation rate and clinical examination
techniques in both countries were completely standardized and
described earlier (Boisen et al., 2004). Human milk samples were
collected from 1 to 3 months post-partum in Denmark and from 1 to 2
months in Finland, as successive aliquots. All mothers were given
oral and written instructions to feed the baby first, and then sample
milk aliquots (hind milk) by manual expression into a glass or porce-
lain container, avoiding the use of mechanical breast pumps. Success-
ive aliquots were frozen in glass bottles [250 ml Pyrex glass bottle
with a Teflon cap (1515/06D), Bibby Sterilin, Staffordshire,
England] and stored in the household freezers. The samples were
delivered frozen to the hospital at the 3 months’ examination and
stored at −20°C. The selected milk samples were thawed at room
temperature for 12 h, heated and shaken for 30 min at 37°C to homo-
genize the samples, then divided into smaller aliquots and refrozen at
−20°C until further chemical analysis. Placentas were collected at
birth by the midwives and frozen as whole placentas in polyethylene
bags at −20°C. Before analysis, placentas were defrosted, mecha-
nically homogenized and aliquoted into 20 ml glass tubes.

From the total biobank, 65 milk samples from each country were
included for organohalogen measurements. The number of samples
determined by funding, as exposure measurements were prospecti-
vely planned to include persistent and non-persistent chemicals (EU
grant QLK4-2001-00 269). Only breast milk samples with a volume
>125 ml were included to ensure that all prospectively planned chemi-
cal analyses could be performed. The samples represent 29 Danish
and 33 Finnish boys with cryptorchidism at birth and 36 and 32 healthy
control boys, respectively. In Denmark, all controls were selected ran-
donoly from the entire birth cohort of healthy boys. In Finland, the
boys were selected prospectively by a case–control design, in which
the boys with cryptorchidism were matched to controls at birth for
maternal parity, smoking (yes/no), diabetes (yes/no), gestational age
(±7 days) and date of birth (±14 days). This design was chosen in Finland
due to lack of sufficient funding to collect and store biological samples from all. Placentas were selected from 112
Finnish mother–child pairs (n = 56 boys with cryptorchidism at
birth, n = 56 controls) and from 168 Danish (n = 39 with cryptor-
chidism, n = 126 controls, n = 3 healthy boys first seen at 3 months
of age). Funding for placenta analysis allowed the inclusion of more samples
from Danish healthy controls. Eighty-six mother–child
pairs (43 Danish and 43 Finnish) were included with both milk and
placenta samples.

The sample preparation, extraction, clean-up and high-resolution
gas chromatography–high-resolution mass spectrometry (HRGC–
HRMS) analyses for organohalogen and chiral compounds of the
Danish and Finnish samples were carried out in the same laboratory.
The methods used have been described in detail previously (Shen
et al., 2005; Damgaard et al., 2006; Main et al., 2006).
Briefly, Na2SO4 (VWR international GmbH, Germany), sea sand
(Riedel-de Haen, Germany), alumina B (ICN Biomedical GmbH,
Germany), florisor and silica gel (Promochem, UK) were heated at
650°C for at least 6 h before use. Wet samples (10 g placenta tissue
or 10 ml milk) were weighed (to a precision of two decimals) and
homogenized with 30 g Na2SO4 and 15 g sea sand. Extraction was
done in a glass column packed with the homogenized matrix and
spiked with 13C internal standards. The extraction solvent was
250 ml acetone and n-hexane (2:1 v/v). The extracts were collected
in flasks weighed in advance and evaporated using a rotary vacuum
evaporator (water bath at 45°C). After evaporation, the flasks
were placed into a desiccator, until stable weight was achieved. Then
the lipid content was calculated on wet weight basis (to a precision
of four decimals). All the results were calculated on the lipid basis
because of the lipophilic properties of the investigated compounds
(Matheson et al., 1990; Needham and Wang, 2002). The lipophilic
residual was redissolved in toluene and the clean-up was done in gel
permeation chromatography (Bio-Beads S-8 column with toluene
eluent at 2 ml/min flow rate) and then in a glass cartridge (packed
with alumina B 0.8 g, Na2SO4 0.3 g, florisor 0.5 g, silica gel 1 g and
Na2SO4 0.5 g from bottom to top). Finally, the sample was condensed
to about 10 μL and 10 μL 1,2,3,4-tetrachlorodibenzo-p-dioxin
was added as a recovery standard.

The organohalogens were measured by HRGC–HRMS and quanti-
fied by an isotope dilution method. HP5890 high-resolution gas chromatography equipped with 60 m DB-XLB column (0.25 mm
internal diameter 0.25 μm film) was used to separate organochlorines.
MAT95 high-resolution mass spectrometer was used to detect organo-
chlorines. A 30 m DB-XLB column was used to analyse bromobi-
phenyl (BB) congeners including HeBB and PeBB. 13C labelled
polychlorobiphenyl (PCB) congeners were used as internal standards to calibrate PBBs and HeBB in Finnish placenta samples (BB-1, -3, -4 and -15 by PCB28; BB-18, -31 and -37 by PCB 153; BB-52 and -49 by PCB138; BB-80, -77, -103, -101, PeBB and HeBB by PCB180; BB-155, -153, -169 and -209 by PCB209). For the analysis of Danish placenta samples and the milk samples, $^{13}$C-BB-77 and $^{13}$C-BB-126 had become available and were used as internal standards (BB-103, -101, -126, -155, -169 and -209 were calculated based on $^{13}$C-BB-126; all the other PBB congeners including HeBB and PeBB were according to $^{13}$C-BB-77). Calculations were based on the relative signal responses of different congeners compared with internal standards, which were stable during the same-instrument operation conditions. A signal corresponding to 3 times noise was defined as the limit of detection for all data. All the other organohalo-gens were calculated by the isotope dilution method. All the results were calculated on the lipid base because of the lipophilic properties of the investigated compounds (Matheson et al., 1990; Needham and Wang, 2002).

The study was conducted according to the Helsinki II declaration after informed oral and written consent of the parents. It was approved by the ethics committees (Finland: 7/1996, Denmark: kF01-030/97) and the Danish Data Protection Agency (1997-1200-074).

Statistics
Lilliefors test was used to check the goodness of fit to a normal distribution of the set of data. Differences in maternal and infant population characteristics were tested by Mann–Whitney U-test. SPSS (13.0) was used for multiple regression analyses to examine determinants (including country of lipid (w/w %) and pesticide concentration (ng/g lipid) in placenta and milk. Analyses on chemical concentrations were performed on log-transformed data to achieve normal distribution and values < LOD were not included. These analyses were only carried out for the eight most prevalent organochlorines and the two most abundant PBB congeners. The following factors were included as covariates: country (Denmark = 1, Finland = 2), parity, maternal smoking (yes = 1, no = 0), gestational age (days), maternal prepregnancy body mass index (BMI, kg/m²), infant date of birth and maternal age at delivery (years). Infant date of birth was transformed into a continuous variable (days): 1 year was defined as 365 days and the earliest collected samples were assigned 0 for each cohort separately.

Results
The characteristics of the study subjects are shown in Table I. There were significant differences between Danish and Finnish mothers in age, parity and smoking habits, with Danish mothers being slightly older, more likely to have a first pregnancy, and more likely to be smoking than Finnish mothers. The BMI of the mothers, the duration of the pregnancy and the birthweight of the child did not differ between the Danish and Finnish subjects.

Lipid concentration
The total lipid concentration in placenta and milk data showed near normal distribution with normal-fitting probabilities of 0.77, 0.53, 0.73 and 0.81 for Danish milk, Finnish milk, Danish placenta and Finnish placenta, respectively. The lipid contents were significantly higher in the Finnish samples than in the Danish for both placenta and breast milk ($P < 0.0001$). This country difference in lipid content remained significant after adjustment for parity, maternal age, maternal BMI, length of pregnancy and maternal smoking (Table II).

In addition, milk lipid content was significantly ($P < 0.0001$) correlated to maternal BMI ($r = -0.17$), but independent of parity, maternal smoking and length of pregnancy (gestational age). Placental lipid content was significantly ($P < 0.0001$) associated with length of pregnancy ($r = -0.32$), but independent of parity, maternal BMI and maternal smoking.

Organochlorine compounds
Heptachlor, aldrin, e-HCH, END-II and t-HE were undetectable in most samples. The concentrations of the other organochlorines measured in Danish and Finnish human milk and placenta samples are shown in Table II. Most of these compounds were detectable in all samples from both countries. There was no difference between the two countries with respect to the percentage of samples with concentrations above the detection limit for the individual chemicals. $p,p'$-DDE, HCB, $\beta$-HCH, dieldrin, $p,p'$-DDT, END-I, OXC and c-HE were the eight major pollutants detected (together they accounted for 89%, 95%, 98% and 98% of the total pesticide concentration in Finnish placenta, Danish placenta, Finnish milk and Danish milk, respectively). The unadjusted concentrations of these eight chemicals were higher in placenta and milk from Denmark than in the samples from Finland. For $p,p'$-DDE, $p,p'$-DDT, $\beta$-HCH, HCB, dieldrin, c-HE and OXC, the levels remained significantly higher in Danish milk samples after adjustment for possible confounders (maternal age, parity, gestational length and date of birth) (Tables II and III) and for $p,p'$-DDE, $p,p'$-DDT, $\beta$-HCH, HCB, dieldrin and c-HE, the levels remained significantly higher in Danish placenta samples after adjustment (Tables II and IV).

In a multiple linear regression analysis, lipid concentration, maternal smoking and maternal BMI did not show any significant correlation to the organochlorine concentrations in milk, whereas maternal age was positively associated and length of pregnancy, infant date of birth and parity were negatively associated with the concentrations of these eight compounds in milk (Table III). Placenta levels of these eight compounds were independent of maternal smoking, but positively associated with maternal age and negatively associated with infant.
Table II. Organochlorine compound concentrations in placenta and breast milk samples from Denmark and Finland (ng/g lipid)\textsuperscript{a}.

<table>
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<tr>
<th></th>
<th>Danish placenta</th>
<th>Finnish placenta\textsuperscript{b}</th>
<th>Danish milk</th>
<th>Finnish milk</th>
<th>P-value\textsuperscript{d}</th>
<th>Median (range)</th>
<th>LOD</th>
<th>N\textsuperscript{c}</th>
<th>Median (range)</th>
<th>LOD</th>
<th>N\textsuperscript{c}</th>
<th>Median (range)</th>
<th>LOD</th>
<th>N\textsuperscript{c}</th>
<th>Median (range)</th>
<th>LOD</th>
<th>N\textsuperscript{c}</th>
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<tbody>
<tr>
<td>Lipid (%)</td>
<td>1.1 (0.55–1.5)</td>
<td>1.22 (0.93–1.52)</td>
<td>112</td>
<td>&lt;0.0001</td>
<td>2.84 (0.36–7.33)</td>
<td>65</td>
<td>4.26 (0.95–10.14)</td>
<td>65</td>
<td>&lt;0.0001</td>
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<td>PeCB</td>
<td>0.47 (0.05–3.45)</td>
<td>0.49 (0.17–171.71)</td>
<td>112</td>
<td>0.24</td>
<td>0.32 (0.13–1.41)</td>
<td>65</td>
<td>0.25 (0.08–1.17)</td>
<td>65</td>
<td>&lt;0.0001</td>
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<tr>
<td>α-HCH</td>
<td>0.52 (0.04–8.92)</td>
<td>0.55 (0.18–692.79)</td>
<td>112</td>
<td>0.01</td>
<td>0.26 (0.05–3.45)</td>
<td>65</td>
<td>0.16 (0.04–0.77)</td>
<td>65</td>
<td>&lt;0.0001</td>
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<td>β-HCH</td>
<td>8.73 (2.87–47.76)</td>
<td>4.55 (1.48–45.54)</td>
<td>112</td>
<td>&lt;0.0001</td>
<td>16.85 (5.97–66.23)</td>
<td>65</td>
<td>10.93 (2.74–30.89)</td>
<td>65</td>
<td>&lt;0.00001</td>
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<td>γ-HCH</td>
<td>0.79 (0.1–2.04)</td>
<td>0.82 (0.39–246.87)</td>
<td>112</td>
<td>0.14</td>
<td>0.65 (0.23–3.34)</td>
<td>65</td>
<td>0.40 (0.08–4.05)</td>
<td>65</td>
<td>0.0002</td>
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<tr>
<td>δ-HCH</td>
<td>0.05 (0.01–0.7)</td>
<td>0.17 (0.00–726.74)</td>
<td>99</td>
<td>&lt;0.0001</td>
<td>0.04 (0.01–0.27)</td>
<td>41</td>
<td>0.03 (0.01–0.16)</td>
<td>25</td>
<td>0.002</td>
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<td>PCA</td>
<td>0.13 (0.03–2.7)</td>
<td>0.15 (0.04–1.7)</td>
<td>112</td>
<td>0.49</td>
<td>0.09 (0.02–0.79)</td>
<td>65</td>
<td>0.03 (0.01–0.11)</td>
<td>64</td>
<td>&lt;0.0001</td>
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<td>HCB</td>
<td>7.68 (2.17–26.49)</td>
<td>4.52 (1.77–14.59)</td>
<td>112</td>
<td>&lt;0.0001</td>
<td>12.4 (6.01–24.56)</td>
<td>65</td>
<td>7.95 (2.94–18.55)</td>
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<td>&lt;0.0001</td>
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<td>OC\textsuperscript{S}</td>
<td>0.10 (0.02–0.39)</td>
<td>0.12 (0.04–0.55)</td>
<td>112</td>
<td>0.0001</td>
<td>4.74 (2.26–12.01)</td>
<td>65</td>
<td>3.56 (0.91–9.4)</td>
<td>65</td>
<td>0.0001</td>
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<td>OXC</td>
<td>1.15 (0.15–3.99)</td>
<td>0.88 (0.22–2.76)</td>
<td>112</td>
<td>0.02</td>
<td>2.87 (1.25–10.82)</td>
<td>65</td>
<td>2.04 (0.63–17.02)</td>
<td>65</td>
<td>&lt;0.0001</td>
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<td>c-HE</td>
<td>0.91 (0.3–3.1)</td>
<td>0.69 (0.21–2.01)</td>
<td>112</td>
<td>&lt;0.0001</td>
<td>0.08 (0.02–0.28)</td>
<td>65</td>
<td>0.04 (0.01–0.14)</td>
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<td>&lt;0.0001</td>
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<tr>
<td>o,p\textsuperscript{-}DDE</td>
<td>0.02 (0.01–0.2)</td>
<td>0.02 (0.01–0.6)</td>
<td>110</td>
<td>0.45</td>
<td>133.76 (24.59–427.55)</td>
<td>65</td>
<td>59.05 (18.95–331.16)</td>
<td>65</td>
<td>&lt;0.0001</td>
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<td>p,p\textsuperscript{-}DDE</td>
<td>40.98 (9.52–269.83)</td>
<td>18.04 (3.1–79.21)</td>
<td>112</td>
<td>&lt;0.0001</td>
<td>0.02 (0.00–0.07)</td>
<td>63</td>
<td>0.02 (0.00–0.17)</td>
<td>65</td>
<td>&lt;0.0001</td>
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<tr>
<td>p,p\textsuperscript{-}DDD</td>
<td>0.05 (0.01–0.36)</td>
<td>0.05 (0.01–0.38)</td>
<td>112</td>
<td>0.37</td>
<td>0.36 (0.1–2.2)</td>
<td>63</td>
<td>0.31 (0.1–3.36)</td>
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<td>&lt;0.0001</td>
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<tr>
<td>p,p\textsuperscript{-}DDE</td>
<td>0.67 (0.16–6.35)</td>
<td>0.48 (0.2–2.4)</td>
<td>112</td>
<td>&lt;0.00001</td>
<td>0.46 (0.13–1.83)</td>
<td>64</td>
<td>0.27 (0.04–1.21)</td>
<td>64</td>
<td>0.0001</td>
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<tr>
<td>o,p\textsuperscript{-}DDT</td>
<td>0.05 (0.01–0.26)</td>
<td>0.05 (0.01–0.92)</td>
<td>112</td>
<td>0.65</td>
<td>5.68 (1.62–37.88)</td>
<td>65</td>
<td>3.43 (1.46–12.9)</td>
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<td>&lt;0.0001</td>
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<tr>
<td>o,p\textsuperscript{-}DDE</td>
<td>0.56 (0.07–4.65)</td>
<td>0.27 (0.12–3.38)</td>
<td>112</td>
<td>&lt;0.00001</td>
<td>20.49 (4.36–106.66)</td>
<td>65</td>
<td>16.58 (8.1–42.51)</td>
<td>65</td>
<td>0.001</td>
<td></td>
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<tr>
<td>DDE/\textsuperscript{E}</td>
<td>87.94 (11.51–380.04)</td>
<td>62.54 (2.78–304.65)</td>
<td>112</td>
<td>&lt;0.00001</td>
<td>55</td>
<td>55</td>
<td>65</td>
<td>0.005</td>
<td></td>
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</tr>
<tr>
<td>DDT</td>
<td>0.01 (0.00–0.18)</td>
<td>0.03 (0.00–0.67)</td>
<td>32</td>
<td>0.30</td>
<td>0.05 (0.01–0.35)</td>
<td>55</td>
<td>0.03 (0.01–0.11)</td>
<td>53</td>
<td>0.005</td>
<td></td>
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<tr>
<td>c-CHL</td>
<td>0.01 (0.00–0.19)</td>
<td>0.03 (0.00–0.10)</td>
<td>31</td>
<td>0.16</td>
<td>0.03 (0.01–0.07)</td>
<td>32</td>
<td>0.02 (0.00–0.05)</td>
<td>32</td>
<td>0.03</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>γ-HE</td>
<td>1.97 (0.27–6.87)</td>
<td>1.75 (0.26–8.78)</td>
<td>112</td>
<td>0.56</td>
<td>7.43 (1.92–18.05)</td>
<td>65</td>
<td>6.40 (1.19–22.66)</td>
<td>65</td>
<td>0.0001</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>END-I</td>
<td>2.46 (0.42–19.57)</td>
<td>1.25 (0.44–4.32)</td>
<td>112</td>
<td>&lt;0.0001</td>
<td>4.88 (1.74–35.5)</td>
<td>65</td>
<td>2.37 (0.77–7.19)</td>
<td>65</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dieldrin</td>
<td>0.06 (0.02–7.79)</td>
<td>0.04 (0.01–1.14)</td>
<td>112</td>
<td>0.0003</td>
<td>0.04 (0.00–0.43)</td>
<td>65</td>
<td>0.06 (0.02–1.12)</td>
<td>64</td>
<td>0.0002</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mirex</td>
<td>0.14 (0.01–1.08)</td>
<td>0.18 (0.02–2.08)</td>
<td>112</td>
<td>0.02</td>
<td>0.21 (0.03–0.66)</td>
<td>65</td>
<td>0.26 (0.02–1.54)</td>
<td>64</td>
<td>0.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

\textsuperscript{a}Samples with values \textless LOD were excluded from the calculation.

\textsuperscript{b}For Finnish placenta data see also our previous report (Shen et al., 2005).

\textsuperscript{c}Number of samples \textgreater LOD. Total numbers of Danish and Finnish placenta samples were 168 and 112, respectively, and those of Danish and Finnish milk samples were 65 and 65, respectively.

\textsuperscript{d}Country differences were tested by multivariate regression controlling for confounders.
date of birth, and parity (Table IV). HCB, c-HE and dieldrin levels in placenta were positively associated with maternal BMI, whereas END-I levels were negatively associated with maternal BMI (Table IV).

**Organobrominated compounds**

The concentrations of BB congeners, including HeBB and PeBB, are listed in Table V. Compared with the levels of organochlorines, much lower levels of organobromines were found and more samples had unmeasurable levels. BB-153 and -155 were the most frequently found congeners. Of these two congeners, BB-153 was generally the more abundant, but in some placenta samples, the levels of BB-155 were higher than BB-153, and in some samples only BB-155, but not BB-153 was detectable. The levels of BB-153 were significantly higher in Danish milk samples compared with Finnish samples ($P < 0.01$), whereas the levels of BB-155 were significantly higher in Finnish milk compared with Danish ($P = 0.016$). None of the other confounders (maternal age, parity, maternal BMI, gestational length and date of birth) was related to the level of these two congeners in milk.

Most of the samples had a very low BB-155/BB-153 ratio (Table V). However, 16 Finnish placenta and 11 Danish placenta samples had a BB-155/BB-153 ratio above the average ($>0.3$); in these samples, also other congeners could frequently be found (they were one or more of BB-31, -37, -52, -49, -80, -77, -103 and -101). In contrast, only two Finnish milk samples had a BB-155/BB-153 ratio close to 0.3. Only in six paired milk and placenta samples could BB-155 and BB-153 be detected in both matrices. In these pairs, there was a strong linear correlation between the BB-155/BB-153 ratio in placenta and in milk samples ($R^2 = 0.99; P < 0.0001; N = 6$) but the ratio was ~60 times higher in the placenta samples than in the milk samples.

**Enantiomeric ratios of chiral compounds**

The ERs with a relative error less than 20% are presented (Table VI). Only the ERs for α-HCH, c-HE and OXC will be discussed because the ERs for these three compounds could be determined for most of the samples.

No significant difference in the ERs between Finnish and Danish samples for α-HCH, c-HE and OXC was observed, but for α-HCH and c-HE the ERs changed with the concentration of the isomers. At high concentrations of the (−)-isomer of α-HCH, the ER was close to the racemic ratio (ER = 1), whereas at a low concentration the ER ratio decreased (Fig. 1). For c-HE, the ER increased with lower concentrations of the (+)-isomer (Fig. 2). There were only a few samples with extreme ER values (for c-HE values close to 1 and for α-HCH values much larger than 1). In contrast to α-HCH and c-HE, no changes in ER with changes in the isomer concentration were found for chiral OXC.
Table V. Concentration of PBB congeners, HeBB and PeBB (pg/g lipid) in Danish and Finnish breast milk and placentas samples.

<table>
<thead>
<tr>
<th></th>
<th>Danish milk</th>
<th>Finnish milk</th>
<th>Danish placenta</th>
<th>Finnish placenta</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Min–Max</td>
<td>Mean (SD)a</td>
<td>N</td>
</tr>
<tr>
<td>BB-4</td>
<td>0 (0%)</td>
<td>—</td>
<td>—</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>BB-31</td>
<td>9 (14.1%)</td>
<td>2–31</td>
<td>8 (9)</td>
<td>2 (1.2%)</td>
</tr>
<tr>
<td>BB-49</td>
<td>11 (17.2%)</td>
<td>1–4</td>
<td>3 (1)</td>
<td>5 (3.0%)</td>
</tr>
<tr>
<td>BB-52</td>
<td>16 (25.0%)</td>
<td>1–7</td>
<td>4 (2)</td>
<td>6 (3.6%)</td>
</tr>
<tr>
<td>BB-77</td>
<td>39 (60.9%)</td>
<td>3–47</td>
<td>13 (9)</td>
<td>20 (11.9%)</td>
</tr>
<tr>
<td>BB-103</td>
<td>0 (0%)</td>
<td>—</td>
<td>—</td>
<td>2 (1.2%)</td>
</tr>
<tr>
<td>BB-101</td>
<td>19 (29.7%)</td>
<td>2–66</td>
<td>15 (17)</td>
<td>6 (3.6%)</td>
</tr>
<tr>
<td>BB-126</td>
<td>0 (0%)</td>
<td>—</td>
<td>—</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>BB-155</td>
<td>49 (76.6%)</td>
<td>0.01–0.2</td>
<td>0.05 (0.03)</td>
<td>10 (6.0%)</td>
</tr>
<tr>
<td>BB-169</td>
<td>1 (1.6%)</td>
<td>4</td>
<td>4</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>PeBB</td>
<td>27 (42.2%)</td>
<td>3–18</td>
<td>7 (4)</td>
<td>4 (2.4%)</td>
</tr>
<tr>
<td>HeBB</td>
<td>49 (76.6%)</td>
<td>5–500</td>
<td>50 (75)</td>
<td>31 (18.4%)</td>
</tr>
</tbody>
</table>

N is the number of samples with detectable levels and SD the standard deviation.

aSamples with values < LOD were excluded from the calculation. Total numbers of Danish and Finnish placenta samples were 168 and 112, respectively, and those of Danish and Finnish milk samples were 65 and 65, respectively.

bFinnish placenta and Danish placenta data may not be directly comparable because of different calibration methods.
Comparison of ER in paired milk and placenta samples

For α-HCH, ER deviated more from the racemic ratio (ER = 1) in human milk than in placenta (Danish samples: average milk ER = 0.59, average placenta ER = 0.82, P < 0.0001, N = 41; Finnish samples: average milk ER = 0.47, average placenta ER = 0.78, P < 0.0001, N = 43). For c-HE, the ER observed in the placenta deviated more from the racemic ratio than the ER observed in milk, although the differences did not reach statistical significance in the Finnish milk–placenta paired samples (Danish samples: average milk ER = 1.95, average placenta ER = 2.24, P = 0.018, N = 40; Finnish samples: average milk ER = 1.98, average placenta ER = 2.13, P = 0.19, N = 31). The ERs of OXC were nearly the same in the paired placenta and milk samples (Danish samples: average milk ER = 1.30, average placenta ER = 1.37, P = 0.37, N = 38; Finnish samples: average milk ER = 1.39, average placenta ER = 1.23, P = 0.14, N = 28).

Discussion

Our study revealed significant geographical differences in the levels of organochlorine compounds in human placenta and milk samples between two Nordic countries. Danish samples had higher concentrations than Finnish samples for most compounds. This country difference remained highly significant after adjustment for possible confounders. The findings suggest that despite close vicinity and comparable lifestyles, exposure may differ significantly between regions. The levels of p,p′-DDE, p,p′-DDT and HCB in human milk from Sweden, another Nordic country located between Denmark and Finland, have been reported (Noren and Meironyte, 2006).
The levels reported in the Swedish human milk samples (collected in 1997) were comparable with the levels we observed in the Danish milk (collected between 1997 and 2001).

Several factors, e.g. maternal age, parity and length of previous lactation, have been reported to affect the exposure of the infant (Harris et al., 2001). In our study, pollutant levels in human milk and placenta decreased with increasing parity for the majority of the eight most prevalent compounds. This provides additional evidence that previous deliveries and lactation may contribute to the clearance of persistent pollutants from the mothers’ fatty stores. The concentration of the most prevalent pollutants in milk and placenta was positively correlated to maternal age, which may reflect differences in exposure through dietary habits, metabolism or bioaccumulating properties (Brunetto et al., 1996; Czaja et al., 1997; Covaci et al., 2002).

It has been reported that the clearance rates for DDT, dichloro-di-phenyl-trichloro-ethene (DDE), dieldrin and HCB were $-0.162$, $-0.113$, $-0.117$ and $-0.118$ year$^{-1}$, respectively (Noren and Meironyte, 2000). We also observed that the levels of many of the measured pollutants were negatively correlated with the date of infant delivery. Additionally, we found slightly decreasing levels of OCs during the brief study period, in line with other reports (Smith, 1999; Solomon and Weiss, 2002). The correlations between maternal BMI and pollutant levels were not consistent and it remains to be determined whether these associations with maternal BMI are genuine or chance findings due to mass significance.

The observed significant negative correlation of gestational age with the concentrations of c-HE, END-I and dieldrin in milk samples suggests that a shorter gestational period may lead to a higher mobilization of chemicals stored in maternal fat tissue during breastfeeding instead of during the third trimester when fetal fat accumulation is greatest. For placental samples gestational age was significantly and positively associated with HCB. We suggest that in parallel to the rapid increase in fetal fat stores from around gestational week 20, more pollutants may be released from the mother’s adipose depots and transferred to the fetus via placental regulation of fatty acid delivery (Haggarty, 2002). Infants born preterm may be less exposed than mature babies to persistent compounds during pregnancy, but then their exposure during breastfeeding may be higher. Thus, the overall exposure appears to be predominantly related to the prenatal maternal body burden. These results should, however, be interpreted with caution as only one of the eight most abundant compounds in placenta correlated with gestational age and thus this may also be a chance finding.

Although the lipid contents measured were in the normal range compared with previously reported data on lipid content in placenta (1–1.5%) and milk (1–6%) (DeKoning and Karmaus, 2000), we observed a significantly higher lipid content in Finnish than in Danish samples. Despite methodological differences in breast milk sample collection periods and timing between the two countries, the nearly normal lipid distributions suggest that the selection was random. It has been reported that maternal nutritional status, including long-term dietary habits and current diet (Harzer et al., 1984; Specker et al., 1987), as well as obese or lean body composition (Rocquelin et al., 1998; Anderson et al., 2005; Marin et al., 2005) affect the lipid content of breast milk. The content and composition of milk lipids is mainly determined by three sources of fatty acids: diet, mobilization of body fat depots and de-novo synthesis of fatty acids by the mammary gland (Ortiz-Olaya et al., 1996). Thus, our observation may reflect differences in dietary habits between the two countries.

We have also considered the possible dilution effect of the fat content on OCs. However, the linear regression models showed that lipid concentration had no significant correlation with the OC concentrations in milk. Along with milk samples, the placenta samples, which have lower fat concentrations, also showed lower OC concentrations for the more persistent compounds. Furthermore, concerning the most abundant compounds, the OC concentrations in placenta samples correlated with OC concentrations in milk samples (Shen et al., 2007). This indicates that lipophilic compounds tend to be concentrated in tissues that have a higher fat concentration. In addition, the lipid content of placenta and milk is much lower than the total lipid burden in the adipose tissue of the mother, thereby reflecting only a minute fraction of the total lipid content relevant to the establishment of equilibrium. Therefore, we think that the country differences in OC concentrations are unlikely to reflect differences in the lipid concentration of the samples.

The fatty acid composition in placenta is determined by the fatty acid content of maternal liver and plasma (Graham et al., 2004). The negative correlation between gestational age and placental lipid concentration found in our study may be the result of the exponentially increasing fatty acid delivery to the fetus from week 20, which decreases the storage of lipid between the microvillous membrane and the basal membrane of the placenta (Haggarty, 2002).

The ERs of chiral compounds are independent of physical processes (leaching, volatilization and atmospheric deposition) and abiotic reactions (hydrolysis and photolysis) (Bidleman and Falconer, 1999), but are sensitive to biotransformation or biodegradation because these are enantiomeric selective processes. The present ER-concentration patterns in Danish placental samples as well as in Danish and Finnish milk samples were similar to previously reported results in Finnish placental samples (Shen et al., 2006). No significant country differences in ERs could be observed, indicating similar historical and recent exposure patterns in the two countries, even though the absolute levels differed.

Little is known about the variation of environmental ERs (ERen). Our data suggest that ERen may be close to 1 for α-HCH and 1.5 for c-HE because ERs measured in samples with high levels approached these values. ERs close to the presumed ERen at low levels may indicate a recent exposure, whereas ERs deviating from the presumed ERen may indicate historical exposure (Shen et al., 2006). The abnormal ER values for a few samples may reflect a special exposure source or different enzyme activity of the individual mother.

ERs close to the presumed ERen at low levels may indicate recent low-level exposure, whereas ERs deviating from the
presumed ER<sub>α</sub> may indicate release of historical exposures stored in adipose depots. The abnormal ER values for a few samples may reflect a special exposure source or different enzyme activity of the individual mother.

Tissue-specific ERs for certain chiral pollutants can result from different enzyme activities (e.g. in kidney and liver) or enantiomeric selective enrichment (e.g. in the blood–brain barrier-protected brain) (Kallenborn and Hüfnerfuss, 2001). For c-HE, there was a linear correlation between the absolute concentrations measured in milk and placenta (Shen et al., 2007), indicating a balanced distribution of c-HE between the two tissues. However, the ERs for c-HE deviated more from the racemic ER in the placenta than in milk indicating that the placenta contributes to the metabolism of this compound. This supports the assumption that placenta can act as a filter towards the fetus by metabolizing foreign compounds. In contrast, ERs for α-HCH found in the placenta was close to the ER<sub>α</sub> and it seemed to be metabolized more in breast tissue. The ER<sub>α</sub> of OXC may be the mixed results of the metabolism and it seemed to be metabolized more in breast tissue. PBBs can be transferred across the human placenta (Eyster et al., 1983; Jacobson et al., 1984). The widely used commercial hexaBBS (HxBB) constitute ~87% of the total PBBs and BB-153 is the principal component (~60%) of HxBB, whereas BB-155 only contributed ~0.5% (Hardy, 2002; EHC 152, 1994). It was therefore expected that BB-153 was the most abundant PBB congener in our study. In some samples, the concentrations of BB-155 were higher than expected. Comparable levels of BB-155 have previously been reported from Europe, whereas data from USA report much lower and generally undetectable levels of BB-155 (EHC 152, 1994; de Boer et al., 1998; Luross et al., 2002). It has been suggested that photochemical debromination of DBB may be a source for environmental BB-155 (EHC 152, 1994). Thus, the difference in BB-155 levels between Europe and USA may be due to the more recent cessation of production of OBB and DBB in Europe compared with the USA (Hardy, 2002). On the basis of our observed ratios of BB-155/BB-153, we suggest that BB-153 is more readily metabolized than BB-155 (Hakk and Letcher, 2003) in the human placenta.

In conclusion, multiple factors contribute to the pre- and post-natal exposure of infants via exposure of the mother to persistent organochlorines. Our study confirms that maternal age is positively, and parity negatively, correlated with higher concentrations of persistent organochlorines. In addition, in this bi-national Nordic study, we found a significant country difference. This indicates that environmental and lifestyle differences between these two countries exist, leading to a higher exposure of the Danish population. Recently, an association between congenital cryptorchidism and the combined exposure to the eight most common organochlorines in human milk was indicated (Damgaard et al., 2006).

In the present study, the observed country difference in exposure levels may have been affected by the selection of samples from cryptorchid and healthy boys. The prevalence of cryptorchidism in this subsample is not representative of the true population prevalence (Boisen et al., 2004), neither is the distribution of cases and controls exactly 50:50. However, the observed exposure difference for organochlorines may play a role for the higher prevalence of male reproductive problems such as testicular cancer, decreased sperm count and cryptorchidism in Denmark when compared with Finland.

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References


Damgaard IN, Skakkebaek NE, Toppari J, Virtanen HE, Shen H, Schramm KW, Petersen JH, Jensen TK, Main KM, The Nordic...


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