international journal of andrology ISSN 0105-6263

ORIGINAL ARTICLE

Country-specific chemical signatures of persistent environmental compounds in breast milk

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Keywords:
breast milk, chemical signature, endocrine disrupting chemicals, geographical differences, semen quality, testicular dysgenesis syndrome

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Received 6 July 2009; revised 18 August 2009; accepted 19 August 2009

doi:10.1111/j.1365-2605.2009.00996.x

Summary

Recent reports have confirmed a worldwide increasing trend of testicular cancer incidence, and a conspicuously high prevalence of this disease and other male reproductive disorders, including cryptorchidism and hypospadias, in Denmark. In contrast, Finland, a similarly industrialized Nordic country, exhibits much lower incidences of these disorders. The reasons behind the observed trends are unexplained, but environmental endocrine disrupting chemicals (EDCs) that affect foetal testis development are probably involved. Levels of persistent chemicals in breast milk can be considered a proxy for exposure of the foetus to such agents. Therefore, we undertook a comprehensive ecological study of 121 EDCs, including the persistent compounds dioxins, polychlorinated biphenyls (PCBs), pesticides and flame retardants, and non-persistent phthalates, in 68 breast milk samples from Denmark and Finland to compare exposure of mothers to this environmental mixture of EDCs. Using sophisticated, bioinformatic tools in our analysis, we reveal, for the first time, distinct country-specific chemical signatures of EDCs with Danes having generally higher exposure than Finns to persistent bioaccumulative chemicals, whereas there was no country-specific pattern with regard to the non-persistent phthalates. Importantly, EDC levels, including some dioxins, PCBs and some pesticides (hexachlorobenzene and dieldrin) were significantly higher in Denmark than in Finland. As these classes of EDCs have been implicated in testicular cancer or in adversely affecting development of the foetal testis in humans and animals, our findings reinforce the view that environmental exposure to EDCs may explain some of the temporal and between-country differences in incidence of male reproductive disorders.

Introduction

A considerable increase in testicular cancer incidence among young men during the last century has taken place worldwide and large scale geographical differences in the incidence of this disease exist (Bray *et al.*, 2006). There is a remarkable three to fourfold higher incidence of testicular cancer in Denmark in comparison with the nearby country Finland. We previously tested the hypothesis that testicular cancer incidence may be a ‘whistleblower’ for occurrence of other reproductive health problems in a population by carrying out large, coordinated, prospective studies of cohorts of newborn boys and their mothers in Denmark and Finland. These showed that the incidence of cryptorchidism and hypospadias was also three to fourfold higher in Denmark than in Finland (Boisen *et al.*, 2004, 2005). Even among healthy newborn boys, there were significant differences as Finnish boys had larger testes than Danish and higher levels of inhibin B, a marker of testicular Sertoli cells (Main *et al.*, 2006b). Prospective studies of the general adult populations have also revealed higher sperm counts in Finnish than in Danish men (Jørgensen *et al.*, 2002).

A crucial question is to what extent these conspicuous differences in occurrence of reproductive problems between two Nordic countries are because of environmental factors. Studies of immigrants' testicular cancer risk have shown that second generation immigrants have similar risk of cancer to that of the host country population (Hemminki & Li, 2002; Myrup *et al.*, 2008). This, together with the increasing trends of male reproductive health problems, strongly suggests that environmental rather than genetic factors play a major role. As humans have been widely exposed over the same time period to man-made persistent EDCs, their aetiological involvement has been suspected. In reality, humans are exposed not to single EDCs, but to complex mixtures and the latest evidence from animal studies shows that such mixtures can have profound effects on male reproductive development at concentrations at which the individual EDCs have no effect (Christiansen *et al.*, 2008; Kortenkamp, 2008; Rider *et al.*, 2009).

Therefore, we have undertaken an ecological study to examine whether exposures to EDC pollutants is higher in Denmark than in Finland. We measured 121 chemicals (listed in Tables S1 and S2) in 68 breast milk samples from 36 Danish and 32 Finnish women who gave birth to healthy boys. Chemicals studied included flame retardants, such as polybrominated diphenyl ethers (PBDE) and biphenyls (PBB), organochlorine pesticides (OC), polychlorinated dibenzo-*p*-dioxins (PCDD/F dioxins) and biphenyls (PCB), and phthalates, all known for their potential as endocrine disruptors. Breast milk was chosen because concentrations of pollutants in milk fat are considered to represent human exposures (Smith, 1999; Wang & Needham, 2007).

Materials and methods

The data set for this analysis was obtained from a joint prospective bi-national study of pregnant women and their offspring between 1997 and 2001. This study aimed at assessing the current prevalence of congenital cryptorchidism and hypospadias in Denmark and Finland as well as identifying environmental and lifestyle factors possibly associated with testis development and function. Questionnaires and breast milk samples were obtained. The design of the study was previously described, as well as details on breast milk sample collection and selection for chemical analysis; some of the data were included in other investigations (Main *et al.*, 2006a; Shen *et al.*, 2006, 2007).

The original data set consisted of 130 breast milk samples from mothers of newborn children. Sixty-eight of the newborns (36 Danish and 32 Finnish) were healthy and without signs of reproductive malformations and 62 were born cryptorchid. As breast milk from women who delivered a boy with cryptorchidism may be a major confounder in an analysis of the general exposure levels to EDCs in a population, we only included breast milk of the 68 mothers who gave birth to healthy boys. A total of 121 chemicals were analysed; however, 12 chemicals with non-detectable levels in all samples were excluded from the final statistical analysis (Tables S1 and S2).

During all chemical analyses, the laboratories and technicians were blinded for country of origin. All laboratories participated in external quality control programmes. Pesticides including enantiomeric compounds and polybrominated biphenyls were analysed at the Institute of Ecological Chemistry, Neuherberg, Germany (Damgaard *et al.*, 2006) and polybrominated diphenyl ethers, dioxins, PCB's and furans at the Department of Environmental Health, National Public Health Institute, Kuopio, Finland (Main *et al.*, 2007). All phthalate analyses were performed at chemical laboratory at the Department of Growth and Reproduction, Copenhagen, Denmark (Mortensen *et al.*, 2005; Main *et al.*, 2006a).

The Danish mothers were slightly younger than the Finnish and more of them participated with their first child in the study. Moreover, Danish samples were collected on average 1.8 years later than Finnish samples. These potential confounders, and others, were adjusted for in the analysis.

To assess the extent of differences in exposure to individual chemicals between Denmark and Finland, chemical concentrations in breast milk samples were analysed using linear multiple regression. The *p*-values were corrected for multiple testing by the method of Bonferroni. Potential confounders, known to affect the level of chemicals in breast milk samples, were added as covariates in the analysis, including maternal age, maternal body mass index (BMI), year of milk sampling, maternal smoking (yes/no), maternal diabetes (yes/no) and parity.

We investigated the differences in combined chemical exposures between the two countries using machine-learning classifiers, which simultaneously take all chemical concentrations into account. These classifiers can detect any combination or pattern of chemicals which discriminates between Danish and Finnish samples. Such patterns may describe for example if the sum of two or more chemicals must be above a certain level, or the level of one chemical is high whilst the level of another chemical is low. Three Machine Learning Classifiers were applied for comparison, two of which were linear methods [Partial Least Squares (PLS) (Wold, 1966) and Sequential Minimal Optimization (SMO) with 1st order polynomial kernel], and one was a non-linear method (Multilayer Perceptron with one hidden layer of 5 nodes).

For the machine learning classifiers, analysis was performed both with and without adjusting for confounders. Confounders were adjusted for by interpolating the data using regression coefficients. In addition, confounders were adjusted for in the analysis with PLS by adding the confounding factors, along with country, as response-variables.

Two different software programs were used; Simca-P 10.5 (by Umetrics Inc., Umeå, Sweden) was used for performing PLS, and Weka 3.5.3 (Witten & Frank, 2005) was used for performing analysis with the other classifiers. One fourth of the samples (17 samples) in the original data were randomly removed and used as a test set for external validation. A balanced number of samples from each country were included in the test set.

Non-detectable sample measurements were treated in three separate ways: they were either set to 0, to half the Limit of Quantification (LOQ), or to LOQ. All analyses presented were repeated for each case. In addition to the measured values of the chemicals, we analysed sums and toxic equivalencies (TEQs) of PCBs, PBDEs and PBBs. Finally, as phthalates differ in their chemical properties and exposure routes compared with all the other compounds in our data set, they were analysed together in a separate model.

Results

After correcting for multiple testing, six chemicals exhibited significant differences between the two countries and all were higher in Danes than in Finns (Table 1, Fig. 1). Without statistical correction for multiple testing, higher concentrations in Danish samples were observed for the vast majority (54 out of 58) of chemicals that exhibited a significant between-country difference (Tables 2A and 2B). Chemicals which did not differ significantly are listed in Table 2C (resulting *p*-values for all chemicals, including TEQs, can be found in Tables S3 and S4).

Analyses with the machine-learning classifiers showed that the chemical exposures in the two countries were so distinct that perfect, or near perfect, separation of samples with respect to country of origin was possible (Fig. 2). Obviously, not all chemicals contribute equally to this difference. To examine the importance of each chemical, we examined the weights of each chemical in the models and performed feature selection by training the different machine-learning methods using only a subset of chemicals (Tables S5–S8 for performance of the machine learning analysis for various models). Each of the methods achieved perfect separation of Danish and Finnish samples using a slightly different set of chemicals (Tables S9–S11). Several of the chemicals were used by two machine-learning methods, and two chemicals, 1,2,3,4,7,8-HCDD and 1,2,3,6,7,8-HCDD, were consistently selected by all three methods. Indeed, the combination of these two chemicals alone perfectly separated the Danish and Finnish samples (Fig. 3). Moreover, the clear between-country difference was robust and did not disappear if either of these two chemicals was left out of the analyses. These results did not change when the chemical levels below the limit of quantification were assigned to 0, one half of LOQ or LOQ.

As phthalates differ from the persistent compounds in their chemical properties, exposure routes and persistence, they were also analysed in a separate model. The phthalate levels alone did not exhibit any strong separation between the two nations.

Discussion

Our comprehensive analysis of more than one-hundred environmental chemicals in contemporary breast milk from Finland and Denmark revealed conspicuous

Table 1 Chemicals with significantly higher concentrations in Danish than in Finnish breast milk samples in a linear multiple regression analysis after correction for multiple testing. Percentiles show unadjusted concentrations

	Percentile, Denmark			Percentile, Finland				
Chemical	25th	50th	75th	25th	50th	75th	*p*-value	Higher in
1,2,3,4,7,8-HCDD	2.39e-3	3.32e-3	4.76e-3	0.78e-3	1.06e-3	1.28e-3	2.18e-4	Denmark
PCB 209	0.088	0.11	0.16	0.045	0.061	0.092	3.27e-5	Denmark
PCB 156	4.21	5.66	8.62	2.83	3.59	4.83	1.07e-2	Denmark
PCB 157	0.70	0.86	1.27	0.44	0.60	0.80	2.25e-2	Denmark
Dieldrin	3.06	4.66	5.98	1.86	2.21	3.10	2.30e-4	Denmark
Hexachlorobenzene	8.80	11.78	14.16	6.87	7.60	8.55	1.32e-4	Denmark

Levels below LOQ were assigned the value 0. Data are given as mean ± SD. LOQ, Limit of Quantification.

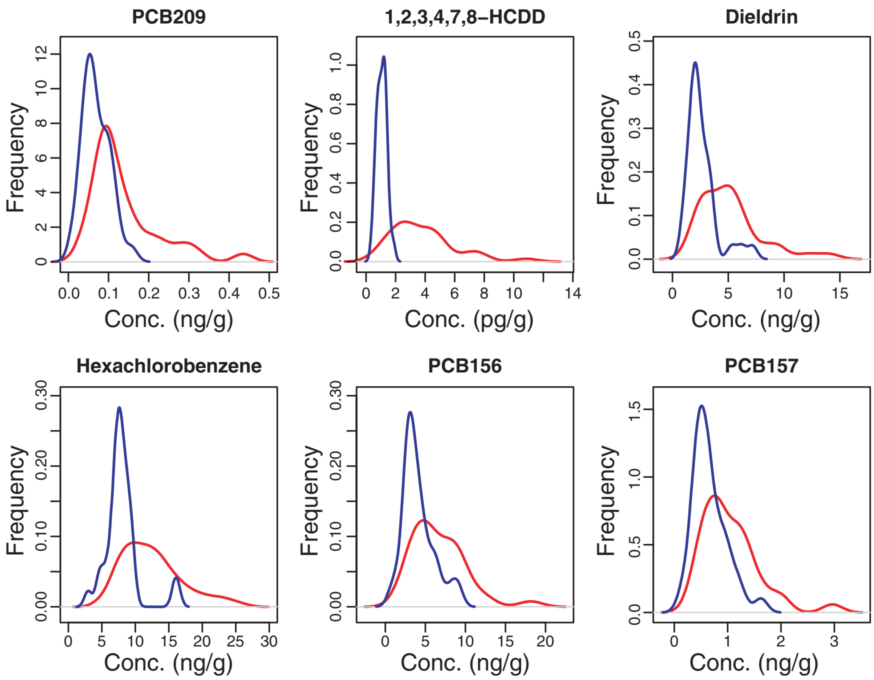

Figure 1 Plots show the distribution of concentrations of chemicals which exhibited significant differences between Denmark (red) and Finland (blue) in a linear multiple regression. Chemicals were measured in breast milk samples from Danish and Finnish mothers of healthy boys.

Table 2A Chemicals that had significantly higher concentrations in Danish than in Finnish breast milk samples in a linear multiple regression analysis *before* correction for multiple testing ($p < 0.05$). Chemicals that still differed significantly after correction are shown in bold (see supplementary Tables S3 and S4 for details)

Class of chemicals	Congeners
PBDE	BDE-153
PBB	2-BB, 4-BB, 22′-BB, 344′-BB, 33′44′-BB, 22′45′6-BB, 33′44′5-BB, 33′44′55′-BB
Organochlorine pesticides	Hexachlorocyclohexanes [(+)-α-HCH, (–)-α-HCH, α-HCH (sum of enantiomers), β-HCH, ε-HCH]
	o,p′-DDT, 1,1,1-trichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane
	p,p′-DDT, 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
	o,p′-DDE, 1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethene
	p,p′-DDE, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethene
	Dieldrin, **Hexachlorobenzene (HCB)**, Aldrin, Trans-chlordane, Heptachlor, (+)-Oxychlordane (OXC), (–)-Oxychlordane, (OXC), Oxychlordane (Sum of enantiomers), (+)-Cis-heptachlor epoxide (HE), (–)-Cis-heptachlor epoxide (HE), Trans-heptachlor epoxide, Endosulfan-I, Pentachlorobenzene
PCB	PCB-28/31, -49, -60, -66, -74, -77, -81, -99, -110, -114, -128, -138, -153, **-156**, **-157**, -167, -169, -170, -180, -183, -187, -189, -194, -206, **-209**, Sum of PCBs, WHO-TEQ
PCDD/PCDF's	2378-TCDD, 12378-PD, 123478-HF, 123678-HF, **123478-HD**, 123789-HD

PBDE, polybrominated diphenyl ethers; PBB, polybrominated biphenyls; PCDD/F dioxins, polychlorinated dibenzo-*p*-dioxins; PCB, polychlorinated biphenyls.

differences, particularly with regard to concentrations of persistent organic pollutants (POPs). In fact, an analysis of only two dioxins could totally separate the Danish breast milk from the Finnish breast milk. Another important finding was that the levels of chemicals were generally higher in the Danish samples, where the concentration range of POPs was also much broader and included some quite high values. Thus, taken together, the exposure levels of the examined chemicals and their mixture pattern seemed quite different in Denmark and Finland.

Three classes of chemicals were represented by the compounds that were found in significantly higher concentrations and with broader distribution spectrums in Danish samples; PCBs, organochlorine pesticides and PCDDs. Several of these have been implicated in impairment of foetal testis development or testis cancer

Table 2B Chemicals that had significantly higher concentrations in Finnish than in Danish breast milk samples in a linear multiple regression analysis *before* correction for multiple testing ($p < 0.05$). No chemical was significantly higher in Finland after correction for multiple testing (see supplementary Tables S3 and S4 for details)

Class of chemicals	Congeners
Phthalate Monoesters	Mono-butylphthalate, mono-benzylphthalate
Organochlorine pesticides	Methoxychlor
PCB	PCB-51

PCB, polychlorinated biphenyls.

(Toppari *et al.*, 1996; Hardell *et al.*, 2003; Main *et al.*, 2006a; Fowler *et al.*, 2007; Andersen *et al.*, 2008; Damgaard *et al.*, 2008; McGlynn *et al.*, 2008).

An important question is whether the apparent different exposure levels can explain the marked differences in male reproductive health problems between the two countries. Most previous studies examining links between exposures and reproductive health problems, including our own previous investigations, have focused on possible effects of single agents or a group of related agents at a time (Main *et al.*, 2007; Kortenkamp, 2008). However, it seems important that evaluations of effects of chemicals on human health should include as many as possible of the agents constituting the 'total pollution cocktail' to estimate the combined effect (Christiansen *et al.*, 2008). Recent animal studies have, in fact, shown that combined exposures to multiple chemicals had significant adverse effects, although previous dose–response studies had shown no effects when the chemicals were administered one at the time at low concentrations (Christiansen *et al.*, 2008; Rider *et al.*, 2008). In the present study, we therefore made use of advanced bioinformatics software programs to extract the total information of all analysed chemicals in all breast milk samples.

Why more POPs in Danish milk?

We were unable to find data which could explain the generally higher levels of EDCs in Danish samples. The major source of human exposure to POPs is from fatty foods (Wang & Needham, 2007). According to the National Danish Implementation Plan of the Stockholm Convention, Miljøstyrelsen (Danish Ministry of the Environment) (2006), the levels of POPs in Danish foodstuff should not raise cause for concern. Furthermore, regulation of the most significant chemicals in Table 1 show no striking differences between Finland and Denmark. Differences in regulatory practices may therefore not account for the specific chemical signatures of the two populations.

Polychlorinated biphenyls have been produced since 1929 and used in many applications such as in paints, plastizisers and dielectric fluids in capacitors and transformers. The sale of PCBs and PCB containing products was banned in Denmark in 1986. In Finland, the manufacture and use of PCB containing products was banned in the early 1990's [Finnish Environment Institute, 2006; Miljøstyrelsen (Danish Ministry of the Environment), 2006].

Polychlorinated dibenzo-*p*-dioxins are unintentionally produced as byproducts in many industrial processes (e.g. paper bleaching), traffic and waste combustion. In Denmark, the total emission of chlorinated dioxins into air (including PCDD and PCDF) in 2000–2002 was estimated to be 11–162 g I-TEQ/year (Hansen & Hansen, 2003). In Finland, the corresponding number was

Table 2C Chemicals that did not differ significantly ($p > 0.05$) between Danish and Finnish breast milk samples in a linear multiple regression analysis of their concentrations (see supplementary Tables S3 and S4 for details)

Class of chemicals	Congeners
PBDE	BDE-28, -47, -66, -75, -77, -85, -99, -100, -119, -138, -154, -183, Sum of PBDEs
PBB	2-BB, 4-BB, 22′-BB, 24′5-BB, 344′-BB, 22′55′-BB, 22′45′-BB, 33′55′-BB, 22′45′6-BB, 22′455′-BB, 33′44′5-BB, 22′44′66′-BB, 22′44′55′-BB, 33′44′55′-BB, Pentabromobenzene (PeBB), Hexabromobenzene (HeBB)
Phthalate monoesters	Mono-methylphthalate, mono-ethylphthalate, mono-2-ethylhexylphthalate, mono-isononylphthalate
Organochlorine pesticides	Hexachlorocyclohexanes (γ-HCH, δ-HCH, ε-HCH) o,p′-DDD, 1,1-dichloro-2-(2-chlorophenyl)-2-(4-chlorophenyl)ethane p,p′-DDD, 1,1-dichloro-2,2-bis(4-chlorophenyl)ethane Octachlorostyrene (OCS), Pentachloroanisole (PCA), Aldrin, Cis-chlordane, Heptachlor, Trans-heptachlor epoxide, Mirex, Endosulfan-II
PCB	PCB-18, -33, -47, -52, -101, -105, -118, -122, -123, -126, -141
PCDD/PCDF's	2378-TCDF, 12378-PF, 23478-PF, 1234678-F, 1234789-F, 234678-HF, 123789-HF, 123678-HD, 1234678-D, OCDF, OCDD, WHO-TEQ, Sum PCDD/F

PBDE, polybrominated diphenyl ethers; PBB, polybrominated biphenyls; PCDD/F dioxins, polychlorinated dibenzo-*p*-dioxins; PCB, polychlorinated biphenyls.

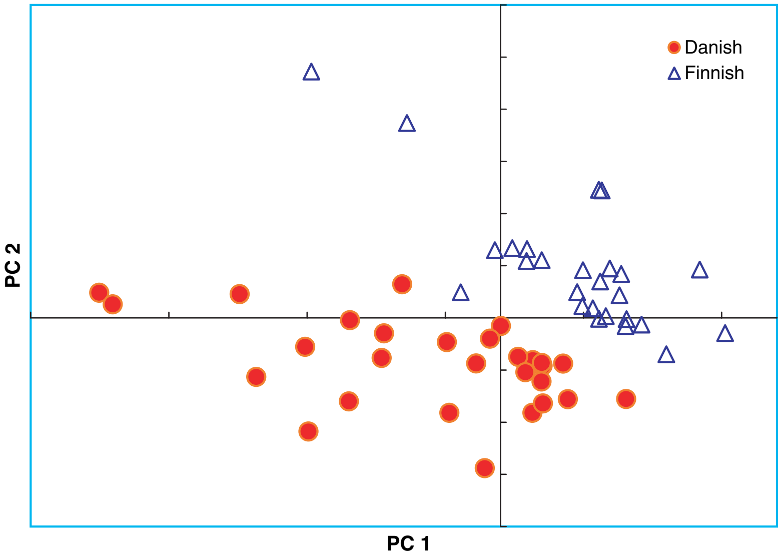

Figure 2 Scatter plot generated from the Partial Least Squares (PLS) model. Each point represents one milk sample, blue: Finnish, red: Danish. The location of each point is a reflection of the chemical concentration profile in the breast milk. The y- and x-axis are the first and second principal components, respectively, which are linear combinations of the concentration of the chemicals. The top 10 most important chemicals in each of the two principal components are listed as follows: PCI: 1,2,3,4,7,8-HCDD, PCB 209, PCB 156, PCB 189, PCB 170, PCB 157, PCB 194, PCB 180, o.p′-DDE, PCB 81. PC2: 1,2,3,6,7,8-HCDD, 1,2,3,4,6,7,8-HepCDD, Mirex, 1,2,3,4,6,7,8-HepCDF, OCDD, PeBB, BDe 154,1,2,3,4,7,8-HCDD, PCB 49, Octachlorostyrene.

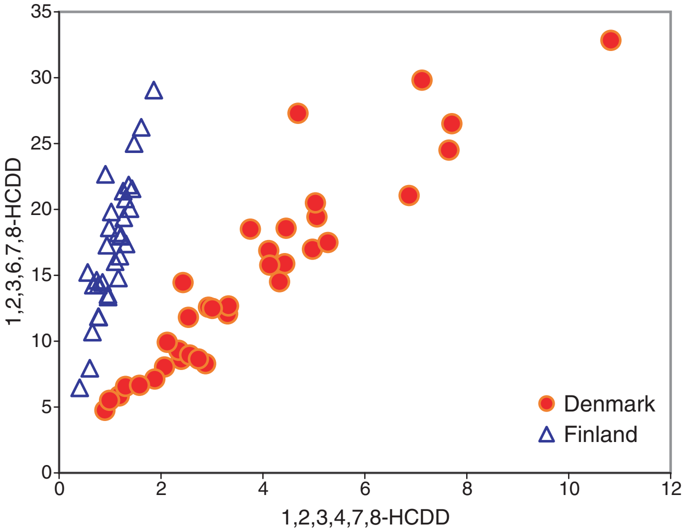

Figure 3 Two-dimensional scatter plot shows the concentration of the two chemicals 1,2,3,4,7,8-HCDD (x-axis) and 1,2,3,6,7,8-HCDD (y-axis) in each breast milk sample. The Danish (red) and Finnish (blue) samples are completely separated into two distinct groups. In each country, the two chemicals show clear linear correlations but with different slopes.

estimated to be 32.24 g I-TEQ/year in 2002 (Finnish Ministry of the Environment, 2006). As the Danish estimate is imprecise, we are unable to assess in which country the emission is higher. In our dataset, seven different PCDD congeners were present including 2,3,7,8-TCDD, which has the highest TEQ factor of all PCDDs and therefore considered the most toxic PCDD. Among the PCDDs measured, only 1,2,3,4,7,8-HCDD differed significantly between the nations and the levels of this compound was somewhat higher than the similar 2,3,7,8-TCDD. However, different PCDD-congeners vary in their biological effects (Niittynen *et al.*, 2007) and therefore the TEQ factors for the compounds do not necessarily reflect their endocrine disrupting potential and their effect on male reproductive health.

Hexachlorobenzene and dieldrin are both organochlorine pesticides that were introduced at about the same time in the 1940s. In Denmark and Finland, this hexachlorobenzene was banned from use as a pesticide in 1993 and 1996 respectively and was totally banned in 2003 and 2002, indicating that the regulation has been similar in both countries [Finnish Environment Institute, 2006; Miljøstyrelsen (Danish Ministry of the Environment), 2006]. Dieldrin, which recently has been shown to be toxic to foetal Leydig cells at low concentrations (Fowler *et al.*, 2007) has been used in Denmark in small amounts between 1956 and 1988 [Miljøstyrelsen (Danish Ministry of the Environment), 2006]. In Finland, dieldrin was stopped being used as a pesticide in 1970, but it was still manufactured for treatment of plywood for export until 2002. An extensive study of possibly contaminated sites in Finland in the early 1990s indicated that levels of dieldrin were very low (Finnish Ministry of the Environment, 2006).

Limitations and strengths

The participating lactating women had a narrow age distribution and were mainly from higher social classes in both countries. Therefore, they may not represent the populations in general. However, as most of the EDCs that we have measured are widely distributed in the environment and/or tend to accumulate in fat and in the food chain, it is likely that our findings in general are applicable to the wider population. Danish samples were on average collected 1.8 years later than Finnish samples, which is a confounder as environmental concentrations of the measured POPs have generally been declining during the past decade (Zietz *et al.*, 2008). However, as we detected higher levels of POPs in Danish samples, the true differences in exposures between Danes and Finns are likely to be even greater than we observed.

In addition, although our study included more than a hundred reproductive toxicants, it should be remembered that current environmental exposures involve many thousand chemicals which were not included in our study, but could still be part of the problem. These include perfluorinated compounds and several non-persistent chemicals for example currently used pesticides and industrial chemicals like bisphenol A, several phthalates and phthalate metabolites not included in our study, certain sun blockers, phytoestrogens and mycotoxins. Furthermore, we know little about the genetic variations in the metabolism of, and susceptibility to, these drugs. Therefore, although our study was extensive, future studies relating chemical exposures to diseases should aim at including an even larger list of these ubiquitous chemicals along with genotype data. Thus, exposure to the chemicals we analysed here may not alone explain the difference in incidence of male reproductive problems between the two nations.

Implications

Persistent chemicals obviously give rise to exposure of newborn babies through breastfeeding after birth. However, the levels of chemicals in breast milk can also be considered a proxy for exposure of the foetus during pregnancy by transfer across the placenta, as these persistent chemicals with very long metabolic half lives in the body show strong correlations between levels in breast milk and concentrations in maternal and foetal serum (Wang & Needham, 2007). Although little is known about the possible reproductive effects in the foetus of most of the measured chemicals, a number of them have already been implicated in such effects in animal and human studies. Additionally, more and more data suggest that the foetal testis is inherently more susceptible to endocrine-disrupting effects than the adult gonad (Andersen *et al.*, 2008; Welsh *et al.*, 2008). Our findings of distinctly different chemical exposure patterns and significantly higher levels of persistent compounds in samples from Denmark than from Finland could therefore play a causal role in the (yet unexplained) higher Danish incidence of male reproductive disorders.

Our data reinforce current thinking of the need to minimize human exposure to EDCs on precautionary grounds. In this regard, it should be noted that some such compounds (e.g. dioxins and flame retardants) are still being unintentionally or intentionally produced and released. Although human exposure to most POPs has decreased, this may not yet be discernible in incidence rates of testis cancer or other male reproductive disorders. For reasons of the persistent nature of these compounds, exposure will be passed on to the next one or even two generations and there is a long latency between perinatal exposure and (adult) manifestations of many reproductive disorders.

Conclusion

This comprehensive study on endocrine disrupting chemicals in Danish and Finnish breast milk samples revealed conspicuous differences: specific chemical signatures were found in the two countries. In addition, the levels of persistent compounds were significantly higher in samples from Denmark, where higher incidences of testis cancer, cryptorchidism, hypospadias and poor semen quality are present. Our findings are important, as these compounds are known for their endocrine disrupting abilities. Furthermore, animal studies, as well as recent human studies, have shown associations between some of the same agents and male reproductive problems.

Funding

This work was made possible by grants from Villum Kann Rasmussen Foundation, The Danish Medical Research Council, The Novo Nordisk Foundation, The Svend Andersen's Foundation, The program commission on Nanoscience, Biotechnology and IT (NABITT), EU (EDEN, DEER, EXPORED), Academy of Finland, Sigrid Jusélius Foundation, Pediatric Research Foundation, Turku University Hospital. The study sponsors played no role in the planning and execution of the research work. All researchers were independent from the study sponsors.

Competing interest declaration

None declared.

Ethical approval

The study was approved by the ethical committees in Finland and Denmark, which included the Joint Commission on Ethics of the Turku University and the Turku University Central Hospital, Turku, Finland; the Ethical Committees of Copenhagen and Frederiksberg County, Copenhagen, Denmark; and the Danish Data Protection Agency, Copenhagen, Denmark. The study complied with the Helsinki II declaration (World Medical Association 2004), after informed oral and written consent of the parents.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 All chemicals which were measured in mother's breast milk.

Table S2 Chemicals excluded from analysis, due to low or non-detectable levels.

Tables S3 (a–c) Results from investigating the country difference of each chemical individually, using multiple regression analysis. *p*-values have been corrected for multiple testing.

Tables S4 (a–c) Results for investigating the country difference of each chemical individually, using multiple regression analysis.

Tables S5 (a–c) Matthews Correlation Coefficients calculated from the performance of the machine learning classifiers on raw data.

Tables S6 (a–c) Matthews Correlation Coefficients calculated from the performance of the machine learning classifiers on interpolated data using regression coefficients.

Tables S7 (a–c) Matthews Correlation Coefficients calculated from the performance of the PLS classifier with country and all confounders as response variables.

Table S8 Performance when only including phthalates in the Machine Learning analyses.

Tables S9 (a–c) Chemicals selected as most important by the machine learning classifiers applied on raw data.

Tables S10 (a–c) Chemicals selected as most important by the machine learning classifiers applied on interpolated data using regression coefficients.

Tables S11 (a–c) Chemicals selected as most important by the PLS with classifier with country and confounders included as response variables.