Environment International 157 (2021) 106815

Contents lists available at ScienceDirect

Environment International

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

A case-cohort study of perinatal exposure to potential endocrine disrupters and the risk of cryptorchidism in the Norwegian HUMIS study

Anteneh Assefa Desalegn [a,b], Nina Iszatt [a], Hein Stigum [a], Tina K. Jensen [c], Merete Eggesbø [a,*]

[a] Norwegian Institute of Public Health, P.O. Box 222, Skøyen, 0213 Oslo, Norway
[b] Faculty of Medicine, University of Oslo, 0372 Oslo, Norway
[c] Department of Clinical Pharmacology, Pharmacy and Environmental Medicine, University of Southern Denmark, 5000 Odense, Denmark

ARTICLE INFO

Handling Editor: Olga-Ioanna Kalantzi

Keywords:
Congenital cryptorchidism
Recurrent cryptorchidism
Endocrine-disrupting chemicals
Human breast milk
Persistent organic chemicals
Variable selection

ABSTRACT

Background: Exposure to endocrine-disrupting chemicals (EDCs) during the critical period of testicular descent may increase the risk of cryptorchidism and male fertility.
Objective: To investigate 27 potential EDCs measured in breast milk as a proxy for perinatal exposure and the risk of cryptorchidism in a prospective cohort.
Method: The Norwegian Human Milk Study (2002–2009) enrolled 2606 mother-infant pairs, of which 1326 were mother-son pairs. In a case-cohort design, we studied 641 male infants who had 27 EDCs already quantified in milk samples: 5 organochlorine pesticides, 14 polychlorinated biphenyls (PCBs), 6 brominated flame retardants, and 2 poly- and perfluoroalkyl substances. We defined cases of congenital, recurrent, persistent and ever-reported cryptorchidism based on questionnaires mothers completed when children were 1, 6, 12 and 24 months old. Variable selection via elastic net logistic regression identified the best cryptorchidism predictors while multivariable logistic regression models determined their effect estimates.
Results: The prevalence of reported congenital cryptorchidism was 6.1%, with half spontaneously descending within six months of birth, after which prevalence stabilized between 2.2 and 2.4%. The ever-reported prevalence of cryptorchidism at 1, 6, 12, or 24 months was 12.2%. Elastic net models identified PCB-74 (OR = 1.31, 95% CI: 1.001–1.703), PCB-114 (OR = 1.36, 95% CI: 1.05–1.77), PCB-194 (OR = 1.28, 95% CI: 1.03–1.53) and β-HCH (OR = 1.26, 95% CI: 1.03–1.53 (per interquartile range increase in concentration of EDCs) as best predictors of congenital cryptorchidism. No EDCs were selected for either recurrent or persistent cryptorchidism, and only PCB-194 was selected by elastic net for ever-reported cryptorchidism (OR = 1.23, 95% CI: 1.01–1.51), in contrast to unpenalized multivariable logistic regression, where most of the individual congeners of PCBs showed significant associations.
Conclusion: In the largest multi-pollutant analysis to date considering potential confounding from co-exposure to other chemicals, perinatal exposure to PCB-74, PCB-114, PCB-194 and β-HCH were associated with increased odds of congenital cryptorchidism. Many PCBs may falsely be associated with cryptorchidism when assessed individually, due to confounding by highly correlated chemicals. Experimental studies are warranted to confirm our findings.

1. Introduction

Cryptorchidism, undescended testes, is one of the most common urogenital abnormalities in newborn males (Batra et al., 2021). It represents the failure of either one or both testes to fully descend to a normal position at the scrotum's base and is strongly associated with

Abbreviations: BDE, brominated diphenyl ether; CPP, The U.S. Collaborative Perinatal Project; DAG, directed acyclic graph; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane; HUMIS, The Norwegian Human Milk Study; IQR, interquartile range; LOD, limit of detection; LOQ, limit of quantification; NDL, (non)-dioxin-like; P, percentile; PBDE, Polybrominated Diphenyl Ether; PCB, polychlorinated biphenyl; PFASs, poly- and perfluoroalkyl substances; PFHxS, perfluorohexane sulfonate; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate; β-HCH, Beta-hexachlorocyclohexane.

* Corresponding author at: Norwegian Institute of Public Health, P.O. Box 222, Skøyen, 0213 Oslo, Norway.
E-mail address: merete.eggesbo@fhi.no (M. Eggesbø).

https://doi.org/10.1016/j.envint.2021.106815
Received 25 February 2021; Received in revised form 29 July 2021; Accepted 2 August 2021
Available online 10 August 2021

reduced male fertility and testicular cancer later in life (Gurney et al., 2017). The prevalence of cryptorchidism in children born full-term varies and can be influenced by the age of diagnosis (ranging from birth to one year) or the diagnostic criteria used to assess the spectrum of disease severity (retractile testis for example). Overall, 1–9% of full-terms baby boys are born with cryptorchidic testis, while the prevalence estimates are higher in preterm deliveries (1–45%) (Sijstermans et al., 2008, Virtanen et al., 2007). A spontaneous descent of testis occurs during the first 3 months of life in approximately half of the cases (Berkowitz et al., 1993, Gurney et al., 2017).

Prospective clinical studies have reported an increasing trend in the prevalence of congenital cryptorchidism in the UK (2.7–4.1%) and Denmark (1.8–8.4%) since the 1950s, which correlates with an increase in persistent chemicals in the environment (Juul et al., 2014, Virtanen and Toppari, 2008). In Norway, the prevalence of cryptorchidism, based on the Medical Birth Registry of Norway (MBRN), has been approximately 0.3% since the early 1970s, although registry-based estimates are prone to underreporting (Brantsæter et al., 2016).

While the exact cause of cryptorchidism remains unknown, identified risk factors include maternal smoking during pregnancy, birth weight, gestational age, family history of cryptorchidism, and genetics, and, to a lesser extent, maternal age, alcohol or caffeine consumption during pregnancy, pregnancy medications, recreational drug use, analgesics, parity (primiparous mothers) (Gurney et al., 2017). In addition, exposure to endogenous hormones and environmental endocrine disrupting chemicals (EDCs), have been suggested as risk factors for cryptorchidism (Gurney et al., 2017). EDCs with estrogenic effects (bisphenol A (BPA), DDT, some PCBs, phyto-estrogens, and phenols), and anti-androgenic effects (DDE, phthalate, and vinclozolin) have been directly linked with cryptorchidism in experimental animal studies (Skakkebæk, 2002, Virtanen and Adamsson, 2012). In humans, levels of some EDCs (BPA, phthalates, PFOS) were shown to correlate with insulin-like factor 3 (INSL3), regulator of testicular descent (Chevalier et al., 2015, Toft et al., 2016).

Several epidemiologic studies show an association between cryptorchidism in humans and, for example, prenatal exposure to diethylstilbestrol (DES) (Virtanen and Adamsson, 2012), maternal exposure to phthalates (Wagner-Mahler et al., 2011), BPA (Komarowska et al., 2015), and maternal residential pesticide (atrazine) exposure (Jørgensen et al., 2014; Agopian et al., 2013). Other studies found no association when they investigated individuals EDCs, but did find effects for a sum or combination of organochlorine pesticides (Damgaard et al., 2006), sum of PCB congeners (Brucker-Davis et al., 2008, Koskenniemi et al., 2015), and sum of PBDEs (Main et al., 2007), while others did not find any association for the sum of 11 PCBs in maternal serum from third trimester (McGlynn et al., 2009), placental levels of 37 PCBs or 17 dioxins (Virtanen et al., 2012). Furthermore, a meta-analysis that summarized 10 case-referent studies investigating different EDC exposures found no increased risk of cryptorchidism and EDC exposure (Bonde et al., 2016). Most of the studies to date have focused on a limited number or class of chemicals as exposure, used mainly congenital cryptorchidism as an outcome, or used single pollutant analysis, which is prone to potential confounding from co-exposure to other chemicals.

We therefore simultaneously investigated the association between exposure to 27 potential EDCs (four class of chemicals) measured in breast milk as a proxy for perinatal exposure and the risk of congenital, recurrent, persistent, and ever-reported cryptorchidism among Norwegian boys in the HUMIS birth cohort using both multi-pollutant analysis (elastic net penalized logistic regression) and single pollutant analysis (multivariable logistic regression)

2. Methods

2.1. Study population

The Norwegian Human Milk Study (HUMIS, 2002–2009) is a prospective multi-center birth cohort of 2,606 mother-infant pairs. It was established to measure levels of persistent organic pollutants (POPs) in breastmilk and to investigate possible health effects associated with high levels. The HUMIS cohort has previously been described in detail (Eggesbø et al., 2009). Briefly, public health nurses recruited new mothers between 2003 and 2009 during routine postnatal care home visits around two weeks postpartum in seven counties across Norway (see Supplementary Table S1 for details of the counties). 21.5% of the mother–child pairs were recruited in 2002–2005 by a pediatrician at the maternity ward in Østfold hospital in Southern Norway, two term births for every preterm birth (Eggesbø et al., 2009). Mothers from all counties followed the same protocol and completed the same questionnaires, regardless of the recruitment procedure.

Among the 2,606 participants enrolled in the HUMIS study, the present study included 1,262 mother-son pairs for prevalence estimates after excluding mother-daughter pairs, uncertain or missing outcomes, and non-singletons. For the study of cryptorchidism with potential EDCs exposure, we used a case-cohort design, restricting our analysis to a subset of 641 mother-son pairs where up to 27 chemicals had been measured in the mothers' breast milk samples (Fig. 1).

The study was approved by the Norwegian Data Inspectorate (ref. 2002/1398), and the Regional Ethics Committee for Medical Research (ref. S-02122). Informed consent was also obtained from all participating women prior to enrollment.

2.2. Outcome assessment

Cryptorchidism was mapped from self-administered questionnaires filled out by the mothers at 1, 6, 12, 24 months. For the purpose of this study, cryptorchidism was defined based on the timing of the presentation as either:

1. Congenital cryptorchidism
   - Cryptorchidism based on mother's report at one month after birth
2. Recurrent cryptorchidism
   - Cryptorchidism at birth that spontaneously descends and then re-ascends
3. Persistent cryptorchidism
   - Cryptorchidism reported both at age 1 and 2 years, including receipt of orchiopexy
4. Ever-reported cryptorchidism
   - Cryptorchidism reported at 1, 6, 12 or 24 months.

2.3. EDCs exposure assessment

The mothers were asked to collect 25 mL of breastmilk each morning on eight consecutive days two weeks after birth and before the child reached two months of age, in line with the WHO recommendation (WHO, 2007). Minor deviations in this sampling protocol, such as collection by breast pump, were accepted. The pooled milk samples were stored frozen in a 250 mL natural High-Density Polyethylene (HDPE) packaging container. The date and time of collection were recorded, as well as whether a breast pump had been used. When the packaging container had been filled, participants mailed it by regular mail, except in the county of Østfold (n = 171, 26.7% in this cryptorchidism study), where the milk samples were collected by study personnel and kept frozen during transport to the Norwegian Institute of Public Health biobank. The different modes of transport of milk samples was not expected to affect EDCs concentration as persistent chemicals are able to withstand severe conditions. The HDPE packaging container (Cat. No.: 967-21244, Thermo Scientific Nalgene®) was made from food-grade high-purity resins. Moreover, the containers were recently tested for potential migration of chemicals (Collet et al. 2020).

Milk was sampled at a median of 33 (10th–90th percentile: 18–57) days after delivery in all counties. More than 90% of the samples were sampled after two weeks and before the child turned 2 months of age.

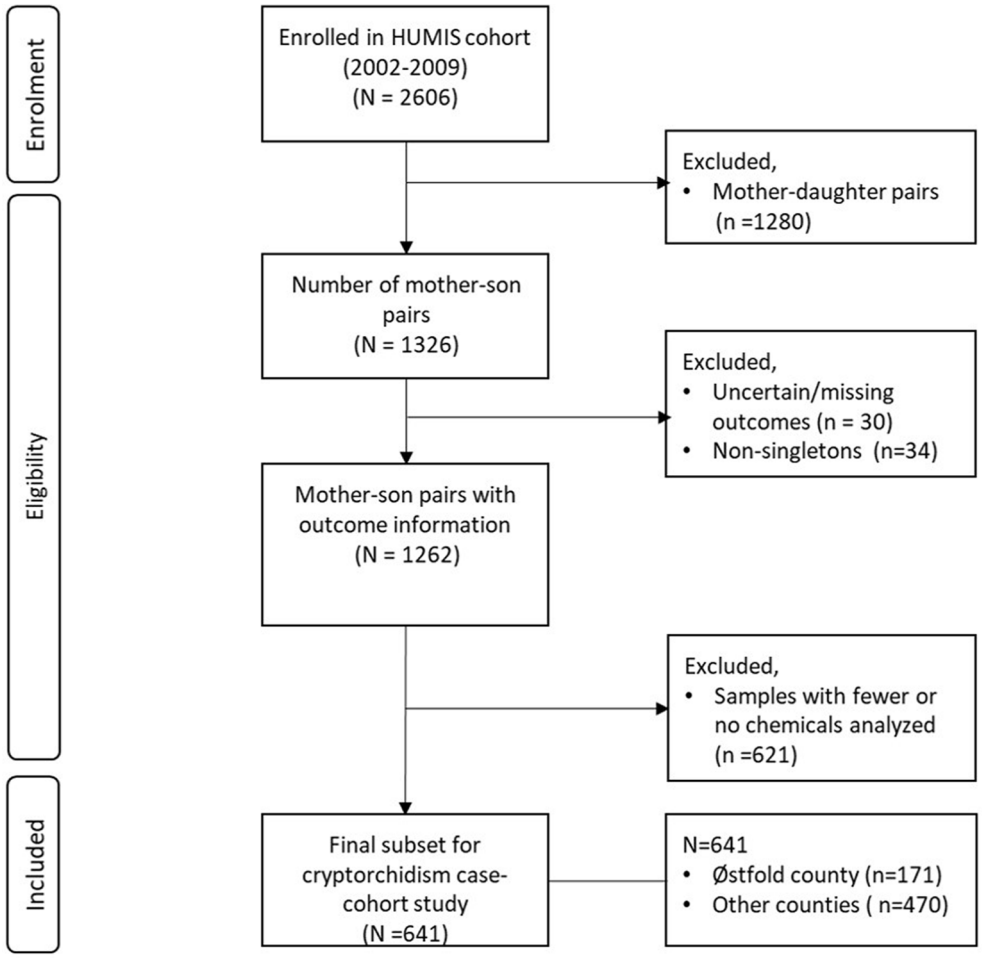

Fig. 1. Flow chart showing selection of the participants into the cryptorchidism study from the HUMIS cohort (2002–2009, Norway).

The median age (10th–90th percentile) in days at the time of breast milk sampling was 29 (10–65) and 34 (22–56) days in Østfold and other counties, respectively. The concentrations of 27 potential EDCs: 5 organochlorine pesticides (OCPs; β-HCH, HCB, p,p′-DDE, p,p′-DDT), 14 polychlorinated biphenyls (PCBs; PCB-105, PCB-114, PCB-118, PCB-156, PCB-157, PCB-167, PCB-189, PCB-74, PCB-99, PCB-153, PCB-170, PCB-180, PCB-194, and PCB-138), 6 polybrominateddiphenyl ethers ((P)BDEs; BDE-28, BDE-47, BDE-99, BDE-100, BDE-153, and BDE-154), and 2 poly- and perfluoroalkyl substances (PFASs; PFOA, PFOS) were quantified in breast milk samples. Four laboratories took part in the chemical analyses for the PFASs, PBDEs, PCBs, and OCPs, as previously described (Forns et al., 2015, Polder et al., 2009, Thomsen et al., 2010, Forns et al., 2016, Cechova et al., 2017, Čechová et al., 2017): the Department of Environmental Exposure and Epidemiology, Norwegian Institute of Public Health (Oslo, Norway), the Department of Environmental Sciences, Norwegian University of Life Sciences (Ås, Norway), the Institute for Environmental Studies, Faculty of Earth and Life Sciences, VU University (Amsterdam, the Netherlands), and the Research Centre for Toxic Compounds in the Environment, Masaryk University (Brno, Czech Republic). Breast milk lipid levels were quantified gravimetrically during chemical analysis. See Supplementary Methods for detailed analytical methods. Exposure values falling below limit of detection (LOD) were replaced by randomly generated numbers between zero and LOD. The concentrations of the EDCs in breast milk are lipid adjusted (ng/g), except for PFASs where concentrations are wet weight (ng/L).

2.4. Covariates

We obtained information on potential confounders, mediators and other covariates from questionnaires that the mothers completed at 1, 6, 12, and 24 months postpartum and the Medical Birth Registry of Norway. Continuous variables included maternal age (years), pre-pregnancy BMI (kg/m^2), parity, birth weight (g), and gestational age (days). Preterm (yes/no), maternal education (low/medium/high), smoking status (no smoking/occasional smoking/daily smoker less than or equal to 10 cigarettes/daily smoker more than 10 cigarettes), gestational diabetes (yes/no), and preeclampsia (yes/no) were categorical variables. Information on child's sex, gestational age, birth weight, and maternal smoking during pregnancy were obtained from the Medical Birth Registry of Norway (Skjaerven et al., 2000).

2.5. Statistical analysis

2.5.1. Adjustment models

We estimated the effect of EDC exposure on the development of cryptorchidism for both unadjusted (crude estimate) and controlling for appropriate confounders (adjusted model) identified by a directed acyclic graph (DAG). Adjustment for the total effect included the following cofounders: maternal education (low, medium, high), maternal age (continuous), pre-pregnancy BMI (kg/m^2), smoking (yes/no), and nulliparity (yes/no) (Supplementary Fig. S1).

We used multiple imputation by chained equations with predictive mean matching (Buuren and Groothuis-Oudshoorn, 2010;(White et al., 2011) to impute missing exposure data (missing due to no chemical

analysis of milk samples: ≤3.3% for 13 chemicals, 13–18% for 12 chemicals, 25–28% for 2 chemicals, and missing covariate data (≤2.8%) up to the full sample size of 641. Details of the missing summary is presented in Supplementary Table S4. We generated 100 multiply imputed data sets, which we used for all analyses.

2.5.2. Single and multi-pollutant variable selection and effect estimation

We used separate ordinary least squares logistic regression models for the single pollutant analysis to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for congenital, recurrent, persistent and ever-reported cryptorchidism, controlling for the potential confounders identified by the DAG. For the multi-pollutant analysis, we used elastic net logistic regression (Zou and Hastie, 2005), a variable selection method to identify important predictors among a high number of correlated exposures, reducing the potential for multicollinearity (Lenters et al., 2018). High correlation between exposures is a challenge in conventional regression methods as they cannot differentiate between true predictors from correlated variables (Agier et al., 2016). Elastic net logistic regression model as a variable selection method that outperforms prediction of an outcome in the presence of strongly correlated predictors (chemical exposures) compared to conventional logistic regression (Lenters et al., 2018), or other regularization and variable selection methods such as Lasso (Zou and Hastie, 2005). The potential confounders were forced into the elastic net model unpenalized while the optimal level of penalization for the correlated 27 chemical exposures was determined using the default 10-fold cross-validation. The analysis was repeatedly performed in each of the multiply imputed datasets (n = 100) and an exposure considered selected if it was retained in at least 50% of the imputed sets. In the second step to obtain unbiased estimates, we then refit the selected subset of chemicals in an ordinary multi-pollutant logistic regression model. We used Stata (version 16.0; Stata Corp LP, College Station, Texas, USA) for all statistical analyses.

2.5.3. Sensitivity analyses

For the final adjusted model, we conducted a number of sensitivity analyses. We ran the model on the complete case dataset for comparison with the multiple imputation dataset. Furthermore, we assessed, individually, the effect of further adjusting for preterm birth (yes/no), use of breast pumps for milk collection (yes/no), timing of milk collection (days), and other reported congenital anomalies (including hypospadias, yes/no). For the elastic net selected EDCs, we also assessed potential effect modification by maternal education, nulliparity, and preterm birth in models including main effects and cross-product terms, with a Wald test p-value of <0.20 suggestive of an interaction.

3. Results

3.1. Maternal and son characteristics of the entire eligible HUMIS cohort and the cryptorchidism case-cohort study participants.

The maternal and son characteristics of the cryptorchidism study participants (n = 641) was representative of the entire HUMIS cohort (n = 2606) with respect to median maternal age (29 vs 30), high maternal education (74.1% vs 74.9%), nulliparity (43.5% vs 42%), small for gestational age (11.1% vs 10.0%), and preterm births (9.7% vs 9.3%) respectively (Table S1).

Among the entire eligible HUMIS cohort participants (n = 1262), the prevalence of reported congenital cryptorchidism was 6.1%, while the prevalence for recurrent cryptorchidism was 8%, 1.6% for persistent cryptorchidism, and 12.2% for ever-reported cryptorchidism at any one of the time points (1, 6, 12, 24 months) (Table S2). 56% of the congenital cryptorchidism cases descended spontaneously within the first 6 months of birth, after which the prevalence stabilized between 2.2 and 2.4%. There were twice as many cases of unilateral cryptorchidism (1.4%) than bilateral cryptorchidism (0.7%) according to the report at 12 months of age. Orchiopexy was planned or performed in three out of 12 babies with reported persistent cryptorchidism up to two years of age despite the recommendation for operation between six and 18 months of age (Batra et al., 2021). The rate of surgery in the recommended age range is also less than one-third in Australia & other populations (Schneuer et al., 2016).

Table 1 shows the socio-demographic characteristics for congenital, recurrent, persistent, and ever-reported cryptorchidism for the case-cohort study subset (n = 641). For ever-reported cryptorchidism, 73.6% of the mothers were between 25 and 35 years of age at delivery, 74% had higher education, close to 4% smoked cigarettes daily, and about 10% of the mothers were obese prior to pregnancy. Approximately 10% of the children were small for gestational age, and or preterm, due to the oversampling of preterm in Østfold County

Fig. 2 shows the boxplot distributions of the 27 EDCs measured in breast milk of mothers in the case-cohort cryptorchidism study, while numerical details (median, IQR, maximum) comparing levels from mothers with and without a cryptorchidic son is described in Supplementary Table S3. The highest breast milk concentrations within each of the four chemical classes were observed for PCB-118 (62.2 ng/g, among mono-ortho DL-PCBs), PCB-153 (296 ng/g, among non-DL PCBs), DDE (1280 ng/g), BDE-47 (73.6 ng/g) and PFOS (484.5 ng/L) (Supplementary Table S3).

The Pairwise Pearson correlations between the 27 EDCs showed 26.5% strong ($r_p \geq 0.75$), 15.1% moderate ($0.5 \leq r_p < 0.75$), and 10.6% low ($0.25 \leq r_p < 0.50$) correlations. In general, there was clustering by chemical class with moderate to strong correlation within chemical classes, low to moderate correlation between PCBs and OCPs, and weak or no correlations between BDEs and PFASs (see Fig. S2).

3.2. Association with cryptorchidism

In the ordinary least squares logistic regression, individual congeners of some of the seven DL-PCBs were significantly associated with congenital cryptorchidism (PCB-114, PCB-156, PCB-157), recurrent cryptorchidism (PCB-114, PCB-118, PCB-167), and ever-reported cryptorchidism (PCB-114). Likewise, some NDL-PCBs were also significantly associated with congenital cryptorchidism (PCB-74, PCB-194), recurrent cryptorchidism (PCB-153, PCB-170, PCB-180, PCB-138), and ever-reported cryptorchidism (PCB-194). None of the PCBs were associated with persistent cryptorchidism (Fig. 3). Among OCPs, β-HCH was the only one significantly associated with congenital cryptorchidism. There were no association for individual congeners of BDEs or PFASs with any of the cryptorchidism definitions. Details of the ordinary least squares regression results for each of the four outcome definitions with each of the 27 chemicals for crude estimate (unadjusted) and adjustment for confounders (total effect) are shown in Supplementary Tables S5–S8.

3.3. Variable selection result

The multipollutant analysis based on elastic net logistic regression selected PCB-74, PCB-114, PCB-194 and β-HCH in at least 50% of the imputed datasets (n = 100 imputations) as the best predictors among the 27 chemicals for congenital cryptorchidism. In the non-penalised multipollutant logistic regression model, the effect estimates were: OR = 1.31, 95% CI: 1.00–1.70 (p = 0.049) for PCB-74, OR = 1.36, 95% CI: 1.05–1.77 for PCB-114, OR = 1.28, 95% CI: 1.03–1.59 for PCB–194 and OR = 1.26, 95% CI: 1.03–1.53 for β-HCH per IQR increase in breast milk concentrations. No EDCs were selected for either recurrent or persistent cryptorchidism while PCB-194 (OR = 1.19, 95% CI: 1.01–1.41) was selected as the best predictor of ever-reported cryptorchidism in 52% of the imputed datasets (Table S9). The IQR varied from 81 to 124% change in concentration, see Supplementary Table S3 for units corresponding to IQR.

Table 1
Socio-demographic characteristics of mother-son pairs (N (%)[a] or median (IQR)[b]) by cryptorchidism case definitions included in the case-cohort study of EDCs and cryptorchidism in the HUMIS cohort (n = 641, 2002–2009, Norway).

		Total N = 641	Congenital cryptorchidism N = 26/485[c]	Recurrent cryptorchidism N = 11/185	Persistent cryptorchidism N = 9/500	Ever-reported cryptorchidism N = 77/641
Maternal age (years)						
	<25	101	5 (1.0%)	1 (0.5%)	2 (0.4%)	10 (1.6%)
	25–35	472	18 (3.7%)	8 (4.3%)	6 (1.2%)	58 (9.0%)
	>35	68	3 (0.6%)	2 (1.1%)	1 (0.2%)	9 (1.4%)
Maternal education (years)						
	<12 yrs	59	3 (0.6%)	0 (0.0%)	1 (0.2%)	9 (1.4%)
	12 years	92	4 (0.8%)	1 (0.5%)	3 (0.6%)	11 (1.7%)
	>12 years	475	19 (3.9%)	9 (4.9%)	5 (1%)	54 (8.4%)
	Missing	15	0 (0.0%)	1 (0.5%)	0 (0.0%)	3 (0.5%)
Birth weight (grams)						
	<2500	33	0 (0.0%)	3 (1.6%)	3 (0.6%)	6 (0.9%)
	2500–4000	437	22 (4.5%)	7 (3.8%)	5 (1%)	54 (8.4%)
	>4000	171	4 (0.8%)	1 (0.5%)	1(0.2%)	17 (2.7%)
Nulliparity		276	14 (2.9%)	7 (3.8%)	3 (0.6%)	28 (4.4%)
Gestational age (days)		641	283 (276–290)	282 (257–284)	271 (255–272)	280 (271–288)
Small-for gestational age		71	3 (0.6%)	1 (0.5%)	2 (0.4%)	8 (1.2%)
Preterm		62	1 (0.2%)	3 (1.6%)	3 (0.6%)	11 (1.7%)
Caesarean section		107	4 (0.8%)	2 (1.1%)	3 (0.6%)	10 (1.6%)
Smoking		78	3 (0.6%)	2(1.1%)	1(0.2%)	7 (1.3%)
Pre-Pregnancy BMI (kg/m^2)						
	Under weight (≤18.4)	22	0 (0.0%)	0 (0.0%)	0 (0%)	0 (0.0%)
	Normal (18.5–24.9)	386	17 (3.5%)	7 (3.8%)	4 (0.8%)	48 (7.5%)
	Overweight (25–29.9)	151	5 (1.0%)	4 (2.2%)	2 (0.4%)	15 (2.3%)
	Obese (≥30)	64	3 (0.6%)	0 (0.0%)	2 (0.4%)	11 (1.7%)
	Missing	18	1 (0.2%)	0 (0.0%)	1(0.2%)	3 (0.5%)
Preeclampsia		29	2 (0.4%)	0 (0.0%)	2 (04%)	8 (1.2%)

Note: BMI: body mass index; IQR: interquartile range.
[a] N (%)) is reported for binary or categorical variables from the total mothers who responded to the questionnaire for each cryptorchidism definition in the column.
[b] Median (IQR) is reported for continuous variables.
[c] The denominator in each column represents the number of mothers who responded to the specific questions related to cryptorchidism presentation.

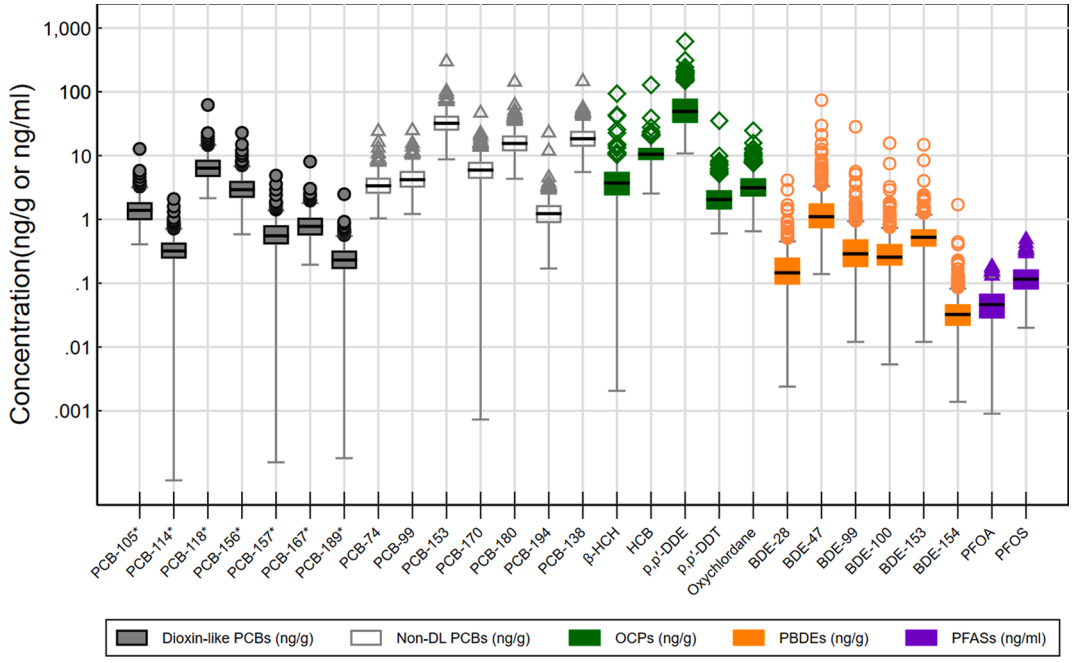

Fig. 2. Boxplot distribution of 27 EDCs found in breast milk among 641 mother-son pairs in the HUMIS cohort (2002–2009, Norway). Horizontal lines correspond to medians, and boxes to the 25th–75th percentiles; whiskers extend to data within the interquartile range times 1.5, and data beyond this are plotted as dots. Wet weight concentrations are presented for PFASs (ng/L) and lipid adjusted concentrations for all other chemicals (ng/g lipid). See Table S3 for numerical values. **Abbreviations:** BDE, brominated diphenyl ether; DDE, dichlorodiphenyldichloroethylene; DDT, dichlorodiphenyltrichloroethane; HCB, hexachlorobenzene; β-HCH, Beta-hexachlorocyclohexane; BDE, Brominated Diphenyl Ether; PCB, polychlorinated biphenyl; PFOA, perfluorooctanoate; PFOS, perfluorooctane sulfonate.

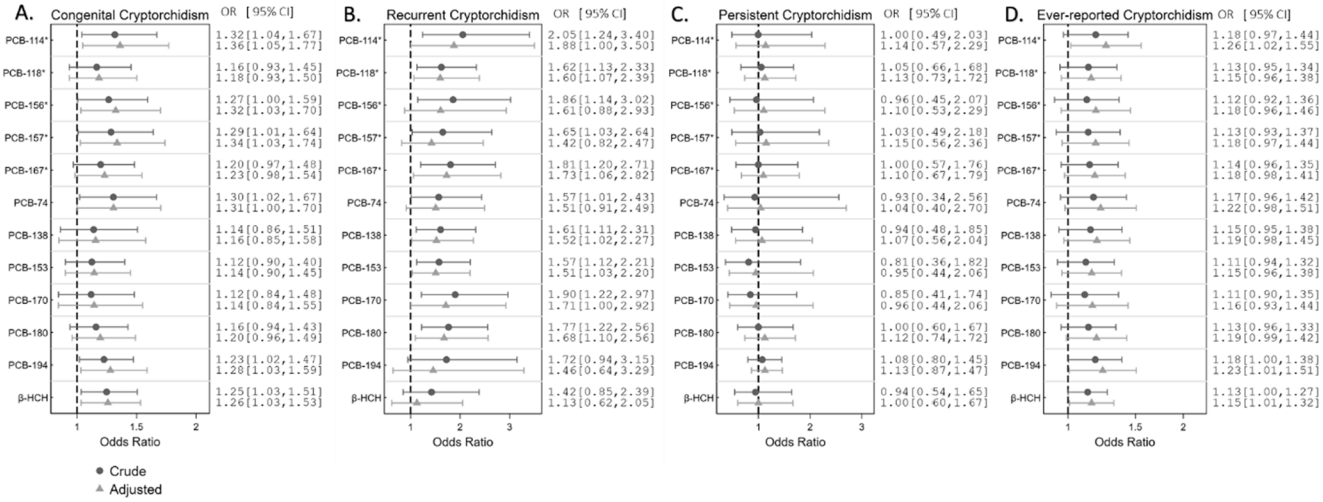

Fig. 3. Forest plot with odds ratio (OR) and 95% confidence interval (CI) per IQR increase in concentration of selected chemicals from 27 potential EDCs that were significantly associated with either congenital cryptorchidism, recurrent cryptorchidism, persistent cryptorchidism or ever-reported cryptorchidism in the case-cohort study in the HUMIS cohort (n = 641, 2002–2009, Norway). DL-PCBs are indicated with asterisk symbol (*) in the figure. Adjusted models included maternal age, education, pre-pregnancy BMI, parity, and smoking. Missing data was multiply imputed (n = 100 imputations).

3.4. Sensitivity analyses

Sensitivity analysis checking the additional effect of adjusting individually for preterm, congenital anomalies including hypospadias, timing of breast milk collection, and use of breast pump for milk collection did not materially alter effect estimates (Supplementary Fig. S3). We also did not detect significant effect modification for the selected EDCs by maternal education, preterm birth and nulliparity with congenital cryptorchidism (p-interaction > 0.30) or ever-reported cryptorchidism (p-interaction > 0.28). Effect estimates for congenital and ever-reported cryptorchidism from complete case analysis were similar to the multiple imputation analysis (Supplementary Fig. S4).

4. Discussion

In this case-cohort study, we found that infants with the highest exposure to breast milk concentrations of PCB-74, PCB-114, PCB-194 and β-HCH had increased odds of congenital cryptorchidism, and ever-reported cryptorchidism (PCB-194 only). The odds of having congenital cryptorchidism was increased by 28–36% for PCBs and 26% for β-HCH per IQR increase in breast milk concentrations. PCB-194 was associated with 23% increased odds of ever-reported cryptorchidism.

In the present study, only 3 PCBs (PCB-74, PCB-114, and PCB-194) were selected as the best predictors of congenital cryptorchidism among the PCBs using variable selection method although more individual PCB congeners were significantly associated with congenital or recurrent cryptorchidism in the unpenalized logistic regression. This highlights the importance of using multipollutant models in order to control for confounding by correlated exposures with shared exposure sources and similarities in kinetics with long half-lives (Zou and Hastie, 2005; Lenters et al., 2018). The epidemiological literature linking prenatal PCB exposure to the risk of cryptorchidism is inconclusive. Prenatal exposure to the sum of 12 dioxin-like PCBs from adipose tissue biopsies were positively associated with congenital cryptorchidism in the Danish-Finnish case-control study (2002–2006, n = 44 cases/38 controls) (Koskenniemi et al., 2015). Similarly, a French case-control study examining 151 cord blood (n = 67 cryptorchidism/84 controls) and 125 colostrum samples (n = 56/69) found a positive association between cryptorchidism and the total PCB concentrations (sum of 7 congeners) in colostrum, OR = 2.74, 95% CI (1.15–6.53), but not in cord blood, nor any association with individual congeners (Brucker-Davis et al., 2008). In the present study, less than 5% of the breast milk samples were collected within the first two weeks after delivery when colostrum is mainly produced. The U.S. Collaborative Perinatal Project (CPP) involving 12 U.S. medical centres between 1959 and 1965 did not find an association between eleven individual PCB congeners, or their sum, and cryptorchidism in a case-control study (n = 230/593) (McGlynn et al., 2009), nor did a nested case-control study within a joint Danish (n = 39/129) and Finish (n = 56/56) prospective cohort study using placental levels of 37 PCBs (n = 112/168) (Virtanen et al., 2012). Moreover, in the same Danish-Finish cohort using breast milk, Krysiak-Baltyn et al (2012) showed PCBs indicating protective effect within the Danish cohort but contrasting result within the Finish cohort.

More support is found in the literature for the role of organochlorines in the aetiology of cryptorchidism. In the present study, only β-HCH was associated with congenital cryptorchidism, among the organochlorines although the median concentrations of HCB, *p,p′*-DDE, *p,p′*-DDT, and oxychlordane were all higher in infants with cryptorchidism compared to infants without cryptorchidism (Supplementary Table S3). In a previous Norwegian study based on agricultural census, farmer's occupational exposure to OCPs was associated with cryptorchidism registered in the Medical Birth Registry (Kristensen et al., 1997). In accordance with this, a higher prevalence of cryptorchidism was also reported in Denmark amongst boys whose mothers were employed in greenhouses while pregnant (Andersen et al., 2008). In line with our study, β-HCH was associated with cryptorchidism in the US CPP study that compared serum concentration below the 10th and above the 90th percentiles (OR = 1.6, 95% CI: 0.7–2.6) (Pierik et al., 2007). Moreover, even though non-significant, children in Germany with cryptorchidism had higher median concentrations of β-HCH in fat samples obtained from orchidopexy compared to controls (Hosie et al., 2000). In Denmark, a case-control study (n = 62/68) reported an association between a combination of eight of the most abundant persistent OCPs in breast milk and congenital cryptorchidism (p = 0.032), although there was no significant difference when individually analysing 27 OCPs, including β-HCH (Damgaard et al., 2006). The exposure levels in breast milk in their study was two to three times higher than in our study in Norway (Supplementary Table S3). There is also literature suggesting associations between cryptorchidism and maternal serum level of other OCPs such as oxychlordane, *p,p′*-DDT, and *p,p′*-DDE (Trabert et al., 2012, Bhatia et al., 2005, Longnecker et al., 2002). However, not all studies support evidence of association between cryptorchidism and maternal serum levels of OCPs (Waliszewski et al., 2005, Bhatia et al., 2005, Axelsson et al., 2020).

We did not find any associations between brominated flame retardants and cryptorchidism, in line with previous studies (Small et al., 2009, Koskenniemi et al., 2015, Bonde et al., 2016), but in contrast to a case–control study in Canada that found an association with cryptorchidism and PBDEs measured in maternal hair samples (Goodyer et al., 2017). In the prospective Danish-Finnish study, Main et al. (2007) found an association to levels of flame retardants in breast milk, but not in placenta. Also, we did not find an association with PFOS and PFOA, in line with two previous studies (Jensen et al., 2013, Toft et al., 2016). However, PFOS exposure in amniotic fluid has been associated with hormones (steroid hormone and INSL3) that regulate testicle descent (Toft et al., 2016), suggesting that a more sensitive effect biomarker may be necessary to detect sub-clinical effects.

Experimental studies are necessary to confirm our findings of an association between PCB-74, PCB-114, PCB-194 and β-HCH and congenital cryptorchidism. Testicular descent occurs during prenatal life in two distinct phases, the first regulated by the insulin-like factor 3 (INSL3), and the second by androgens and calcitonin gene-related peptides (CGRP) (Hutson et al., 2015). Reduced androgen to estrogen ratio, and interference with androgen or INSL3 secretion during prenatal life, are mechanisms known to induce cryptorchidism in animals (Virtanen and Adamsson, 2012). Elevated serum PCB-74 was significantly associated with lower serum testosterone among the adult Native American population, particularly in females (Goncharov et al., 2009), and prenatal PCB-74 exposure was associated with decreased anogenital distance among boys in a cohort study in Mexico (Loreto-Gómez et al., 2018). The effect of PCB-114, a Dl-PCB congener, may be due to its aryl hydrocarbon receptor (AhR) activation, which exhibits anti-estrogenic or estrogenic effects based on tissue specificity (Warner et al., 2012). On the other hand, the effect of β-HCH on testicular descent may be due to its estrogenic properties (Coosen and van Velsen, 1989), while the probable mechanisms for PCB-194 are not clear.

In this Norwegian birth cohort, the prevalence of congenital cryptorchidism was 6.1%, and remained unchanged when established risk factors, such as preterm births and small for gestational age infants (Gurney et al., 2017), were excluded. Our prevalence estimates are higher than reported in the medical birth registry of Norway, which is, however, prone to underreporting (Brantsæter et al., 2016). More than half of the congenital cryptorchidism cases reported spontaneous descendent within six months of age in accordance with normal physiology, driven by the transient surge in gonadotrophins and consequent rise in testosterone levels (mini puberty), making testicular descent possible until five months of age (Kuiri-Hänninen et al., 2019). The reported prevalence after six months was stable (2.2–2.4%) in our cohort in accordance with the expected low probabilities of spontaneous testicular descent after 6 months of age (Shin and Jeon, 2020).

This case-cohort study has several advantages. The participants in the longitudinal HUMIS cohort are enrolled from across Norway to represent the general population. Chemicals were analysed in breast milk, which represents the real-life perinatal exposure to a mixture of many potential EDCs, and acts as a proxy for dose at the target tissue in the infants. Given the positive correlation between levels of persistent chemicals in breast milk, umbilical cord, and maternal serum, breast milk can be a suitable proxy for prenatal exposure and a good indicator of body burden for persistent compounds (Cerrillo et al., 2005, Skaare et al., 1988). It also represents both prenatal and postnatal exposure, and postnatal exposure may further delay testicular descent that has not occurred by the time of birth. Another strength of the study is the extensive assessment of 27 potential EDCs and the use of a variable selection method for the multi-pollutant analysis to reduce confounding from correlated co-exposure. The outcome ascertainment, cryptorchidism, was defined based on mother's report using repeated questionnaire at 1, 6, 12, and 24 months with several questions per questionnaire to cross-check validity of their report. In addition, having several time points to check cryptorchidism presentation increases the possibility of detecting any cryptorchidism and capture the spectrum of severity (Gurney et al., 2017). For example, measurement only at birth would exclude boys with a normally descended testis that subsequently ascends spontaneously; likewise, late measurements may lose less severe cases of cryptorchidism that descends shortly after birth. Another strength was substantial questionnaire data to enable a thorough assessment of potential confounding using DAGs. The prevalence of cryptorchidism in this study ranges between 1.6 and 8% depending on the time when the questionnaire was sent and severity of cryptorchidism. These prevalence estimates are high compared to the relatively stable prevalence of 0.3–0.4% reported since the early 1970s based on Medical birth registry of Norway (MBRN) (Brantsæter et al., 2016). However, prevalence estimates based on measurement at birth are known to be prone to underreporting since they may exclude boys with an ascending testicle or acquired cryptorchidism (normally descended testis at birth that subsequently ascended).

There are, however, some limitations to our study. One of the main limitations is the outcome ascertainment, which was based on maternal report and can be difficult even for trained observers. However, as mentioned above, multiple questionnaires were administered at different time points with detailed information including performed or planned operation to cross-check validity of their report. In addition, the measurement error arising from maternal report in our study is likely to be non-differential as mothers do not know their chemical exposure, biasing estimates towards the null. Reliability of maternal report among Norwegian mothers has also been validated for important birth and pregnancy parameters compared with registry data, although cryptorchidism was not included (Skulstad et al., 2017). Due to financial constraints measurement of chemical exposure was limited to 641 boys out of 1262, affecting the statistical power of the study. We obtained exposure information postpartum and not during first trimester, when testicles develop. Also, we cannot rule out the role of residual confounding from unmeasured or unknown confounders even though the questionnaire contained extensive list of information and we controlled for more classes of chemicals than any other study. Moreover, for some classes of potential EDCs, fewer samples were measured, leading to varying sample sizes, or had higher numbers of values below the limit of detection. However, multiple imputation was performed to reduce potential bias tied to missing values.

5. Conclusions

In this prospective cohort, PCB-74, PCB-114, PCB-194 and β-HCH were associated with congenital cryptorchidism. Altogether, 27 potential EDCs were analysed in this study making it the largest multi-pollutant study to date on cryptorchidism. We highlight the importance of using appropriate statistical models to handle highly correlated chemicals simultaneously to reduce false positive associations, as we observed marked different results in the single pollutant analysis compared to the elastic net logistic regression model. Further mechanistic studies are necessary to confirm our findings and elucidate how the selected EDCs may influence cryptorchidism.

Funding

This work was supported in part by the Research Council of Norway (NEVRINOR, grant agreement no. 226402), the European Commission's Seventh Framework Program (Developmental Neurotoxicity Assessment of Mixtures in Children (DENAMIC), grant agreement FP7-ENV-2011-282957), and the European Union's Horizon 2020 research and innovation programme (PROTECTED Marie Sklodowska-Curie grant agreement No. 722634).

CRediT authorship contribution statement

Anteneh Assefa Desalegn: Methodology, Investigation, Formal analysis, Investigation, Writing – original draft, Writing – review &

editing. **Nina Iszatt:** Data curation, Methodology, Formal analysis, Investigation, Writing – review & editing, Supervision. **Hein Stigum:** Methodology, Software, Formal analysis, Writing – review & editing, Supervision. **Tina K. Jensen:** Methodology, Writing – review & editing, Supervision. **Merete Eggesbø:** Conceptualization, Data curation, Methodology, Software, Project administration, Resources, Formal analysis, Investigation, Writing – review & editing, Funding acquisition, Supervision.

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.

Acknowledgements

Jan Kuta, Ondřej Audy and Petr Kukučka at the Research Centre for Toxic Compounds in the Environment, Faculty of Science, Masaryk University, Kamenice; 753/5, 625 00 Brno, Czech Republic, are acknowledged for the GC-MS analytical measurements.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106815.

References

Agier, L., Portengen, L., Chadeau-Hyam, M., Basagaña, X., Giorgis-Allemand, L., Siroux, V., Robinson, O., Vlaanderen, J., González, J.R., Nieuwenhuijsen, M.J., Vineis, P., Vrijheid, M., Slama, R., Vermeulen, R., 2016. A Systematic Comparison of Linear Regression-Based Statistical Methods to Assess Exposome-Health Associations. Environ. Health Perspect. 124, 1848–1856.

Agopian, A.J., Lupo, P.J., Canfield, M.A., Langlois, P.H., 2013. Case-control study of maternal residential atrazine exposure and male genital malformations. Am. J. Med. Genet. A 161a, 977–982.

Andersen, H.R., Schmidt, I.M., Grandjean, P., Jensen, T.K., Budtz-Jørgensen, E., Kjaerstad, M.B., Baelum, J., Nielsen, J.B., Skakkebaek, N.E., Main, K.M., 2008. Impaired reproductive development in sons of women occupationally exposed to pesticides during pregnancy. Environ. Health Perspect. 116, 566–572.

Axelsson, J., Scott, K., Dillner, J., Lindh, C.H., Zhang, H., Rylander, L., Rignell-Hydbom, A., 2020. Exposure to polychlorinated compounds and cryptorchidism. A nested case-control study. PLoS One 15, e0236394.

Batra, N.V., Demarco, R.T., Bayne, C.E., 2021. A narrative review of the history and evidence-base for the timing of orchidopexy for cryptorchidism. J. Pediatr. Urol. (21) https://doi.org/10.1016/j.jpurol.2021.01.013. S1477-5131(21)00013-9.

Berkowitz, G.S., Lapinski, R.H., Dolgin, S.E., Gazella, J.G., Bodian, C.A., Holzman, I.R., 1993. Prevalence and natural history of cryptorchidism. Pediatrics 92, 44–49.

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.

Bonde, J.P., Flachs, E.M., Rimborg, S., Glazer, C.H., Giwercman, A., Ramlau-Hansen, C. H., Hougaard, K.S., Høyer, B.B., Hærvig, K.K., Petersen, S.B., Rylander, L., Specht, I. O., Toft, G., Bräuner, E.V., 2016. The epidemiologic evidence linking prenatal and postnatal exposure to endocrine disrupting chemicals with male reproductive disorders: a systematic review and meta-analysis. Hum. Reprod. Update 23, 104–125.

Brantsæter, A.L., Torjusen, H., Meltzer, H.M., Papadopoulou, E., Hoppin, J.A., Alexander, J., Lieblein, G., Roos, G., Holten, J.M., Swartz, J., Haugen, M., 2016. Organic Food Consumption during Pregnancy and Hypospadias and Cryptorchidism at Birth: The Norwegian Mother and Child Cohort Study (MoBa). Environ. Health Perspect. 124, 357–364.

Brucker-Davis, F., Wagner-Mahler, K., Delattre, I., Ducot, B., Ferrari, P., Bongain, A., Kurzenne, J.-Y., Mas, J.-C., Fénichel, P., 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.

Buuren, S.V., Groothuis-Oudshoorn, K., 2010. mice: Multivariate imputation by chained equations in R. J. Stat. Softw. 1–68.

Cechova, E., Scheringer, M., Seifertova, M., Mikes, O., Kroupova, K., Kuta, J., Forns, J., Eggesbo, M., Quaak, I., De Cock, M., Van De Bor, M., Patayova, H., Palkovicova Murinova, L., Kocan, A., 2017. Developmental neurotoxicants in human milk: Comparison of levels and intakes in three European countries. Sci. Total Environ. 579, 637–645.

Čechová, E., Seifertova, M., Kukucka, P., Vojta, S., Quaak, I., de Cock, M., van de Bor, M., Kocan, A., 2017. An effective clean-up technique for GC/EI-HRMS determination of developmental neurotoxicants in human breast milk. Anal. Bioanal. Chem. 409, 1311–1322.

Cerrillo, I., Granada, A., López-Espinosa, M.J., Olmos, B., Jiménez, M., Caño, A., Olea, N., Fátima Olea-Serrano, M., 2005. Endosulfan and its metabolites in fertile women, placenta, cord blood, and human milk. Environ. Res. 98, 233–239.

Chevalier, N., Brucker-Davis, F., Lahlou, N., Coquillard, P., Pugeat, M., Pacini, P., Panaïa-Ferrari, P., Wagner-Mahler, K., Fénichel, P., 2015. A negative correlation between insulin-like peptide 3 and bisphenol A in human cord blood suggests an effect of endocrine disruptors on testicular descent during fetal development. Hum. Reprod. 30, 447–453.

Collet, B., Van Vugt-Lussenburg, B.M.A., Swart, K., Helmus, R., Naderman, M., de Rijke, E., Eggesbø, M., Brouwer, A., van der Burg, B., 2020. Antagonistic activity towards the androgen receptor independent from natural sex hormones in human milk samples from the Norwegian HUMIS cohort. Environ. Int. 143, 10594.

Coosen, R., van Velsen, F.L., 1989. Effects of the β-isomer of hexachlorocyclohexane on estrogen-sensitive human mammary tumor cells. Toxicol. Appl. Pharmacol. 101, 310–318.

Damgaard, I.N., Skakkebaek, N.E., Toppari, J., Virtanen, H.E., Shen, H., Schramm, K.-W., Petersen, J.H., Jensen, T.K., Main, K.M., Nordic Cryptorchidism Study, G., 2006. Persistent pesticides in human breast milk and cryptorchidism. Environ. Health Perspect. 114, 1133–1138.

Eggesbø, M., Stigum, H., Longnecker, M.P., Polder, A., Aldrin, M., Basso, O., Thomsen, C., Skaare, J.U., Becher, G., Magnus, P., 2009. Levels of hexachlorobenzene (HCB) in breast milk in relation to birth weight in a Norwegian cohort. Environ. Res. 109, 559–566.

Forns, J., Iszatt, N., White, R.A., Mandal, S., Sabaredzovic, A., Lamoree, M., Thomsen, C., Haug, L.S., Stigum, H., Eggesbo, M., 2015. Perfluoroalkyl substances measured in breast milk and child neuropsychological development in a Norwegian birth cohort study. Environ. Int. 83, 176–182.

Forns, J., Mandal, S., Iszatt, N., Polder, A., Thomsen, C., Lyche, J.L., Stigum, H., Vermeulen, R., Eggesbo, M., 2016. Novel application of statistical methods for analysis of multiple toxicants identifies DDT as a risk factor for early child behavioral problems. Environ. Res. 151, 91–100.

Goncharov, A., Rej, R., Negoita, S., Schymura, M., Santiago-Rivera, A., Morse, G., Carpenter, D.O., 2009. Lower Serum Testosterone Associated with Elevated Polychlorinated Biphenyl Concentrations in Native American Men. Environ. Health Perspect. 117, 1454–1460.

Goodyer, C.G., Poon, S., Aleksa, K., Hou, L., Atehortua, V., Carnevale, A., Koren, G., Jednak, R., Emil, S., Bagli, D., Dave, S., Hales, B.F., Chevrier, J., 2017. A Case-Control Study of Maternal Polybrominated Diphenyl Ether (PBDE) Exposure and Cryptorchidism in Canadian Populations. Environ. Health Perspect. 125, 057004.

Gurney, J.K., McGlynn, K.A., Stanley, J., Merriman, T., Signal, V., Shaw, C., Edwards, R., Richiardi, L., Hutson, J., Sarfati, D., 2017. Risk factors for cryptorchidism. Nat. Rev. Urol. 14, 534.

Hosie, S., Loff, S., Witt, K., Niessen, K., Waag, K.L., 2000. Is there a correlation between organochlorine compounds and undescended testes? Eur. J. Pediatr. Surg.: Off. J. Austr. Assoc. Pediatr. Surg. [et al] = Zeitschrift fur Kinderchirurgie 10, 304–309.

Hutson, J.M., Li, R., Southwell, B.R., Newgreen, D., Cousinery, M., 2015. Regulation of testicular descent. Pediatr. Surg. Int. 31, 317–325.

Jensen, D.V., Christensen, J., Virtanen, H.E., Skakkebæk, N.E., Main, K.M., Toppari, J., Veje, C.V., Andersson, A.-M., Nielsen, F., Grandjean, P., 2013. No association between exposure to perfluorinated compounds and congenital cryptorchidism: a nested case-control study among 215 boys from Denmark and Finland. Reproduction. REP-13-0444.

Jørgensen, K.T., Jensen, M.S., Toft, G.V., Larsen, A.D., Bonde, J.P., Hougaard, K.S., 2014. Risk of cryptorchidism among sons of horticultural workers and farmers in Denmark. Scand. J. Work. Environ. Health 40, 323–330.

Juul, A., Almstrup, K., Andersson, A.-M., Jensen, T.K., Jorgensen, N., Main, K.M., Meyts, E.R.-D., Toppari, J., Skakkebaek, N.E., 2014. Possible fetal determinants of male infertility. Nat. Rev. Endocrinol. 10, 553–562.

Komarowska, M.D., Hermanowicz, A., Czyzewska, U., Milewski, R., Matuszczak, E., Miltyk, W., Debek, W., 2015. Serum Bisphenol A Level in Boys with Cryptorchidism: A Step to Male Infertility? Int. J. Endocrinol. 2015, 973154.

Koskenniemi, J.J., Virtanen, H.E., Kiviranta, H., Damgaard, I.N., Matomäki, J., Thorup, J.M., Hurme, T., Skakkebaek, N.E., Main, K.M., Toppari, J., 2015. Association between levels of persistent organic pollutants in adipose tissue and cryptorchidism in early childhood: a case-control study. Environ. Health 14, 78.

Kristensen, P., Irgens, L.M., Andersen, A., Bye, A.S., Sundheim, L., 1997. Birth Defects among Offspring of Norwegian Farmers, 1967–1991. Epidemiology 8, 537–544.

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., Audouze, K., Brunak, S., Main, K.M., 2012. Association between chemical pattern in breast milk and congenital cryptorchidism: modelling of complex human exposures. Int. J. Androl. 35, 294–302.

Kuiri-Hänninen, T., Koskenniemi, J., Dunkel, L., Toppari, J., Sankilampi, U., 2019. Postnatal Testicular Activity in Healthy Boys and Boys With Cryptorchidism. Front. Endocrinol. 10, 489.

Lenters, V., Vermeulen, R., Portengen, L., 2018. Performance of variable selection methods for assessing the health effects of correlated exposures in case–control studies. Occup. Environ. Med. 75, 522–529.

Longnecker, M.P., Klebanoff, M.A., Brock, J.W., Zhou, H., Gray, K.A., Needham, L.L., Wilcox, A.J., 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.

Loreto-Gómez, C., Farías, P., Moreno-Macías, H., Guzmán, C., Riojas-Rodríguez, H., 2018. Prenatal exposure to persistent organic compounds and their association with anogenital distance in infants. Reprod. BioMed. Online 37, 732–740.
Main, K.M., Kiviranta, H., Virtanen, H.E., Sundqvist, E., Tuomisto, J.T., Tuomisto, J., Vartiainen, T., Skakkebaek, N.E., Toppari, J., 2007. Flame retardants in placenta and breast milk and cryptorchidism in newborn boys. Environ. Health Perspect. 115, 1519–1526.
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.
Pierik, F.H., Klebanoff, M.A., Brock, J.W., Longnecker, M.P., 2007. Maternal pregnancy serum level of heptachlor epoxide, hexachlorobenzene, and beta-hexachlorocyclohexane and risk of cryptorchidism in offspring. Environ. Res. 105, 364–369.
Polder, A., Skaare, J.U., Skjerve, E., Loken, K.B., Eggesbo, M., 2009. Levels of chlorinated pesticides and polychlorinated biphenyls in Norwegian breast milk (2002–2006), and factors that may predict the level of contamination. Sci. Total Environ. 407, 4584–4590.
Schneuer, F.J., Holland, A.J.A., Pereira, G., Jamieson, S., Bower, C., Nassar, N., 2016. Age at Surgery and Outcomes of an Undescended Testis. Pediatrics 137, e20152768.
Shin, J., Jeon, G.W., 2020. Comparison of diagnostic and treatment guidelines for undescended testis. Clin. Exp. Pediatr. 63, 415–421.
Sijstermans, K., Hack, W.W., Meijer, R.W., Van der Voort-Doedens, L.M., 2008. The frequency of undescended testis from birth to adulthood: a review. Int. J. Androl. 31, 1–11.
Skaare, J.U., Tuveng, J.M., Sande, H.A., 1988. Organochlorine pesticides and polychlorinated biphenyls in maternal adipose tissue, blood, milk, and cord blood from mothers and their infants living in Norway. Arch. Environ. Contam. Toxicol. 17, 55–63.
Skakkebæk, N.E., 2002. Endocrine disrupters and testicular dysgenesis syndrome. Hormone Res. Paediatr. 57, 43.
Skjaerven, R., Gjessing, H.K., Bakketeig, L.S., 2000. Birthweight by gestational age in Norway. Acta Obstet. Gynecol. Scand. 79, 440–449.
Skulstad, S.M., Igland, J., Johannessen, A., Bertelsen, R.J., Lønnebotn, M., Omenaas, E. R., Svanes, C., Real, F.G., 2017. Validation of maternal reported pregnancy and birth characteristics against the Medical Birth Registry of Norway. PLoS ONE 12, e0181794.
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.
Toft, G., Jönsson, B.A.G., Bonde, J.P., Nørgaard-Pedersen, B., Hougaard, D.M., Cohen, A., Lindh, C.H., Ivell, R., Anand-Ivell, 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.
Thomsen, C., Stigum, H., Froshaug, M., Broadwell, S.L., Becher, G., Eggesbo, M., 2010. Determinants of brominated flame retardants in breast milk from a large scale Norwegian study. Environ. Int. 36, 68–74.
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.
Virtanen, H.E., Adamsson, A., 2012. Cryptorchidism and endocrine disrupting chemicals. Mol. Cell. Endocrinol. 355, 208–220.
Virtanen, H.E., Bjerknes, R., Cortes, D., Jørgensen, N., Rajpert-De Meyts, E., Thorsson, A. V., Thorup, J., Main, K.M., 2007. Cryptorchidism: classification, prevalence and long-term consequences. Acta Pædiatr. 96, 611–616.
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.
Virtanen, H.E., Toppari, J., 2008. Epidemiology and pathogenesis of cryptorchidism. Hum Reprod Update 14, 49–58.
Wagner-Mahler, K., Kurzenne, J.-Y., Delattre, I., Bérard, E., Mas, J.-C., Bornebush, L., Tommasi, C., Boda-Buccino, M., Ducot, B., Boullé, C., Ferrari, P., Azuar, P., Bongain, A., Fénichel, P., Brucker-Davis, F., 2011. Prospective study on the prevalence and associated risk factors of cryptorchidism in 6246 newborn boys from Nice area, France. Int. J. Androl. 34, e499–e510.
Waliszewski, S.M., Infanzon, R.M., Arroyo, S.G., Pietrini, R.V., Carvajal, O., Trujillo, P., Hayward-Jones, P.M., 2005. Persistent organochlorine pesticides levels in blood serum lipids in women bearing babies with undescended testis. Bull. Environ. Contam. Toxicol. 75, 952–959.
Warner, J., Osuch, J.R., Karmaus, W., Landgraf, J.R., Taffe, B., O'Keefe, M., Mikucki, D., Haan, P., 2012. Common classification schemes for PCB congeners and the gene expression of CYP17, CYP19, ESR1 and ESR2. Sci. Total Environ. 414, 81–89.
WHO, 2007. Fourth WHO Coordinated Survey of Human Milk for Persistent Organic Pollutants in Cooperation with UNEP. Guidelines for Developing A National Protocol. Available online: https://www.who.int/foodsafety/chem/POPprotocol.pdf?ua=1. Revised 1 October 2007 ed. Geneva, Switzerland WHO.
White, I.R., Royston, P., Wood, A.M., 2011. Multiple imputation using chained equations: Issues and guidance for practice. Stat. Med. 30, 377–399.
Zou, H., Hastie, T., 2005. Regularization and variable selection via the elastic net. J. R. Stat. Soc.: Ser. B (Stat. Methodol.) 67, 301–320.