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Chemosphere 313 (2023) 137343

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Levels of persistent organic pollutants in breast milk samples representing Finnish and Danish boys with and without hypospadias

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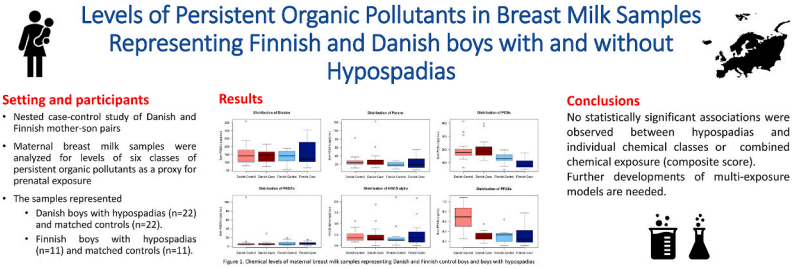
HIGHLIGHTS

- Animal studies suggest causal links between exposure to POPs and hypospadias.
- In this human case-control study composite exposure score was also evaluated.
- No association between hypospadias and exposure to POPs or mixtures was observed.

GRAPHICAL ABSTRACT



ARTICLE INFO

Handling Editor: A. Gies

Keywords:
Endocrine disruptors
Endocrine disrupting chemicals
EDCs
Hypospadias
POPs
Persistent organic pollutants

ABSTRACT

Hypospadias is a congenital malformation of penile urethra with unknown etiology in most cases. Persistent organic pollutant (POP) exposure may disrupt endocrine function during a critical window of development of male genitalia. In animal studies, POPs have been associated with male reproductive disorders, including hypospadias, but only few studies have assessed this relationship in humans. The aim of this study is to investigate the association between hypospadias and POP concentration levels in breast milk, as a proxy for prenatal exposure. This is a nested case-control study of Danish and Finnish mother-son pairs. Maternal breast milk samples were collected between 1997 and 2002, and they represent infant boys born with hypospadias [n = 33 (n = 22 Danish and n = 11 Finnish)] and their 1:1 matched controls. Breast milk samples were analyzed for six classes of POPs [including dioxins, polychlorinated biphenyls, flame retardants and perfluorinated alkylated

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https://doi.org/10.1016/j.chemosphere.2022.137343
Received 26 August 2022; Received in revised form 18 November 2022; Accepted 19 November 2022
Available online 21 November 2022

substances (PFAS)]. We estimated odds ratios (ORs) and 95% confidence intervals (CI) for each chemical class using conditional logistic regression. In addition, a composite exposure score system was used to explore the effect of a POP mixture (four chemical classes): The composite score was categorized as low, moderate, or high exposure, and differences between cases and controls were tested with conditional logistic regression. No statistically significant associations were observed between the sums of the chemical classes and hypospadias in either country. The composite score was unable to detect differences in the risk of hypospadias between the tertiles of POP exposure. Levels of PFAS were significantly higher in Danish than in Finnish breast milk samples. This small study does not provide evidence for an association between hypospadias and exposure to POPs but adds information on quantitative exposures. Further development of multi-exposure models is needed for assessing the potential mixture effect associated with multiple chemical exposures.

List of abbreviations

Ah	aryl hydrocarbon
BMI	body mass index
dl	dioxin-like
EDC	endocrine disrupting chemical
GC-HRMS	gas chromatography coupled to high-resolution mass spectrometry
HBCD	hexabromocyclododecane
JEM	job-exposure matrix
LC-HRMS	liquid chromatography coupled to high-resolution mass spectrometry
LC-MS/MS	liquid chromatography coupled to tandem mass spectrometry
LLE	liquid/liquid extraction
LOD	limit of detection
ndl	non-dioxin-like
PBDE	polybrominated diphenyl ethers
PCB	polychlorinated biphenyls
PCDD	polychlorinated dibenzo-p-dioxins
PCDF	polychlorinated dibenzofurans
PFAS	perfluorinated alkylated substances
PFOA	perfluorooctanoic acid
PFOS	perfluorooctanesulfonic acid
POP	Persistent organic pollutant
SPE	Solid Phase Extraction
WHO-TEq2005	World Health Organization Toxic Equivalent

1. Introduction

Hypospadias is a congenital malformation in males where the urethra opens on the ventral side of the penis, scrotum, or perineum, instead of the tip of the penis (van der Zanden et al., 2012). The mildest forms may not be detected until the foreskin can be retracted (Boisen et al., 2005). The mild glanular and coronal forms are the most common types found in Finnish and Danish infants (Boisen et al., 2005; Virtanen et al., 2001). The etiology of hypospadias is unknown in most human cases, but both genes and the environment are thought to have a role (Thorup et al., 2014; van der Zanden et al., 2012). Hypospadias may be caused by disruptions in balance between androgen and estrogen signaling, such as defects in biosynthesis, metabolism or action of androgens or exposure to estrogenic endocrine disrupting chemicals (EDCs) (Cripps et al., 2019; Cunha et al., 2015; Govers et al., 2019; Mattiske and Pask, 2021; Toppari et al., 2010). There is evidence that exposure to anti-androgens may be one mechanism by which penile abnormality develops (Christiansen et al., 2008; Sinclair et al., 2017). Animal studies have shown that hypospadias is common in male pups exposed in utero to chemicals inhibiting testosterone production or testosterone action via blocking the androgen receptor, such as pesticides and phthalates (Fisher et al., 2003; Gore et al., 2015; Mylchreest et al., 1999).

Many different EDCs found in the environment may have the potential to disrupt the sex hormone balance at a critical window of male genital development. EDCs include for instance persistent organic pollutants (POPs) and other chemicals, which tend to accumulate in adipose tissues and in the food chain (Bergman et al., 2013). Examples of such chemicals are dioxins, [i.e. polychlorinated dibenzo-*p*-dioxins (PCDDs) and related halogenated aromatic hydrocarbons, e.g. polychlorinated dibenzofurans (PCDFs)], polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and perfluorinated alkylated substances (PFAS). These chemicals have the potential to disrupt sex hormone balance through different mechanisms. Dioxins, that act via the aryl hydrocarbon (Ah) receptor, are not known to cause hypospadias on their own, but a combined disruption of the androgen and Ah receptor mediated pathways from exposure to chemical mixtures caused cumulative dose-additive effects on the development of male external genitalia (Rider et al., 2010). Dioxin-like (dl) PCBs also act via the Ah receptor, and non-dioxin-like (ndl) PCBs have anti-androgenic properties (Schrader and Cooke, 2003). PBDEs and perfluorooctanoic acid (PFOA) have been identified as having anti-androgenic potential, for at least some of their congeners (Eggert et al., 2019; Stoker et al., 2005). In utero exposure of rats to perfluorooctanesulfonic acid (PFOS) caused a reduced testosterone production and impairment of fetal Leydig cells (Zhao et al., 2014). PBDEs and HBCD are used as flame retardants. The effects of HBCD have not been well studied, though maternal serum levels of HBCD at third trimester of pregnancy showed a tendency to correlate negatively with serum free testosterone levels in their sons at the age of three months (Meijer et al., 2012). Several POPs have also estrogenic or antiestrogenic properties (De Coster and van Larebeke, 2012; Li et al., 2013). Although many POPs were banned or restricted in 2004 by the Stockholm convention or before, their ability to persist and bioaccumulate requires more research to fully understand their effects (Bergman et al., 2013).

Previous studies have shown that Danish male infants are exposed to larger concentrations of many EDCs than their Finnish counterparts (Antignac et al., 2016; Krysiak-Baltyn et al., 2010; Shen et al., 2008; Virtanen et al., 2012). Likewise, according to our joint prospective longitudinal cohort study, the prevalence of hypospadias was significantly higher in Denmark (1.03% of boys were diagnosed to have hypospadias by 3 months of age) than in Finland (0.27% of boys were diagnosed by the same age) (Boisen et al., 2005; Virtanen et al., 2001). An increasing trend of prevalence of hypospadias has been reported in many countries including Denmark (Boisen et al., 2005; Lund et al., 2009; Nissen et al., 2015), but not in Finland (Aho et al., 2000; Virtanen et al., 2001; Yu et al., 2019). In this case-control study, we examined the association between hypospadias and exposure to six groups of POPs. Levels of dioxins (PCDDs and PCDFs), PCBs, PBDEs, HBCD-α, and PFAS (PFOA and PFOS) were analyzed in the breast milk of mothers of

newborn boys in Denmark and Finland.

2. Subjects and methods

2.1. Study design and study population

This study involved the collaboration of Finnish and Danish research groups at two hospitals, Turku University Hospital and the University Hospital of Copenhagen (Rigshopitalet and Hvidovre Hospital). All the breast milk samples were collected in the Danish-Finnish prospective cohort study on the prevalence and risk factors of congenital cryptorchidism and hypospadias 1997–2002 (Boisen et al., 2004, 2005; Virtanen et al., 2001). For the current study a matched case-control design was used in both countries. Maternal breast milk chemical levels served as a proxy for prenatal exposure of newborn boys. In both countries, all hypospadias cases whose mothers succeeded in collection of sufficient amounts of breast milk sample were included in the current study. The boys were evaluated for hypospadias at birth and/or at 3 months. The standardized recruitment criteria, examination technique, and the observed prevalence rates of hypospadias have been published previously (Boisen et al., 2005; Virtanen et al., 2001). Results on chemical levels found in Finnish breast milk samples have been published previously except for PFAS, and the Danish data reported here has been published in part (Antignac et al., 2016). However, evaluation of case-control differences in maternal breast milk chemical levels between boys with and without hypospadias has not been reported before (Antignac et al., 2016).

The Danish group included n = 44 breast milk samples, representing 22 boys with hypospadias and 22 matched control boys. The Finnish group included n = 22 breast milk samples representing 11 boys with hypospadias and 11 matched controls. The matching criteria for each country was gestational age (± 7 days), maternal diabetes (yes/no), maternal smoking (yes/no), and parity, and date of birth (± 1 year).

The study was performed according to the Helsinki II declaration and was approved by the local Finnish and Danish ethics committees (KF 01-030/97/KF 01276357 in Copenhagen and 7/1996, 6/2001 in Turku) and the Danish Data Protection Agency (1997-1200-074). Oral and written informed consent was given by the parents.

2.2. Breast milk sample collection, storage, and measurements

Breast milk samples were recommended to be taken as hindmilk after feeding the child and without using breast pumps. All milk samples were collected as several small aliquots between 1- and 3-months post-partum for a sample size of 150 mL (up to a maximum of 200 mL) and frozen at each household in 250 mL Pyrex glass bottles (1515/06D; Bibby Sterilin, Staffordshire, UK) with Teflon coated caps at −20 °C until analysis.

The methodologies applied to isolate, detect, and quantify the targeted 6 POP chemical classes, including dioxins (17 PCDD/F congeners), PCBs (12 dl and 6 ndl congeners), 7 PBDE congeners, HBCD-α, and perfluoroalkylated substances (PFOA and PFOS) have been previously described (Antignac et al., 2009, 2013, 2016; Costera et al., 2006; Kadar et al., 2011), see complete list of the congeners in Supplementary Table S1. Briefly, the process of PCDD/Fs, PCBs, and HBCD analysis involved first extraction, then purification, and lastly quantification. Initially, ^{13}C-labelled internal standards were added to the breast milk samples and liquid/liquid extraction was performed with pentane. By gravimetric determination, the fat content was measured from extracts and hexane was added for the subsequent purification process which involved three chromatographic steps from silica to Florisil and lastly carbon columns. PCDD/F, PCB, PBDE measurements were performed by gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS) and HBCD measurements were performed by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Whereas the semi-persistent PFAS compounds were analyzed by a separate method described elsewhere (Kadar et al., 2011). This included a liquid/liquid extraction (LLE) for preliminary protein precipitation followed by two stages of purification by Solid Phase Extraction (SPE) first in Oasis® HLB and then carbon graphitized (Evanicarb®) cartridges. The extract was reconstituted in 200 μL of a fluorometholone solution as external standard in a methanol/water mixture (30/70 *v/v*). PFAS measurements were performed by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS).

These procedures were validated according to current European standards in the field of regular control of foodstuff of animal origin and the ISO 17025 standard. Quality Assurance/Quality Control (QA/QC) procedures included systematic analysis of negative (blank, n = 3) and positive (standard reference material, n = 1) control samples in all batches of analyzed samples, and several interlaboratory assays were completed at national and international level to confirm the method robustness and accuracy.

2.3. Statistics

Population characteristics are given as numbers or medians and ranges. Differences between boys with and without hypospadias were analyzed by country of origin with the Wilcoxon-Signed Rank test. As the samples were matched on gestational age, maternal diabetes, maternal smoking, and parity, the case-control differences were not tested for these variables.

In order to control for the time trends in exposure to POPs during the study period 1997–2002, a variable was created where each child was ranked by birth date and year by setting the first child born in this study to zero, and each child thereafter was ranked by the number of days that had elapsed since the first child's birthdate. This was considered the variable "rank of birthdate". This was calculated within each country and also for the two countries combined.

Conditional logistic regression was used when estimating the magnitude of associations between exposure to POPs and hypospadias in Finland and Denmark separately and combined. The sums of the congeners in each of 6 chemical classes [PCDDs, PCDFs, PCBs (seven indicator PCBs included: ndl congeners PCB-28, -52, −101, −138, −153, −180 and dl congener PCB-118), PBDEs, α-HBCD, and PFAS] were analyzed as independent continuous variables. This analysis also included the following subgroups: WHO-TEq$_{2005}$ PCDD/F, WHO-TEq$_{2005}$ dl-PCB, Total WHO-TEq$_{2005}$, coplanar PCBs, and non-coplanar PCBs. When the chemical measurement was below the lower limit of detection (LOD) the value was set as zero (lower bound approach). When evaluating for case-control differences, a stepwise conditional logistic regression with backward elimination was completed. Maternal body mass index (BMI), maternal age, weight for gestational age, and rank of birthdate in the cohort were included in the full model. Although not significant, the final adjusted regression model included only maternal BMI and maternal age. Multicollinearity was assessed for all continuous predictors and the correlations between exposure levels and other predictors were evaluated with Spearman's rho and Pearson's r. All analyses used untransformed data.

To assess the degree of exposure to mixtures of POPs a scoring system was created based on another poly-exposure classification model (Brucker-Davis et al., 2008). For each country, the sum of each chemical class for which 100% of samples were analyzed (PCDDs, PCDFs, PCBs, PBDEs) was divided into tertiles and scored - 1 was assigned for the first tertile, 2 for the second tertile, and 3 for the third tertile. The composite score for each subject (case or control) was calculated by adding the individual score numbers of the 4 chemical classes. The composite scores of all subjects were then divided into tertiles for low, moderate, and high exposure to the mixture. Differences in exposure levels between cases and controls were evaluated by country and also countries combined with simple conditional logistic regression using the low exposure as the reference group.

Differences in breast milk PFAS levels between Finland and Denmark were explored using Mann-Whitney-U test. The criterion for statistical

significance was a two-tailed p-value of <0.05. Correction for multiple comparisons testing was not applied. All statistics were performed using R and RStudio (v.3.3.2).

3. Results

The range of severity of cases with hypospadias consisted of 1 penoscrotal case, 1 coronal/penile case, and 31 glanular or coronal (mild) cases. Five of the cases had also congenital cryptorchidism. Three of the hypospadias cases had a first degree relative with hypospadias. Two hypospadias cases (one from Finland and one from Denmark) had a brother with hypospadias and one Danish case had a father with hypospadias. There were no significant differences between the cases and controls in maternal age, BMI, weight for gestational age, or birth weight in either country (Table 1) or combined (data not shown).

The chemical concentrations in the case and control groups are shown in Table 2. There were no significant differences in exposure levels between the cases and controls analyzed for the countries combined or separated (Fig. 1 and Supplementary Tables S2 and S3, respectively). There was no significant difference between cases and controls for the PCB congeners with the highest concentrations: PCB-118, -138, −153, −156, and −180 (Supplementary Table S4).

The composite score for the mixture of exposures is shown in Table 3. There was no difference between cases and controls when analyzing tertiles of composite exposure levels (countries combined or separately).

When comparing the Danish and Finnish data for their country-specific chemical signature, the sum of PFAS was significantly higher in Danish than in Finnish breast milk samples, median (range) 0.47 (0.16–1.08) ng/mL vs. 0.28 (0.11–0.77) ng/mL, respectively, p = 0.002.

4. Discussion

The hypothesis that prenatal exposure to POPs is associated with an increased risk of hypospadias (Skakkebaek et al., 2016) was not supported by our study. There was no association seen with individual chemical classes, or with combined chemical exposure (composite score). Human exposure to environmental chemicals occurs rarely in isolation and therefore evaluation of effects of chemical exposure using a composite score was done. This allowed us to consider the differences in the magnitude of chemical exposure between the two countries, with Finland having a significantly lower burden of exposure than Denmark for many POPs.

Human studies investigating the association between hypospadias and early exposure to chemicals are scarce, and of those, few studies use quantitative measurements from human tissues. This study adds to the epidemiologic evidence by including individual measurements of chemical levels in human breast milk. Many epidemiologic studies rely instead on retrospective surveys, questionnaires, interviews about past exposure through job-exposure matrices (JEMs) and exposure estimates based on residential information. Thus, they may be prone to misclassification bias. Some, but not all, studies have found associations between maternal residential exposure to EDCs (Agopian et al., 2013; Bougnères et al., 2021; Carmichael et al., 2013; Cognez et al., 2019; García et al., 2017; Meyer et al., 2006; Rappazzo et al., 2016; Sheth et al., 2019; Winston et al., 2016) or maternal and paternal job exposures to EDCs (especially to pesticides) and hypospadias (Estors Sastre et al., 2019; Giordano et al., 2010; Haraux et al., 2016; Kalfa et al., 2015; Morales-Surez-Varela et al., 2011; Nassar et al., 2010; Rocheleau et al., 2009, 2011; Vrijheid et al., 2003).

Breast milk chemical levels were used as a proxy for fetal exposure in our study (Damgaard et al., 2006). Other studies have investigated exposure to POPs, including additional chemicals that were not included in our analysis, by analyzing chemical levels in boys' serum, maternal serum, amniotic fluid or hair samples (Bhatia et al., 2005; Carmichael et al., 2010; Giordano et al., 2010; Koren et al., 2019; Longnecker et al., 2002; McGlynn et al., 2009; Poon et al., 2018; Rignell-Hydbom et al., 2012; Shekharyadav et al., 2011; Small et al., 2009; Toft et al., 2016; Trabert et al., 2012). Boys' serum levels of the pesticide hexachlorohexane and DDT metabolite DDE were significantly increased in hypospadias cases compared to controls in one study (Shekharyadav et al., 2011). High maternal serum levels of hexachlorobenzene were associated with increased risk of hypospadias in two studies (Giordano et al., 2010; Rignell-Hydbom et al., 2012). However, other studies evaluating maternal serum levels of pesticides, PCBs and flame retardants (PBDEs and polybrominated biphenyls) found no significant association with hypospadias or the results were inconsistent (Bhatia et al., 2005; Carmichael et al., 2010; Longnecker et al., 2002; McGlynn et al., 2009; Small et al., 2009; Trabert et al., 2012). Our study found no significant association between either PCB or PBDE exposure and hypospadias. However, a larger study evaluating PBDE levels in maternal hair samples reported significantly higher levels among mothers of infants with hypospadias (n = 152) as compared to controls (n = 64) (Koren et al., 2019; Poon et al., 2018). We found no significant association between PFAS exposure and hypospadias, which is in line with a previous study analyzing PFOS levels in amniotic fluid (Toft et al., 2016). We found no significant association between hypospadias and dioxin or HBCD-α exposure. To our knowledge, there are no previous studies evaluating these compounds.

Our previous studies reported higher chemical levels in Danish than in Finnish breast milk, cord blood, and whole placenta samples (Antignac et al., 2016; Jensen et al., 2014; Krysiak-Baltyn et al., 2010; Main et al., 2007; Virtanen et al., 2012) for many, but not all chemical groups presented here. We observed significantly higher PFAS levels in Danish milk samples as compared to Finnish milk samples. Country differences

Table 1
Population characteristics [median (range) or numbers] for Danish and Finnish mothers and infant boys.

	Denmark			Finland		
	Control (n = 22)	Hypospadias (n = 22)	p-value	Control (n = 11)	Hypospadias (n = 11)	p-value
Maternal age (years)	29.8 (23.6–36.3)	30.1 (22.9–36.6)	1.00	32.0 (27.4–44.7)	30.9 (23.8–39.6)	0.46
BMI (kg/m^2)[a]	22.4 (19.4–29.3)	20.8 (17.1–40.6)	0.12	21.2 (18.7–27.2)	23.3 (20.1–26.2)	0.10
Diabetes (yes/no)	0/22	0/22	NA	0/11	0/11	NA
Smoking (Y/N)[b]	4/17	5/17	NA	1/10	1/10	NA
Parity (no.)						
1	18	18	NA	5	4	NA
2	3	3		5	5	
≥ 3	1	1		1	2	
Gestational age (days)	286 (256–294)	285 (249–296)	NA	279 (263–288)	276 (249–303)	NA
Weight for gest. age (%)	3.0 (−10.0 - 15.1)	−4.4 (−31.0 - 44.4)	0.19	−1.5 (−11.0 - 23.0)	4.5 (−30.8 - 38.3)	1.00
Birth weight (kg)	3.8 (3.0–4.3)	3.6 (1.9–4.8)	0.14	3.5 (3.0–4.6)	3.6 (2.0–4.7)	1.00

Wilcoxon-Signed Rank test; BMI, body mass index; NA, not applicable due to matched design.
[a] Maternal BMI data is missing for 5 Danish mothers and 4 Finnish mothers.
[b] Smoking data is missing for 1 Danish mother.

Table 2
Breast milk chemical concentrations and odd ratios for the association with hypospadias.

Countries Combined											
		Control (n = 33)			Hypospadias (n = 33)			Crude[b]		Adjusted[c]	
Chemical Group[a]	Unit	DF (%)	n	Median (range)[a]	DF (%)	n	Median (range)[a]	p-value	OR (95% CI)	p-value	OR (95% CI)
ΣPCDD	pg/g l.w.	100	33	140.60 (55.10–358.10)	100	33	140.80 (66.85–305.70)	0.93	1.00 (0.99–1.01)	0.90	1.00 (0.99–1.01)
ΣPCDF	pg/g l.w.	100	33	22.56 (9.23–83.09)	100	33	21.71 (7.11–121.90)	0.41	1.02 (0.98–1.05)	0.79	1.01 (0.96–1.06)
WHO-TEq$_{2005}$ PCDD/F	pg/g l.w.	100	33	12.86 (3.93–29.50)	100	33	12.33 (3.48–38.59)	0.94	1.00 (0.91–1.10)	0.89	0.99 (0.86–1.14)
ΣPCB[d]	ng/g l.w.	100	33	165.00 (47.72–413.80)	100	33	158.30 (52.35–397.90)	0.53	0.99 (0.99–1.01)	0.53	0.99 (0.99–1.01)
ΣCoplanar PCB[e]	pg/g l.w.	100	33	90.70 (32.33–188.00)	100	33	89.09 (28.40–229.80)	0.45	1.01 (0.99–1.02)	0.38	1.01 (0.99–1.03)
ΣNon-coplanar PCB[f]	pg/g l.w.	100	33	25,550 (7297–60,420)	100	33	24,490 (7719–59,990)	0.57	1.00 (0.99[h] - 1.00)	0.66	1.00 (0.99[h] - 1.00)
WHO-TEq$_{2005}$ dl-PCB	pg/g l.w.	100	33	6.65 (2.28–13.56)	100	33	6.53 (2.01–16.41)	0.66	1.04 (0.86–1.26)	0.70	1.04 (0.83–1.31)
Total WHO-TEq$_{2005}$	pg/g l.w.	100	33	19.68 (6.21–42.93)	100	33	18.08 (5.72–46.24)	0.82	1.01 (0.94–1.08)	0.95	1.00 (0.92–1.10)
ΣPBDE	ng/g l.w.	100	33	4.75 (2.16–111.10)	100	33	5.24 (1.47–28.91)	0.49	0.98 (0.94–1.03)	0.40	0.93 (0.79–1.10)
α-HBCD[g]	ng/g l.w.	100	32	0.32 (0.03–2.19)	94	33	0.31 (0.0–2.15)	0.69	1.36 (0.30–6.15)	0.72	1.35 (0.26–7.14)
ΣPFAS	ng/mL	100	27	0.48 (0.12–1.08)	100	23	0.27 (0.11–0.77)	0.06	0.03 (<0.001–1.10)	0.08	0.01 (<0.001 to1.70)

DF, detection frequency of chemicals in breast milk samples; OR, odds ratio; CI, confidence interval; PCDD/PCDF, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; WHO-Teq, World Health Organization Toxic Equivalent; PCB, polychlorinated biphenyls; dl: dioxin-like; l.w., lipid weight; PBDE, polybrominated diphenyl ethers; HBCD, hexabromocyclododecane; PFAS, perfluorinated alkylated substances.

[a] Untransformed chemical data.
[b] Conditional logistic regression model.
[c] Conditional logistic regression model adjusted for maternal age and maternal BMI, body mass index.
[d] Sum of PCB-28, -52, −101, −118, −138, −153, −180 [e]coplanar PCB-77, -81, 126, −169.
[f] Noncoplanar PCB-105, -114, −118, −123, −156, −157, −167, −189.
[g] One outlier removed.
[h] 0.99999.

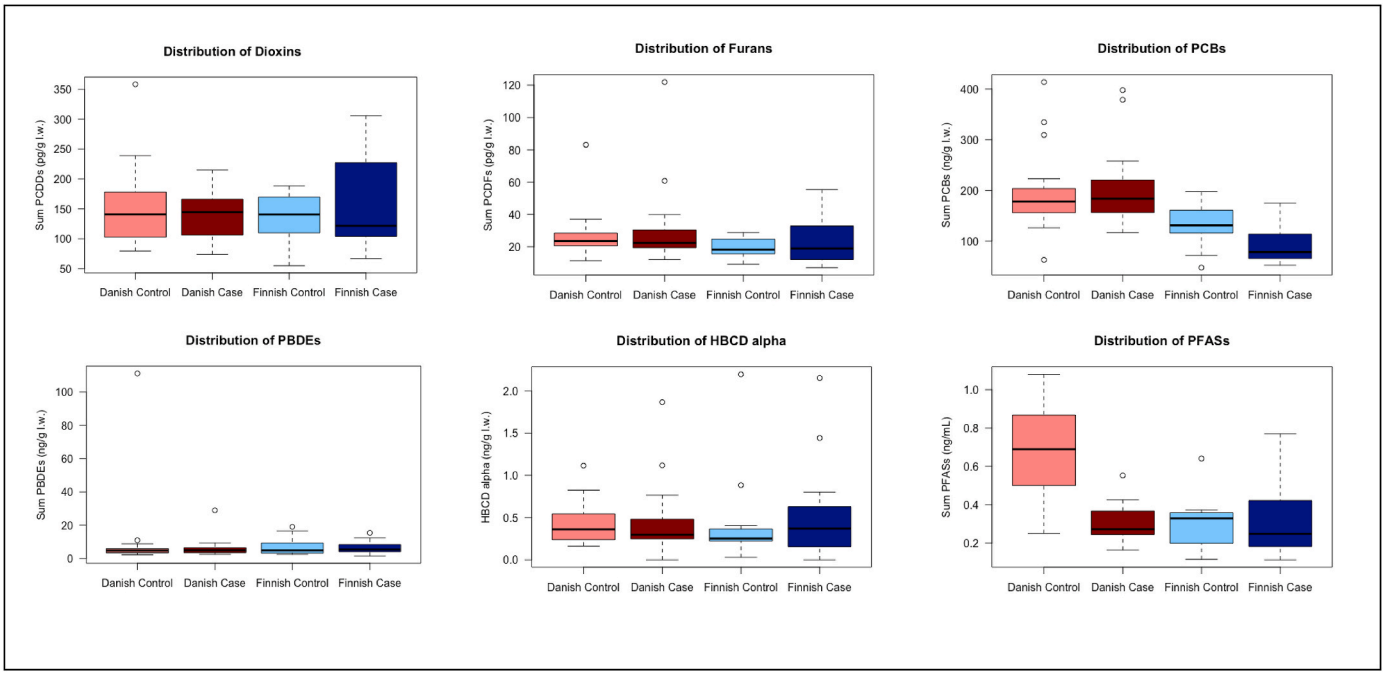

Fig. 1. Distributions of levels of persistent organic pollutants (sum of PCDDs, PCDFs, PCBs, PBDEs, PFASs and HBCD-α) in breast milk samples representing Danish and Finnish control boys and boys with hypospadias. (PCDDs, polychlorinated dibenzo-p-dioxins; PCDFs, polychlorinated dibenzofurans; PCBs, polychlorinated biphenyls; PBDEs, polybrominated diphenyl ethers; HBCD-α, hexabromocyclododecane alpha; PFASs, perfluorinated alkylated substances).

Table 3
Composite scores of exposure and odds ratios for the associations with hypospadias.

Countries Combined Composite Score						
Composite Score	Score	Total	Controls	Hypospadias	*p*-value[a]	OR (95% CI)
4 chemical classes[b]	low (4-6)	23	10	13	reference	reference
	moderate (7-9)	26	16	10	0.27	0.49 (0.14–1.75)
	high (10-12)	17	7	10	0.83	0.85 (0.20–3.63)
Danish Composite Score						
Composite Score	**Score**	**Total**	**Controls**	**Hypospadias**	***p*-value**[a]	**OR (95% CI)**
4 chemical classes[b]	low (4-6)	15	6	9	reference	reference
	moderate (7-9)	17	11	6	0.27	0.48 (0.13–1.75)
	high (10-12)	12	5	7	0.90	0.90 (0.19–4.28)
Finnish Composite Score						
Composite Score	**Score**	**Total**	**Controls**	**Hypospadias**	***p*-value**[a]	**OR (95% CI)**
4 chemical classes[b]	low (4-6)	8	4	4	reference	reference
	moderate (7-9)	9	5	4	0.66	0.67 (0.11–3.99)
	high (10-12)	5	2	3	NA	NA

OR, odds ratio; CI, confidence interval; NA not applicable because not accurately estimated.
For each country, the sum of each chemical class for which 100% of samples were analyzed (PCDDs, PCDFs, PCBs, PBDEs) was divided into tertiles and scored: 1 was assigned for the first tertile, 2 for the second tertile, and 3 for the third tertile. The composite score for each subject was obtained by summing up the individual score numbers of the 4 chemical classes (range 4–12). The composite scores of all subjects were then divided into tertiles for low, moderate, and high exposure.
[a] Simple conditional logistic regression.
[b] Chemical groups include: ΣPCDD, ΣPCDF, ΣPCB, ΣPBDE.

in exposure to PFASs were also observed in cord blood samples during this time period (1997–2002), with Denmark having an approximately two-fold higher exposure than Finland (Jensen et al., 2014).

Hypospadias is a rare condition and it is therefore difficult to collect enough cases to reach statistical power within longitudinal cohort studies (Skakkebaek et al., 2016). Many studies that found non-significant results often also had small samples sizes and/or they included cases of hypospadias combined with cases of cryptorchidism. Our study represents a small dataset (33 cases) and may thus be underpowered to show a true difference between cases and controls. In addition, we cannot exclude some variation in timing of breast milk sample collection (collection time up to two months), which may affect chemical levels (Thomsen et al., 2010). Furthermore, POPs have been regulated in the past several decades and subsequently, their production and release has declined as observed by time trends in air and human tissue samples (Hardell et al., 2010; Hung et al., 2016), which may have reduced our potential to find a significant association.

5. Conclusions

In conclusion, our study found no evidence for an association between hypospadias and prenatal exposure to POPs for individual chemical groups or a composite exposure score for a mixture of PCDDs, PCDFs, PCBs and PBDEs. Further developments of multi-exposure models are needed for assessing the potential mixture effect associated to the multiple chemical exposures.

Credit author statement

Marie Tysman: Formal analysis, Writing – original draft, review & editing. Jorma Toppari: Conceptualization, Formal analysis, Writing – review & editing. Katharina M Main: Conceptualization, Investigation, Writing – review & editing. Annika Adamsson: Investigation, Formal analysis, Writing – original draft, review & editing. Christine Wohlfahrt-Veje: Investigation, Project administration, Writing – review & editing. Jean-Philippe Antignac: Investigation, Methodology, Writing – review & editing. Bruno Le Bizec: Conceptualization, Methodology, Investigation, Writing – review & editing. Eliisa Löyttyniemi: Formal analysis, Writing – review & editing. Niels E Skakkebaek: Conceptualization, Writing – review & editing. Helena E Virtanen: Investigation, Formal analysis, Project administration, Writing – original draft, review & editing.

Data statement

The dataset supporting the conclusions of this article comprises health related participant data and their use is therefore restricted under the regulations on professional secrecy (Act on the Openness of Government Activities, 612/1999) and on sensitive personal data (Personal Data Act, 523/1999, implementing the EU data protection directive 95/46/EC). Due to these legal restrictions, the data from this study cannot be stored in public repositories or otherwise made publicly available. However, data access may be permitted on a case by case basis upon request only. Data sharing outside the research groups is done in collaboration with the research groups and requires a group-specific data transfer agreements (DTA). Investigators can submit an expression of interest to the chairman of the Finnish steering group (Prof Jorma Toppari, University of Turku, Turku, Finland. Email: jorma.toppari@utu.fi).

Funding

The study was funded in the framework of the EU DEER research project (F97-ENV-2007-1; Grant 212,844), and also by Danish Agency for Science, Technology and Innovation (09-067180), Research Fund of Rigshospitalet, Copenhagen University Hospital, Academy of Finland (308065), Sigrid Jusélius Foundation, and Turku University Hospital. We would like to thank Fulbright Finland for this opportunity for collaboration between the U.S. and Finland on environmental health research. The funding sources had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Acknowledgements

We are grateful to the families for their participation in this mother-son cohort study. We thank the research team and technical staff, especially Mrs Johanna Järvi, for their sustained involvement.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2022.137343.

References

Agopian, A., Lupo, P., Canfield, M., Langlois, P., 2013. Case-control study of maternal residential atrazine exposure and male genital malformations. Am. J. Med. Genet. A 161A, 977–982. https://doi.org/10.1002/ajmg.a.35815.

Aho, M., Koivisto, A.M., Tammela, T.L.J., Auvinen, A., 2000. Is the incidence of hypospadias increasing? Analysis of Finnish hospital discharge data 1970-1994. Environ. Health Perspect. 108, 463–465. https://doi.org/10.1289/ehp.00108463.

Antignac, J.P., Veyrand, B., Kadar, H., Marchand, P., Oleko, A., Bizec, B. Le, Vandentorren, S., LeBizec, B., 2013. Occurrence of perfluorinated contaminants in breast milk of French women and relation with socio-demographical and clinical parameters: results of the ELFE pilot study. Chemosphere 91, 802–808. https://doi.org/10.1016/j.chemosphere.2013.01.088.

Antignac, J.P., Cariou, R., Zalko, D., Berrebi, A., Cravedi, J.P., Maume, D., Marchand, P., Monteau, F., Riu, A., Andre, F., Le Bizec, B., 2009. Exposure assessment of French women and their newborn to brominated flame retardants: determination of tri- to deca- polybromodiphenylethers (PBDE) in maternal adipose tissue, serum, breast milk and cord serum. Environ. Pollut. 157, 164–173. https://doi.org/10.1016/j.envpol.2008.07.008.

Antignac, J.P., Main, K.M., Virtanen, H.E., Boquien, C.Y., Marchand, P., Venisseau, A., Guiffard, I., Bichon, E., Wohlfahrt-Veje, C., Legrand, A., Boscher, C., Skakkebæk, N. E., Toppari, J., Le Bizec, B., 2016. Country-specific chemical signatures of persistent organic pollutants (POPs) in breast milk of French, Danish and Finnish women. Environ. Pollut. 218, 728–738. https://doi.org/10.1016/j.envpol.2016.07.069.

Bergman, Å., Heindel, J.J., Jobling, S., Kid, K.A., Zoeller, R.T., 2013. State of the Science of Endocrine Disrupting Chemicals 2012. United Nations Environmental Programme and the World Health Organization IOMC.

Bhatia, R., Shiau, R., Petreas, M., Weintraub, J.M., Farhang, L., Eskenazi, B., 2005. Organochlorine pesticides and male genital anomalies in the child health and development studies. Environ. Health Perspect. 113, 220–224. https://doi.org/10.1289/ehp.7382.

Boisen, K.A., Chellakooty, M., Schmidt, I.M., Kai, C.M., Damgaard, I.N., Suomi, A.M., Toppari, J., Skakkebæk, N.E., Main, K.M., 2005. Hypospadias in a cohort of 1072 Danish newborn boys: prevalence and relationship to placental weight, anthropometrical measurements at birth, and reproductive hormone levels at three months of age. J. Clin. Endocrinol. Metabol. 90, 4041–4046. https://doi.org/10.1210/jc.2005-0302.

Boisen, K.A., Kaleva, M., Main, K.M., Virtanen, H.E., Haavisto, A.M., Schmidt, I.M., Chellakooty, M., Damgaard, I.N., Mau, C., Reunanen, M., Skakkebaek, N.E., Toppari, J., 2004. Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. Lancet 363, 1264–1269. https://doi.org/10.1016/S0140-6736(04)15998-9.

Bougnères, P., Porcher, R., Esterle, L., Baker, D., de la Vaissière, A., Meurisse, S., Valtat, S., Castell, A.L., Mouriquand, P., Valleron, A.J., 2021. Exploring the risk of hypospadias in children born from mothers living close to a vineyard. PLoS One 16, 1–10. https://doi.org/10.1371/journal.pone.0249800.

Brucker-Davis, F., Wagner-Mahler, K., Delattre, I., Ducot, B., Ferrari, P., Bongain, A., Kurzenne, J.Y., Mas, J.C., Fénichel, P., Pacini, P., Boda-Buccino, M., Bornebusch, L., Azuar, P., Dupuy, M., Meneguz, C., Tommasi, C., Pradier, C., Maccagnan, S., Court, F., Lecuyer, A., 2008. Cryptorchidism at birth in Nice area (France) is associated with higher prenatal exposure to PCBs and DDE, as assessed by colostrum concentrations. Hum. Reprod. 23, 1708–1718. https://doi.org/10.1093/humrep/den186.

Carmichael, S.L., Herring, A.H., Sjödin, A., Jones, R., Needham, L., Ma, C., Ding, K., Shaw, G.M., 2010. Hypospadias and halogenated organic pollutant levels in maternal mid-pregnancy serum samples. Chemosphere 80, 641–646. https://doi.org/10.1016/j.chemosphere.2010.04.055.

Carmichael, S.L., Yang, W., Roberts, E.M., Kegley, S.E., Wolff, C., Guo, L., Lammer, E.J., English, P., Shaw, G.M., 2013. Hypospadias and residential proximity to pesticide applications. Pediatrics 132, 1216–1226. https://doi.org/10.1542/peds.2013-1429.

Christiansen, S., Scholze, M., Axelstad, M., Boberg, J., Kortenkamp, A., Hass, U., 2008. Combined exposure to anti-androgens causes markedly increased frequencies of hypospadias in the rat. Int. J. Androl. 31, 241–248. https://doi.org/10.1111/j.1365-2605.2008.00866.x.

Cognez, N., Warembourg, C., Zaros, C., Metten, M.A., Bouvier, G., Garlantézec, R., Charles, M.A., Béranger, R., Chevrier, C., 2019. Residential sources of pesticide exposure during pregnancy and the risks of hypospadias and cryptorchidism: the French ELFE birth cohort. Occup. Environ. Med. 76, 672–679. https://doi.org/10.1136/oemed-2019-105801.

Costera, A., Feidt, C., Marchand, P., Bizec, B.L., Rychen, G., 2006. PCDD/F and PCB transfer to milk in goats exposed to a long-term intake of contaminated hay. Chemosphere 64, 650–657. https://doi.org/10.1016/j.chemosphere.2005.10.052.

Cripps, S.M., Mattiske, D.M., Black, J.R., Risbridger, G.P., Govers, L.C., Phillips, T.R., Pask, A.J., 2019. A loss of estrogen signaling in the aromatase deficient mouse penis results in mild hypospadias. Differentiation 109, 42–52. https://doi.org/10.1016/j.diff.2019.09.001.

Cunha, G.R., Sinclair, A., Risbridger, G., Hutson, J., Baskin, L.S., 2015. Current understanding of hypospadias: relevance of animal models. Nat. Rev. Urol. 12, 271–280. https://doi.org/10.1038/nrurol.2015.57.

Damgaard, I.N., Skakkebæk, N.E., Toppari, J., Virtanen, H.E., Shen, H., Schramm, K.W., Petersen, J.H., Jensen, T.K., Main, K.M., the Nordic Cryptorchidism Study Group, 2006. Persistent pesticides in human breast milk and cryptorchidism. Environ. Health Perspect. 114, 1133–1138. https://doi.org/10.1289/ehp.8741.

De Coster, S., van Larebeke, N., 2012. Endocrine-disrupting chemicals: associated disorders and mechanisms of action. J. Environ. Public Health. https://doi.org/10.1155/2012/713696.

Eggert, A., Cisneros-Montalvo, S., Anandan, S., Musilli, S., Stukenborg, J.B., Adamsson, A., Nurmio, M., Toppari, J., 2019. The effects of perfluorooctanoic acid (PFOA) on fetal and adult rat testis. Reprod. Toxicol. 90, 68–76. https://doi.org/10.1016/j.reprotox.2019.08.005.

Estors Sastre, B., Campillo Artero, C., González Ruiz, Y., Fernández Atuan, R., Rodríguez Bragagnini, P., Frontera Juan, G., Gracia Romero, J., 2019. Occupational exposure to endocrine-disrupting chemicals and other parental risk factors in hypospadias and cryptorchidism development: a case–control study. J. Pediatr. Urol. 15, 520.e1–520.e8. https://doi.org/10.1016/j.jpurol.2019.07.001.

Fisher, J.S., Macpherson, S., Marchetti, N., Sharpe, R.M., 2003. Human "testicular dysgenesis syndrome": a possible model using in-utero exposure of the rat to dibutyl phthalate. Hum. Reprod. 18, 1383–1394. https://doi.org/10.1093/humrep/deg273.

García, J., Ventura, M.I., Requena, M., Hernández, A.F., Parrón, T., Alarcón, R., 2017. Association of reproductive disorders and male congenital anomalies with environmental exposure to endocrine active pesticides. Reprod. Toxicol. 71, 95–100. https://doi.org/10.1016/j.reprotox.2017.04.011.

Giordano, F., Abballe, A., De Felip, E., Di Domenico, A., Ferro, F., Grammatico, P., Ingelido, A.M., Marra, V., Marrocco, G., Vallasciani, S., Figà-Talamanca, I., 2010. Maternal exposures to endocrine disrupting chemicals and hypospadias in offspring. Birth Defects Res. A 88, 241–250. https://doi.org/10.1002/bdra.20657.

Gore, A.C., Chappell, V.A., Fenton, S.E., Flaws, J.A., Nadal, A., Prins, G.S., Toppari, J., Zoeller, R.T., 2015. EDC-2: the endocrine society's second scientific statement on endocrine-disrupting chemicals. Endocr. Rev. 36, 1–150. https://doi.org/10.1210/er.2015-1010.

Govers, L.C., Phillips, T.R., Mattiske, D.M., Rashoo, N., Black, J.R., Sinclair, A., Baskin, L. S., Risbridger, G.P., Pask, A.J., 2019. A critical role for estrogen signaling in penis development. Faseb. J. 33, 10383–10392. https://doi.org/10.1096/fj.201802586RR.

Haraux, E., Braun, K., Buisson, P., Stéphan-Blanchard, E., Devauchelle, C., Ricard, J., Boudailliez, B., Tourneux, P., Gouron, R., Chardon, K., 2016. Maternal exposure to domestic hair cosmetics and occupational endocrine disruptors is associated with a higher risk of hypospadias in the offspring. Int. J. Environ. Res. Publ. Health 14, 27. https://doi.org/10.3390/ijerph14010027.

Hardell, E., Carlberg, M., Nordström, M., van Bavel, B., 2010. Time trends of persistent organic pollutants in Sweden during 1993-2007 and relation to age, gender, body mass index, breast-feeding and parity. Sci. Total Environ. 408, 4412–4419. https://doi.org/10.1016/j.scitotenv.2010.06.029.

Hung, H., Katsoyiannis, A.A., Brorström-Lundén, E., Olafsdottir, K., Aas, W., Breivik, K., Bohlin-Nizzetto, P., Sigurdsson, A., Hakola, H., Bossi, R., Skov, H., Sverko, E., Barresi, E., Fellin, P., Wilson, S., 2016. Temporal trends of persistent organic pollutants (POPs) in arctic air: 20 years of monitoring under the Arctic Monitoring and Assessment Programme (AMAP). Environ. Pollut. 217, 52–61. https://doi.org/10.1016/j.envpol.2016.01.079.

Jensen, D.V., Christensen, J., Virtanen, H.E., Skakkebæk, N.E., Main, K.M., Toppari, J., Veje, C.W., Andersson, A.M., Nielsen, F., Grandjean, P., Jensen, T.K., 2014. No association between exposure to perfluorinated compounds and congenital cryptorchidism: a nested case-control study among 215 Boys from Denmark and Finland. Reproduction 147, 411–417. https://doi.org/10.1530/REP-13-0444.

Kadar, H., Veyrand, B., Barbarossa, A., Pagliuca, G., Legrand, A., Bosher, C., Boquien, C. Y., Durand, S., Monteau, F., Antignac, J.P., Le Bizec, B., 2011. Development of an analytical strategy based on liquid chromatography-high resolution mass spectrometry for measuring perfluorinated compounds in human breast milk: application to the generation of preliminary data regarding perinatal exposure in France. Chemosphere 85, 473–480. https://doi.org/10.1016/j.chemosphere.2011.07.077.

Kalfa, N., Paris, F., Philibert, P., Orsini, M., Broussous, S., Fauconnet-Servant, N., Audran, F., Gaspari, L., Lehors, H., Haddad, M., Guys, J.M., Reynaud, R., Alessandrini, P., Merrot, T., Wagner, K., Kurzenne, J.Y., Bastiani, F., Bréaud, J., Valla, J.S., Lacombe, G.M., Dobremez, E., Zahhaf, A., Daures, J.P., Sultan, C., 2015. Is hypospadias associated with prenatal exposure to endocrine disruptors? A French collaborative controlled study of a cohort of 300 consecutive children without genetic defect. Eur. Urol. 68, 1023–1030. https://doi.org/10.1016/j.eururo.2015.05.008.

Koren, G., Carnevale, A., Ling, J., Ozsarfati, J., Kapur, B., Bagli, D., 2019. Fetal exposure to polybrominated diphenyl ethers and the risk of hypospadias: focus on the

congeners involved. J. Pediatr. Urol. 15, 405.e1–405.e6. https://doi.org/10.1016/j.jpurol.2019.05.023.
Krysiak-Baltyn, K., Toppari, J., Skakkebaek, N.E., Jensen, T.S., Virtanen, H.E., Schramm, K.W., Shen, H., Vartiainen, T., Kiviranta, H., Taboureau, O., Brunak, S., Main, K.M., 2010. Country-specific chemical signatures of persistent environmental compounds in breast milk. Int. J. Androl. 33, 270–278. https://doi.org/10.1111/j.1365-2605.2009.00996.x.
Li, X., Gao, Y., Guo, L.-H., Jiang, G., 2013. Structure-dependent activities of hydroxylated polybrominated diphenyl ethers on human estrogen receptor. Toxicology 309, 15–22. https://doi.org/10.1016/j.tox.2013.04.001.
Longnecker, M.P., Klebanoff, M.A., Brock, J.W., Zhou, H., Gray, K.A., Larry, L., 2002. Maternal serum level of 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene and risk of cryptorchidism, hypospadias, and polythelia among male offspring. Am. J. Epidemiol. 155, 313–322. https://doi.org/10.1093/aje/155.4.313.
Lund, L., Engebjerg, M., Pedersen, L., Ehrenstein, V., Nørgaard, M., Sørensen, H., 2009. Prevalence of hypospadias in Danish boys: a longitudinal study, 1977-2005. Eur. Urol. 55, 1022–1026. https://doi.org/10.1016/j.eururo.2009.01.005.
Main, K.M., Kiviranta, H., Virtanen, H.E., Sundqvist, E., Tuomisto, J.T., Tuomisto, J., Vartiainen, T., Skakkebæk, N.E., Topparl, J., 2007. Flame retardants in placenta and breast milk and cryptorchildism in newborn boys. Environ. Health Perspect. 115, 1519–1526. https://doi.org/10.1289/ehp.9924.
Mattiske, D.M., Pask, A.J., 2021. Endocrine disrupting chemicals in the pathogenesis of hypospadias; developmental and toxicological perspectives. Curr. Res. Toxicol. 2, 179–191. https://doi.org/10.1016/j.crtox.2021.03.004.
McGlynn, K.A., Guo, X., Graubard, B.I., Brock, J.W., Klebanoff, M.A., Longnecker, M.P., 2009. Maternal pregnancy levels of polychlorinated biphenyls and risk of hypospadias and cryptorchidism in male offspring. Environ. Health Perspect. 117, 1472–1476. https://doi.org/10.1289/ehp.0800389.
Meijer, L., Martijn, A., Melessen, J., Brouwer, A., Weiss, J., De Jong, F.H., Sauer, P.J.J., 2012. Influence of prenatal organohalogen levels on infant male sexual development: sex hormone levels, testes volume and penile length. Hum. Reprod. 27, 867–872. https://doi.org/10.1093/humrep/der426.
Meyer, K.J., Reif, J.S., Rao Veeramachaneni, D.N., Luben, T.J., Mosley, B.S., Nuckols, J. R., 2006. Agricultural pesticide use and hypospadias in Eastern Arkansas. Environ. Health Perspect. 114, 1589–1595. https://doi.org/10.1289/ehp.9146.
Morales-Surez-Varela, M.M., Toft, G.V., Jensen, M.S., Ramlau-Hansen, C., Kaerlev, L., Thulstrup, A.-M., Llopis-Gonzlez, A., Olsen, J., Bonde, J.P., 2011. Parental occupational exposure to endocrine disrupting chemicals and male genital malformations: a study in the Danish national birth cohort study. Environ. Health 10, 1–9. https://doi.org/10.1186/1476-069X-10-3.
Mylchreest, E., Sar, M., Cattley, R., Foster, P., 1999. Disruption of androgen-regulated male reproductive development by di(n-butyl)phthalate during late gestation in rats is different from flutamide. Toxicol. Appl. Pharmacol. 156, 81–95. https://doi.org/10.1006/taap.1999.8643.
Nassar, N., Abeywardana, P., Barker, A., Bower, C., 2010. Parental occupational exposure to potential endocrine disrupting chemicals and risk of hypospadias in infants. Occup. Environ. Med. 67, 585–589. https://doi.org/10.1136/oem.2009.048272.
Nissen, K.B., Udesen, A., Garne, E., 2015. Hypospadias: prevalence, birthweight and associated major congenital anomalies. Congenital. Anom. 55, 37–41. https://doi.org/10.1111/cga.12071.
Poon, S., Koren, G., Carnevale, A., Aleksa, K., Ling, J., Ozsarfati, J., Kapur, B.M., Bagli, D., 2018. Association of in utero exposure to polybrominated diphenyl ethers with the risk of hypospadias. JAMA Pediatr. 172, 851–856. https://doi.org/10.1001/jamapediatrics.2018.1492.
Rappazzo, K., Warren, J., Meyer, R., Herring, A., Sanders, A., Brownstein, N.C., Luben, T. J., 2016. Maternal residential exposure to agricultural pesticides and birth defects in a 2003-2005 North Carolina birth cohort. Birth Defects Res. A 106, 240–249. https://doi.org/10.1002/bdra.23479.
Rider, C.V., Furr, J.R., Wilson, V.S., Earl Gray Jr., L., 2010. Cumulative effects of in utero administration of mixures of reproductive toxicants that disrupt common target tissues via diverse mechanisms of toxicity. Int. J. Androl. 33, 443–462. https://doi.org/10.1111/j.1365-2605.2009.01049.x.
Rignell-Hydbom, A., Lindh, C.H., Dillner, J., Jönsson, B.A.G., Rylander, L., 2012. A nested case-control study of intrauterine exposure to persistent organochlorine pollutants and the risk of hypospadias. PLoS One 7, 1–5. https://doi.org/10.1371/journal.pone.0044767.
Rocheleau, C.M., Romitti, P.A., Dennis, L.K., 2009. Pesticides and hypospadias: a meta-analysis. J. Pediatr. Urol. 5, 17–24. https://doi.org/10.1016/j.jpurol.2008.08.006.
Rocheleau, C.M., Romitti, P.A., Sanderson, W.T., Sun, L., Lawson, C.C., Waters, M.A., Stewart, P.A., Olney, R.S., Reefhuis, J., 2011. Maternal occupational pesticide exposure and risk of hypospadias in the national birth defects prevention study. Birth Defects Res. A 91, 927–936. https://doi.org/10.1002/bdra.22860.
Schrader, T.J., Cooke, G.M., 2003. Effects of aroclors and individual PCB congeners on activation of the human androgen receptor in vitro. Reprod. Toxicol. 17, 15–23. https://doi.org/10.1016/s0890-6238(02)00076-x.
Shekharyadav, C., Bajpai, M., Kumar, V., Ahmed, R.S., Gupta, P., Banerjee, B.D., 2011. Polymorphism in CYP1A1, GSTMI,GSTT1 genes and organochlorine pesticides in the etiology of hypospadias. Hum. Exp. Toxicol. 30, 1464–1474. https://doi.org/10.1177/0960327110392402.
Shen, H., Main, K.M., Andersson, A.-M., Damgaard, I.N., Virtanen, H.E., Skakkebaek, N. E., Toppari, J., Schramm, K.-W., 2008. Concentrations of persistent organochlorine compounds in human milk and placenta are higher in Denmark than in Finland. Hum. Reprod. 23, 201–210. https://doi.org/10.1093/humrep/dem199.
Sheth, K.R., Kovar, E., White, J.T., Chambers, T.M., Peckham-Gregory, E.C., O'Neill, M., Langlois, P.H., Seth, A., Scheurer, M.E., Lupo, P.J., Jorgez, C.J., 2019. Hypospadias risk is increased with maternal residential exposure to hormonally active hazardous air pollutants. Birth Defects Res. A 111, 345–352. https://doi.org/10.1002/bdr2.1461.
Sinclair, A.W., Cao, M., Pask, A., Baskin, L., Cunha, G.R., 2017. Flutamide-induced hypospadias in rats: a critical assessment. Differentiation 94, 37–57. https://doi.org/10.1016/j.diff.2016.12.001.
Skakkebaek, N.E., Rajpert-De Meyts, E., Buck Louis, G.M., Toppari, J., Andersson, A.-M., Eisenberg, M.L., Jensen, T.K., Jorgensen, N., Swan, S.H., Sapra, K.J., Ziebe, S., Priskorn, L., Juul, A., 2016. Male reproductive disorders and fertility trends: influences of environment and genetic susceptibility. Physiol. Rev. 96, 55–97. https://doi.org/10.1152/physrev.00017.2015.
Small, C.M., DeCaro, J.J., Terrell, M.L., Dominguez, C., Cameron, L.L., Wirth, J., Marcus, M., 2009. Maternal exposure to a brominated flame retardant and genitourinary conditions in male offspring. Environ. Health Perspect. 117, 1175–1179. https://doi.org/10.1289/ehp.0800058.
Stoker, T.E., Cooper, R.L., Lambright, C.S., Wilson, V.S., Furr, J., Gray, L.E., 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. https://doi.org/10.1016/j.taap.2005.05.010.
Thomsen, C., Haug, L.S., Stigum, H., Frøshaug, M., Broadwell, S.L., Becher, G., 2010. Changes in concentrations of perfluorinated compounds, polybrominated diphenyl ethers, and polychlorinated biphenyls in Norwegian breast-milk during twelve months of lactation. Environ. Sci. Technol. 44, 9550–9556. https://doi.org/10.1021/es1021922.
Thorup, J., Nordenskjöld, A., Hutson, J.M., 2014. Genetic and environmental origins of hypospadias. Curr. Opin. Endocrinol. 21, 227–232. https://doi.org/10.1097/MED.0000000000000063.
Toft, G., Jönsson, B.A.G., Bonde, J.P., Norgaard-Pedersen, B., Hougaard, D.M., Cohen, A., Lindh, C.H., Ivell, R., Anand-Ivel, R., Lindhard, M.S., 2016. Perfluorooctane sulfonate concentrations in amniotic fluid, biomarkers of fetal leydig cell function, and cryptorchidism and hypospadias in Danish boys (1980-1996). Environ. Health Perspect. 124, 151–156. https://doi.org/10.1289/ehp.1409288.
Toppari, J., Virtanen, H.E., Main, K.M., Skakkebaek, N.E., 2010. Cryptorchidism and hypospadias as a sign of testicular dysgenesis syndrome (TDS): environmental connection. Birth Defects Res. A 88, 910–919. https://doi.org/10.1002/bdra.20707.
Trabert, B., Longnecker, M.P., Brock, J.W., Klebanoff, M.A., McGlynn, K.A., 2012. Maternal pregnancy levels of trans-nonachlor and oxychlordane and prevalence of cryptorchidism and hypospadias in boys. Environ. Health Perspect. 120, 478–482. https://doi.org/10.1289/ehp.1103936.
van der Zanden, L.F.M., van Rooij, I.A.L.M., Feitz, W.F.J., Franke, B., Knoers, N.V.A.M., Roeleveld, N., 2012. Aetiology of hypospadias: a systematic review of genes and environment. Hum. Reprod. Update 18, 260–283. https://doi.org/10.1093/humupd/dms002.
Virtanen, H.E., Kaleva, M., Haavisto, A.-M., Schmidt, I.M., Chellakooty, M., Main, K.M., Skakkebaek, N.E., Toppari, J., 2001. The birth rate of hypospadias in the Turku area in Finland. APMIS 109, 96–100. https://doi.org/10.1034/j.1600-0463.2001.d01-109.x.
Virtanen, H.E., Koskenniemi, J.J., Sundqvist, E., Main, K.M., Kiviranta, H., Tuomisto, J. T., Tuomisto, J., Viluksela, M., Vartiainen, T., Skakkebaek, N.E., Toppari, J., 2012. Associations between congenital cryptorchidism in newborn boys and levels of dioxins and PCBs in placenta. Int. J. Androl. 35, 283–293. https://doi.org/10.1111/j.1365-2605.2011.01233.x.
Vrijheid, M., Armstrong, B., Dolk, H., Van Tongeren, M., Botting, B., 2003. Risk of hypospadias in relation to maternal occupational exposure to potential endocrine disrupting chemicals. Occup. Environ. Med. 60, 543–550. https://doi.org/10.1136/oem.60.8.543.
Winston, J.J., Emch, M., Meyer, R.E., Langlois, P., Weyer, P., Mosley, B., Olshan, A.F., Band, L.E., Luben, T.J., 2016. Hypospadias and maternal exposure to atrazine via drinking water in the National Birth Defects Prevention study. Environ. Health 15, 76. https://doi.org/10.1186/s12940-016-0161-9.
Yu, X., Nassar, N., Mastroiacovo, P., Canfield, M., Groisman, B., Bermejo-Sánchez, E., Ritvanen, A., Kiuru-Kuhlefelt, S., Benavides, A., Sipek, A., Pierini, A., Bianchi, F., Källén, K., Gatt, M., Morgan, M., Tucker, D., Canessa, M., Gajardo, R., Mutchinick, O., Szabova, E., Csáky-Szunyogh, M., Tagliabue, G., Cragan, J.D., Nembhard, W.N., Rissmann, A., Goetz, D., Bower, C., Baynam, G., Lowry, R.B., Leon, J.A., Luo, W., Rouleau, J., Zarante, I., Fernandez, N., Amar, E., Dastgiri, S., Contiero, P., Martínez-de-Villarreal, L.E., Borman, B., Bergman, J.E.H., de Walle, H. E.K., Hobbs, H.A., Nance, A.E., Agopian, A.J., 2019. Hypospadias prevalence and trends in international birth defect surveillance systems, 1980-2010. Eur. Urol. 76, 482–490. https://doi.org/10.1016/j.eururo.2019.06.027.
Zhao, B., Li, L., Liu, J., Li, H., Zhang, C., Han, P., Zhang, Y., Yuan, X., Ge, R.S., Chu, Y., 2014. Exposure to perfluorooctane sulfonate in utero reduces testosterone production in rat fetal leydig cells. PLoS One 9, 1–11. https://doi.org/10.1371/journal.pone.0078888.