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Prenatal exposure to phthalates and phenols and infant endocrine-sensitive outcomes: The MIREC study

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ABSTRACT

Background: Anogenital distance (AGD) and the second to fourth finger (2D:4D) digit ratio may be early markers of in utero androgen exposure for the infant. Phthalates and phenols have been identified as endocrine disrupting chemicals.

Objectives: To study the association between prenatal exposure to phthalates, bisphenol A (BPA) and triclosan (TCS) and AGD and the 2D:4D digit ratios.

Methods: Single spot urine samples were collected in the first trimester from the MIREC Study and analyzed for phthalates and phenols. Anogenital distance (n = 394) at birth and 2D:4D digit ratios (n = 420) at 6 months were measured in male and female infants. Associations between maternal concentrations of phenols and phthalate metabolites and these outcomes were estimated using multiple linear regression models.

Results: In females, the anoclitoris distance (ACD) was negatively associated with mono-benzyl phthalate (MBzP) (β = −1.24; 95% CI −1.91, −0.57) and positively associated with mono-ethyl phthalate (MEP) (β = 0.65; 95% CI 0.12, 1.18) (masculinizing). In males, anopenile distance (APD) was positively associated with mono-n-butyl phthalate (MnBP) (β = 1.17; 95% CI 0.02, 2.32) and the molar sum of low molecular weight phthalates (ΣLMW). Female 2D:4D of the right hand was positively associated with MnBP and negatively with total BPA (masculinizing).

Conclusions: Significant associations were only observed for the long AGD metrics. Positive associations were observed between MnBP or LMW phthalates and APD in males. In females, prenatal MEP was associated with a masculinizing effect on ACD, while MBzP was associated with a feminizing effect. No significant associations were observed between prenatal phenols and AGD. Given the paucity of research on digit ratios and prenatal chemical exposures, it is difficult to say whether this metric will be a useful marker of prenatal androgen or anti-androgen exposure. Given the large number of associations examined, the statistical associations observed may have been due to Type 1 error. The inconsistencies in results between studies suggest that this issue is yet to be resolved.

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1. Background

Endocrine disrupting chemicals (EDCs) are "compounds, either natural or synthetic, which, through environmental or inappropriate developmental exposures, alter the hormonal and homeostatic systems that enable the organism to communicate with and respond to its environment" (Diamanti-Kandarakis et al. 2009). These chemicals are most harmful when exposure occurs during critical periods of development; that is, prenatally or during early postnatal development (Bigsby et al. 1999). The development of the reproductive tract and its comprising organs is highly dependent on the function of hormones during fetal growth. Measurement of endocrine sensitive endpoints, especially those that are sexually dimorphic, can be used as sentinels for later adverse health effects arising from prenatal exposure to endocrine disrupting chemicals (Arbuckle et al. 2008). Several studies have shown the sexually dimorphic nature of anogenital distance (AGD) in humans (Liu et al. 2014; Barrett et al. 2014). Anogenital distance is the distance between the genitals and the anus in boys and girls (Sathyanarayana et al. 2015). Longer AGD in women has been associated with higher testosterone levels (Mira-Escolano et al. 2014), possibly representing a masculinization effect in the prenatal period, while shorter AGD in males may imply diminished masculinization of the genitalia (Dean and Sharpe 2013).

Additionally, several studies suggest that the ratio between the length of the second and fourth fingers (2D:4D digit ratio), which also varies by sex (generally in males the 2nd digit is shorter than the 4th resulting in lower digit ratios), may be a useful measure of fetal androgen or anti-androgen exposure (Ventura et al. 2013; Zheng and Cohn 2011). In mice, Zheng and Cohn (2011) reported that during early stages of digit development, the ratio of prenatal androgen to estrogen signaling determined the length of the 4th digit and, ultimately, affected the 2D:4D ratio. Androgen receptor (AR) and estrogen receptor α (ER-α) activity were higher in the 4th digit than in the second. Inactivation of AR decreased growth of digit 4, which caused a higher 2D:4D ratio, whereas inactivation of ER-α increased growth of digit 4, which led to a lower 2D:4D ratio (Zheng and Cohn 2011).

Phthalates and phenols are ubiquitous chemicals with potential endocrine disrupting properties. Phthalates are a group of industrial chemicals used in plastics, paints and coatings, adhesives and sealants, automotive parts, electronics, and personal care products (Government of Canada 2017a). The percentage of the Canadian population with detectable urinary concentrations of phthalates varied from very low to 100% (in 2007–2009), depending on the phthalate metabolite measured (Health Canada 2013). Phthalates are thought to disrupt steroid regulated development of the reproductive tract through dysregulation of steroid synthesis (Witorsch and Thomas 2010) and estrogenic, androgenic or anti-androgenic activity (Thibaut and Porte 2004; Inoshita et al. 2003; Jobling et al. 1995; Harris et al. 1997). For example, benzyl butyl phthalate (BBP) and dibutyl phthalate (DBP) both increased transcription of an estrogen-regulated gene in breast cancer cells (Jobling et al. 1995). Thus, we sought to investigate the relationship between phthalate exposure and steroid dependent endpoints.

Bisphenol A (BPA) is an industrial chemical used widely in the production of polycarbonate plastics, which are used in many consumer products. BPA is also found in epoxy resins, which are used in the protective lining of metal-based food and beverage cans (Government of Canada 2017b). BPA has also been measured in paper currency (Liao and Kannan 2011). Although the primary route of exposure to BPA is through diet (Environment Canada and Health Canada 2008), exposure to BPA can also occur from other sources, such as via handling of thermal paper receipts (Hehn 2016). Approximately 90% and 95% of the Canadian population had measurable concentrations of BPA in their urine in 2007–2009 (age range of 6–79 years) and 2009–2011 (age range of 3–79 years), respectively (Health Canada 2013). A recent review suggests that disruption of the estrogenic pathway is central in the mediation of the effects of BPA on reproductive, neurobehavioral, metabolic functions and the mammary gland (ANSES's Working Group on "Endocrine disruptors" et al. 2018). Although BPA has been identified as an estrogenic endocrine disrupting chemical, the effects of BPA appear to involve complex interactions between multiple signaling systems that vary in a cell type-specific manner (MacKay and Abizaid 2018). Thus, as MacKay and Abizaid (2018) note, any given BPA-induced phenotype should not be expected to be explainable entirely in terms of estrogenic stimulation.

Triclosan (TCS) is used as an antimicrobial agent and preservative in a variety of personal-care and cleaning products (Environment and Climate Change Canada and Health Canada 2016). In Canada, TCS was detectable in about 70% of the urine samples collected as part of the 2009–2011 Canadian Health Measures Survey for those aged 3–79 years (Health Canada 2013). While one review of laboratory studies suggests that triclosan has estrogenic properties (Wang and Tian 2015), another review of mammalian and human studies concludes that there is little evidence that TCS adversely affects the male or female reproductive systems, with the caveat that only a limited number of such studies have been conducted (Witorsch 2014).

Changes in early sexually dimorphic markers, such as AGD and the 2D:4D ratio, may suggest health effects from prenatal exposure to these chemicals. Studies of prenatal phthalate exposure and AGD have been inconsistent with some reporting significant reductions in AGD in male infants that varied by phthalate metabolite (Swan et al. 2005, 2015; Swan 2008; Bustamante-Montes et al. 2013; Suzuki et al. 2012; Bornehag et al. 2015), while others reported effects in female but not male infants (Huang et al. 2009) or observed no effect in male infants (Jensen et al. 2016). One study reported that parental occupational exposure to BPA during pregnancy was associated with shortened AGD in male offspring (Miao et al. 2011) and another reported that higher 1st trimester BPA was associated with significantly shorter AGD in daughters (Barrett et al. 2017). Only one study has examined the association between in utero exposure to TCS and AGD and observed no significant effects in boys or girls (Lassen et al. 2016). Further, no studies have looked at the association between in utero exposure to BPA, TCS or phthalate metabolites and the 2D:4D digit ratio in humans. One study has reported significantly feminized digit ratios in male rats that were prenatally exposed to BPA (Auger et al. 2013).

Animal models have suggested that the critical window for testicular dysgenesis syndrome (TDS) induction, such as the effects of phthalates on AGD, are in the masculinization programming window (van den Driesche et al. 2017), which in humans is estimated to occur between 8 and 14 weeks gestation (Welsh et al. 2014).

The objectives of this study were to investigate the association between maternal urinary levels of several phthalates and phenols in the first trimester (the masculinization programming window), and early markers of reproductive health in Canadian infants, namely AGD and 2D:4D digit ratio in male and female infants.

2. Methods

2.1. Study population and data collection

The Maternal-Infant Research on Environmental Chemicals (MIREC) Study recruited women during the first trimester of pregnancy through prenatal clinics (ultrasound, midwife and/or doctor's clinics) in 10 cities across Canada (Arbuckle et al. 2013). The eligibility criteria included: age 18 years or older; ability to consent and communicate in English or French; planning to deliver at a local hospital; and agreeing to participate in the cord blood collection component of the study. Women with known fetal chromosomal or major malformations in the current pregnancy or with a history of chronic medical conditions (renal disease with altered renal function, epilepsy, any collagen disease such as lupus erythematosus and scleroderma, active and chronic liver disease (hepatitis), heart disease, serious pulmonary disease, cancer, hematologic disorder (patients with anemia or thrombophyllia

were included), threatened spontaneous abortion (women with previous bleeding in first trimester could be included if the site documented a viable fetus at the time of recruitment)) and illicit drug use were excluded.

During the 1st trimester, a detailed questionnaire was administered to collect information on the woman's age, education, race, medical history (obstetrical and non-obstetrical), household income, country of birth, and smoking habits.

As funding was limited and there were delays in obtaining ethics approval at some of the sites, the follow-up study (MIREC-ID) of infants at birth and 6 months of age targeted six of the 10 cities and recruited mothers and singleton infants without any major congenital birth defects or neurological disorders (Supplemental Material Fig. S1). Anthropometric and anogenital measures of the infants were conducted during the neonatal period. When the infant reached 6 months of age (adjusted for gestational age of premature infants), the follow-up visit was scheduled to measure infant growth and digit length, among other assessments.

The study population for this research project consisted of 396 mother-infant pairs that were assessed at birth (from 5 of the 6 cities) and 421 mother-infant pairs assessed at approximately 6 months of age (from the 6 cities). Three hundred and seventeen mother-infant pairs participated in both the birth and 6-month assessments, while 79 participated in the birth assessment only and 104 in the 6-month assessment only.

The study was reviewed by research ethics boards in each recruitment site and at Health Canada and all participants signed informed consent forms. All examiners completed a training session with a pediatrician, endocrinologist, psychologist and pediatric urologist using volunteer infants and were provided with a detailed manual of procedures and presentation materials for review.

Gestational age at birth and infant age at examination were determined from medical charts and questionnaires. Infant weight and length were measured during the physical exam using an infant scale (seca 727) and infantometer (seca 416). Length was measured from the crown of the head to the heel while both legs were stretched simultaneously (with one hand on the baby's knees) and the chin supported perpendicular to the surface. Two measurements were taken and if the difference between two first measurements was > 5 g for weight or 3 mm for length, then a third measurement was taken. The mean of the two measurements was calculated and used for this analysis; if a third measurement was taken, then the mean of the 2 closest measures was calculated and used.

2.2. Urine analyses

During the 1st trimester, each woman provided a spot urine sample that was collected in polypropylene cups, aliquoted into 30-mL Nalgene® tubes, and then frozen at −20 °C until analyzed. Maternal urines were analyzed for 11 phthalate metabolites (by LC-MS/MS) and total BPA (by GC–MS/MS) (Arbuckle et al. 2014), as well as total and free TCS (by ultra-performance liquid chromatography-tandem mass spectrometry using isotope dilution) (Provencher et al. 2014; Arbuckle et al. 2015). The phthalate metabolites were: mono-benzyl phthalate (MBzP; limit of detection (LOD):0.20 μg/L), mono-*n*-butyl phthalate (MnBP; LOD:0.20 μg/L), mono-cyclohexyl phthalate (MCHP; LOD:0.20 μg/L), mono-ethyl phthalate (MEP; LOD:0.50 μg/L), mono-(2-ethylhexyl) phthalate (MEHP; LOD:0.20 μg/L), mono-(2-ethyl-5-hydroxy-hexyl) phthalate (MEHHP; LOD:0.40 μg/L), mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP; LOD:0.20 μg/L), mono-isononyl phthalate (MiNP; LOD:0.40 μg/L), mono-methyl phthalate (MMP; LOD:5.00 μg/L), mono-(3-carboxypropyl) phthalate (MCPP; LOD:0.20 μg/L), and mono-*n*-ocytl phthalate (MnOP; LOD:0.70 μg/L). The LODs for total BPA, total TCS and free TCS in urine were 0.2 μg/L, 0.12 μg/L, and 0.008 μg/L, respectively.

All field blanks were free of phthalate, BPA and TCS contamination and all materials in contact with urine samples were pre-screened and found not to be a source of contamination (Arbuckle et al. 2014, 2015).

Specific gravity was measured in thawed urine samples by a refractometer (UG-1, Atago #3461, Atago U.S.A. Inc., Bellevue, WA). Furthermore, for each participant, several sampling characteristics were noted at the time of urine collection, including: the date and time it was collected (possibly related to diurnal or seasonal phthalate variability) and the number of minutes since last urination.

2.3. Outcome measures

2.3.1. Anogenital distance measurements

Measures were taken shortly after birth (mean 3.5 days) using methods described in Sathyanarayana et al. (2010, 2015) (Supplemental Material Fig. S2). For female infants, the distance (in mm) from the center of the anus to the posterior convergence of the fourchette (anofourchette or AFD) or the clitoris (anoclitoris distance or ACD) was measured. To measure both distances in female infants, the infant was required to be in the dorsal decubitus position. Similarly, for male infants, the distances (in mm) between the base of the scrotum (junction of the smooth perineal skin and the rugated skin of the scrotum) and the mid-anus (anoscrotal distance or ASD) and between the centers of the anus to the cephalad (superior) base of the penis (anopenile distance or APD) were measured. For the measurement of the distances, metric dial Vernier calipers (Scienceware; Bel-Art Products, Pequannock, New Jersey) with modified rounded corners with increments of 0.1 mm were used. The caliper was properly calibrated and set to zero prior to each measurement. Two measurements were taken and reported; if there was a > 2 mm difference between the 2 measures, then a third measurement was taken. For the purpose of this analysis, the mean of the 2 closest measures was calculated and used.

2.3.2. 2D:4D digit ratio

A second outcome of interest was the ratio of the lengths of the second and fourth finger digits (2D:4D digit ratio) (Dean and Sharpe, 2013). The length (in cm) of the second and fourth finger of both the left and right hand were measured during the 6-month assessment of the infant. These measurements were done with a transparent plastic ruler with millimetre increments, assuring the bottom of the ruler was aligned with the basal crease of each finger. Measurements were taken twice for each finger length for each hand. For the purpose of this analysis, the mean finger length for each hand was employed.

2.4. Statistical analysis

The exposures of interest were the urinary concentrations of BPA, TCS and phthalates (μg/L) in pregnant women, as measured by single spot urine samples. The metabolites MMP, MCHP, MiNP and MnOP had > 50% of the samples below the LOD (Table 1) and were excluded from further analysis of individual metabolites. As a result, the following analytes were examined: MnBP, MBzP, MCPP, MEHHP, MEHP, MEOHP, MEP, total BPA, total TCS and free TCS. We also calculated the molar sums of di-2-ethylhexyl phthalate (DEHP) metabolites (MEHP, MEOHP, MEHHP), low molecular weight (MMP, MEP, MnBP, MCHP, and MBzP) and high molecular weight (MCPP, MEHP, MnOP, MEOHP, MiNP, and MEHHP) phthalate metabolites. We also examined the molar sum of medium-chain phthalate metabolites (MnBP, MBzP, MCHP, MiNP, MEHP, MEOHP, MEHHP) as there is some evidence that phthalates in the medium-chain subgroup (where the longest carbon backbone is between 3 and 7) have a common mode of action, producing phthalate-induced androgen insufficiency during male reproductive development in the rat (the “rat phthalate syndrome”) (Environment Canada and Health Canada 2015; Environment and Climate Change Canada and Health Canada 2017). The chemical concentrations were highly skewed and were natural log transformed before being included in the models. These transformations resulted in

Table 1
First trimester maternal urine volumetric concentrations (μg/L) for participants with birth assessments.

Chemical (μg/L)	N	LOD	% < LOD	Min	Max	Geometric Mean	95% Confidence interval		Sample median	Sample 95th percentile
							Lower	Upper		
MMP	370	5	83.24	ND[a]	87.00	ND	ND	ND	ND	9.90
MCHP	370	0.2	94.59	ND	77.00	ND	ND	ND	ND	0.30
MEP	370	0.5	0.27	ND	13,000.00	27.15	23.21	31.75	25.00	420.00
MnBP	370	0.2	0.00	0.36	800.00	10.66	9.43	12.05	12.00	61.00
MBzP	369	0.2	0.54	ND	230.00	5.15	4.52	5.86	5.40	38.00
MEHHP	370	0.4	1.62	ND	520.00	7.62	6.72	8.64	8.35	46.00
MiNP	370	0.4	98.38	ND	6.20	ND	ND	ND	ND	ND
MnOP	370	0.7	97.57	ND	1.10	ND	ND	ND	ND	ND
MCPP	370	0.2	18.65	ND	26.00	0.73	0.63	0.84	0.83	8.10
MEOHP	370	0.2	0.81	ND	290.00	5.39	4.78	6.08	6.00	31.00
MEHP	366	0.2	1.37	ND	110.00	1.95	1.74	2.18	2.05	12.00
Σ DEHP[b]	366	NA[c]	NA	1.38	3021.26	52.89	46.94	59.60	57.50	301.06
Σ LMW[b]	369	NA	NA	22.82	67,239.48	297.23	263.26	335.59	281.19	2447.30
Σ HMW[b]	366	NA	NA	3.72	3028.75	61.76	55.24	69.06	65.55	315.29
Σ MC[b]	366	NA	NA	6.21	3654.55	140.08	124.94	157.05	166.54	745.62
Total BPA	396	0.2	12.12	ND	17.00	0.78	0.69	0.87	0.82	5.00
Total TCS	390	0.12	0.51	ND	2322.96	10.83	8.69	13.51	7.77	649.17
Free TCS	393	0.008	22.90	ND	36.71	0.07	0.05	0.08	0.05	4.73

LMW = low molecular weight; HMW = high molecular weight; MC = medium chain.
[a] ND - Concentration below the level of detection (LOD).
[b] nmol/L.
[c] NA - Not Applicable.

more normally and evenly distributed residuals. All values below the LOD were substituted with 1/2 LOD.

The outcome variables included both the short anogenital distance measure (i.e. the anofourchette and the anoscrotal distances in females and males, respectively), the long anogenital distance measure (i.e. the anoclitoris and the anopenile in females and males, respectively) and the 2D:4D digit ratio in both the left and right hands. We calculated the coefficients of variation (CVs) across repeated measures. All results were stratified by gender. The Levene's test was used to examine the homogeneity of variance across sites. The only significant difference found was in the variance across sites for the anofourchette distance; however, this may have been due to Type 1 error, given the number of outcomes examined.

Potential confounders considered in all models included several demographic and anthropometric measurement variables as well as urine collection parameters that were evaluated in previous analyses of predictors of maternal urinary phthalate and phenol concentrations (Arbuckle et al. 2014, 2015). The following maternal variables were considered: age, race, country of birth (Canada, other), pre-pregnancy body mass index (BMI), education, annual household income, active exposure to tobacco smoke and passive smoke exposure (any time during pregnancy) (Table 2). In addition, we considered season, time of urine collection and time since last void (categorized as in Table 2), recruitment centre, gestational age at birth, infant age, infant weight and length at examination, and lastly, a weight-for-length *z* score which was calculated based on the standards set out by the World Health Organization (2011). Specific gravity of the urine was included in the analysis to account for varying dilution of the urine voids.

To identify potential confounders in the models, the "change-in-estimate" (CIE) method, in which variables are selected based on relative or absolute changes in the estimated exposure effect was used (Greenland, 1989). Automating the CIE strategy was necessary to allow for a better assessment of the confounding variables. The SAS macro %ABE (Augmented backward elimination) (http://cemsiis.meduniwien.ac.at/kb/wf/software/statistischesoftware/abe/) procedure was used with a *p*-value of 0.2 and a change-in-estimate criterion of 0.1 (10%). Furthermore, the CIE criterion was standardized such that its application was independent of the scaling of the explanatory variables and the outcome variable's scale.

Multiple linear regression models were created to examine the relationship between the AGD and the 2D:4D digit ratios and the exposure of interest, including specific gravity and significant confounders as identified from the change-in estimate procedure. The same confounders were kept for all phenol and phthalate models. For all models, regression assumptions were examined using residual plots and were illustrated by plotting a normal probability curve and quantile-quantile plots. Adjusted models were also checked for collinearity using the variance inflation factors (VIFs) of each variable.

3. Results

3.1. Birth assessment

The distribution of prenatal exposure to the chemicals is described in Table 1. MEP, MnBP, MBzP, and the DEHP metabolites were most frequently detected. MMP was rarely detected, which was likely due to the method's high detection limit. As illustrated in Table 2, participants tended to be from higher socio-economic groups, predominantly white, not currently smoking and born in Canada, similar to the original cohort. The mean age of infants at the birth examination was 3.49 days old with an even distribution of 198 males and 198 females.

In females, the mean anoclitoris distance was approximately 33.5 mm, while the mean anofourchette distance was much smaller at 14.7 mm (Table 3). In males, the mean anopenile distance was 43.8 mm, while the mean anoscrotal distance was 22.5 mm. The Spearman's rank correlation between the long and short AGDs was 0.66 ($p < 0.0001$). Results of the multiple regression modelling showed that in females, the anoclitoris distance was positively associated with maternal urinary MEP concentration (masculinizing in effect) and negatively with MBzP (Table 4). None of maternal phenol or phthalate concentrations were significantly associated with anofourchette distances. In males, MnBP and the sum of low molecular weight phthalates was positively associated with anopenile distance; none of the analytes were significantly associated with anoscrotal distance (Table 5).

3.2. 6-Month assessment

Data were available for 420 infants with a mean age of 207 days at

Table 2
Characteristics of mother-infant pairs and urine collection for participants from MIREC-ID birth assessment. (n = 396).

	Frequency	Percentage (%)	Percentage for entire cohort (%)
Recruitment site			
A	17	4.3	
B	87	22.0	
C	105	26.5	
D	107	27.0	
E	80	20.2	
Maternal education			
≤High school	36	9.1	8.8
Some college	20	5.1	5.3
College/trade school diploma	96	24.4	23.6
University degree	242	61.4	62.3
Household income ($)			
≤50,000	80	20.7	17.4
50,001–100,000	189	48.8	39.6
≥100,000	118	30.5	38.2
Mother's country of birth			
Canada	340	85.9	81.3
Other	56	14.1	18.7
Mother's race			
White	361	91.2	
Other	35	8.8	
Mother's smoking status (active)			
Never	255	68.6	61.7
Former	94	25.3	26.3
Current (quit during pregnancy, occasional or daily)	23	6.2	12.0
Mother's exposure to environmental tobacco smoke during pregnancy (passive)			
No	147	38.0	
Yes	240	62.0	
Pre-pregnancy body mass index (kg/m^2)			
< 25.00	220	60.1	63.4
25.00–29.99	77	21.0	22.0
≥30	69	18.8	14.6
Season of urine collection			
Spring	102	25.8	
Summer	87	22.0	
Fall	133	33.6	
Winter	74	18.7	
Time of urine collection			
6:00–11:59	203	51.3	
12:00–14:59	122	30.8	
15:00–24:00	71	17.9	
Time since last urination (min)			
≤75	103	26.9	
76–120	113	29.5	
121–170	61	15.9	
> 170	106	27.7	
Sex of baby			
Female	198	50	47.4
Male	198	50	52.6
	Range	Mean (S.D.)	
Maternal age (years), n = 396	[17,42]	31.37 (4.68)	
Gestational age at birth (weeks), n = 396	[33.4,42]	39.56 (1.44)	
Infant age at exam (days), n = 396	[0,44]	3.49 (4.77)	
Infant weight at exam (kg), n = 341	[1.95,4.59]	3.28 (0.47)	
Infant length at exam (cm), n = 374	[43.05,59.10]	50.43 (2.50)	
Weight-for-length Z-score[a], n = 316	[−5.21,2.83]	−0.46 (1.22)	

[a] World Health Organization, Child Growth Standards. WHO Anthro (version 3.2.2, January 2011) and macros. http://www.who.int/childgrowth/software/en/

examination and weight of 7.9 kg (data not shown). Summary statistics for their 2D:4D digit ratios for both hands are shown in Table 6. The median digit ratios for the right hand tended to be larger than those of the left for both sexes and the ratio was lower in the left hand of males; however, the differences were small. Male 2D:4D ratios were not significantly different than those of females in the same hand. Spearman rank correlations between the long AGDs and 2D:4D ratios were 0.09 for the left and 0.04 for the right hands and were 0.11 for the left and 0.09 for the right hands between the short AGDs and 2D:4D ratios. In females, maternal 1st trimester BPA concentrations were negatively associated with 2D:4D ratios in the right hand (masculinizing in effect), while MnBP was positively associated with the ratio in the right hand (Table 7). None of the chemicals were significantly associated with 2D:4D ratios in males (Table 8).

4. Discussion

Our previous work has demonstrated the high prevalence of exposure to multiple phthalates and phenols in this Canadian pregnant population (Arbuckle et al. 2014; Arbuckle et al. 2015). The current prospective study of prenatal exposure to phthalates and phenols and early potential markers of reproductive health did not find any significant associations between maternal urinary chemical concentrations and shorter AGD in male infants. Similar to our results, two Swedish studies reported no dose-response association between exposure to similar phthalates measured in the 1st trimester in 196 mother-infant pairs and AGD measured at 21 months (Bornehag et al. 2015) or later in pregnancy among 245 mothers (Jensen et al. 2016). However, the earlier Swedish study did report small (4%) reductions in AGD associated with increases in DiNP (diisononyl phthalate) exposure (Bornehag et al. 2015); a phthalate that was not measured in our study. A smaller study of Mexican mother-son pairs (n = 73) only reported significant associations between prenatal phthalates and AGD if all the metabolites were summed (Bustamante-Montes et al. 2013), while a Japanese study (n = 111) only reported an association with MEHP (Suzuki et al. 2012) and no association was observed in a Taiwanese study of 33 male infants (Huang et al. 2009). In contrast, studies of US mothers reported significant associations between a shorter AGD in 134 males and prenatal exposure to MnBP, MBzP, MEP, and MiBP but not DEHP (di-2-ethylhexyl phthalate) metabolites (Swan et al. 2005) but a more recent study (TIDES) (n = 366 males) only reported significant inverse associations with DEHP metabolite concentrations in first trimester urine (Swan et al. 2015). Analysis of a subset of the TIDES population with phthalate measurements in all 3 trimesters observed consistent negative (but not statistically significant) associations with AGD and DEHP metabolites but only in the 1st trimester, which is consistent with rodent data on the critical window for exposure (Martino-Andrade et al. 2016). Another US study also reported significant inverse associations between prenatal MEHP and AGD in 171 boys, which was stronger for African Americans (Wenzel et al. 2018).

Studies of female infants and AGD are limited and inconsistent with a Taiwanese study (n = 32) reporting a negative association with MnBP (Huang et al. 2009), an American study (n = 373) finding no association with any of the phthalates measured (Swan et al. 2015) and our study (n = 196) finding a positive association between ACD and MEP and a negative association with MBzP. A recent American study of 128 female infants has also reported a significant positive association between prenatal MEP and ACD in whites but in African Americans, they observed a significant inverse association (Wenzel et al. 2018).

There are a number of possible explanations for the inconsistencies observed in the literature on phthalates and AGD, including differences in exposure levels (Fig. 1 and Supplemental Material Fig. S3), varying mixtures of phthalates and other potential endocrine disrupting chemicals, timing of urine collection (time of day, gestational age, fasting

Table 3
Summary statistics for anogenital distance outcomes (mm).

Outcome	N	Min	Max	Mean	Standard Deviation	25th Percentile	50th Percentile	75th Percentile	Coefficient of Variation[a]
Anoclitoris distance (females)	195	23.55	52.4	33.5	4.29	30.75	33.55	35.9	4.32
Anofourchette distance (females)	196	6.8	41.55	14.74	4.66	11.8	13.9	16.3	7.06
Anopenile distance (males)	197	30.95	57.55	43.8	4.98	40.45	43.6	47.15	4.01
Anoscrotal distance (males)	198	11.15	39.95	22.48	4.63	19.6	22.25	25.45	6.12

[a] Coefficient of variation across repeated measures.

status), exposure misclassification due to intra-individual variability, and how and at what age AGD was measured. In our study, the research staff were trained by the American team who have led previous AGD studies (e.g., Sathyanarayana et al. 2015), so the AGD methodology should be comparable. Most studies of AGD, including this one, have relatively small sample sizes, each of which tested a large number of potential associations and thus are subject to Type 1 error. Therefore, finding a small number of these associations to be statistically significant by chance alone is not unexpected. In fact, given the 104 associations examined in this paper, if the null hypothesis were true in every case, then we would expect to see 5 to 6 (0.05×104) statistically significant associations.

Maternal stress may also play a role as stress has been shown to modify the negative association between some phthalates and AGD in male infants, with associations only observed when the mother reported no stressful life events during pregnancy (Barrett and Swan 2015; Barrett et al. 2016).

Multigenerational and/or transgenerational effects may also contribute to the disparity in results between human populations. A multigenerational study of mice exposed to a mixture of phthalates similar to human exposure reported a decrease in AGD in F1 (1st generation) females (Zhou et al. 2017a), an increase in anogenital distance in F2 females and a decrease in AGD in F3 females (Zhou et al. 2017b).

We found no significant associations between prenatal BPA exposure and AGD in male or female infants. In a study of occupational exposure to BPA using a job-exposure matrix, a dose-response relationship was observed between increasing estimated BPA exposure levels in pregnancy and shortened AGD in 153 male offspring (Miao et al. 2011). Another study of 137 term male infants reported no significant correlation between AGD and maternal urinary BPA measured in the 3rd trimester (Liu et al. 2016). A small study of newborns reported that high cord blood BPA levels were significantly correlated with shortened anoscrotal distance in 72 males but found no associations in 58 females (Mammadov et al. 2018). However, this latter paper did not detail the steps that should be taken to ensure lack of contamination when measuring BPA in blood (Vandenberg et al. 2014). In animal studies, decreased AGD has been reported following exposure to BPA in pregnancy in both male and female rats (Christiansen et al. 2014) and in first and second generation male rats (Boudalia et al. 2014).

In our cohort, maternal 1st trimester total or free triclosan concentrations in urine were not significantly associated with any AGD metric in male or female infants. A Danish study observed an inverse but insignificant (p-values < 0.10) association in 252 boys between maternal urinary TCS levels and both the long and the short AGD measure and no effects in 179 girls at 3 months of age (Lassen et al. 2016). Although TCS concentrations were lower in the Danish study than in MIREC, the APD beta's were in opposite directions, while the ASD betas were similar (Fig. 1 and see Supplemental Material Fig. S3).

In our exploratory analysis of finger digit ratios, BPA was negatively associated with 2D:4D digit ratio (a masculinizing effect) in females, but only in the right hand. No significant associations were observed in male infants for BPA or for any other chemical examined. No similar human studies could be found. In the Wistar rat, sexual dimorphism of

Table 4
Association between anoclitoris and anofourchette distances in female infants at birth and ln-transformed chemical metabolites in 1st trimester maternal urine, expressed as a beta-coefficient (change in mm) with 95% CI from an adjusted linear regression model.

	Anoclitoris distance[a]				Anofourchette distance[b]			
Chemical	Coefficient	Lower 95% CI	Upper 95% CI	*P* value	Coefficient	Lower 95% CI	Upper 95% CI	*P* value
Total TCS	0.1903	−0.1456	0.5262	0.26	−0.1350	−0.5264	0.2565	0.50
Free TCS	0.0150	−0.3493	0.3791	0.94	−0.1039	−0.536	0.3283	0.64
Total BPA	−0.1301	−0.7786	0.5183	0.69	0.0684	−0.6388	0.7755	0.85
MEP	**0.6507**	**0.1227**	**1.1787**	**0.02**	−0.3194	−0.8180	0.1792	0.21
MnBP	−0.3004	−1.1068	0.5060	0.46	0.0253	−0.7233	0.7740	0.95
MBzP	**−1.2401**	**−1.9080**	**−0.5723**	**0.0004**	−0.4095	−1.0525	0.2335	0.21
MEHHP	−0.3563	−1.2716	0.5589	0.44	0.2599	−0.5925	1.1123	0.55
MCPP	−0.2337	−0.8737	0.4063	0.47	−0.0755	−0.7032	0.5522	0.81
MEOHP	−0.3064	−1.279	0.6663	0.53	0.3153	−0.5815	1.2122	0.49
MEHP	−0.3923	−1.4511	0.6665	0.46	0.5348	−0.4041	1.4737	0.26
Σ DEHP[c]	−0.3676	−1.3583	0.6231	0.46	0.3332	−0.5794	1.2458	0.47
Σ LMW[d]	0.5731	−0.1465	1.2928	0.12	−0.3763	−1.0625	0.3099	0.28
Σ HMW[e]	−0.5249	−1.6279	0.5781	0.35	0.3583	−0.6534	1.3699	0.48
Σ MC[f]	−0.5447	−1.4875	0.3981	0.25	0.1428	−0.7266	1.0122	0.75

The numerical figures in bold are statistically significant.

[a] Phenols controlled for: specific gravity (SG), site, time of urine collection, maternal age, gestational age, weight-for-length z-score; Phthalates controlled for: SG, education, mother born in Canada, gestational age, maternal age, weight-for-length z-score.

[b] Phenols controlled for: SG, income, smoking status, maternal race, weight-for-length z-score; Phthalates controlled for: SG, smoking status, maternal race, weight-for-length z-score.

[c] Molar sum of DEHP metabolites: MEHP, MEOHP and MEHHP.

[d] Molar sum of low molecular weight phthalate metabolites: MMP, MEP, MnBP, MCHP, MCPP and MBzP.

[e] Molar sum of high molecular weight phthalate metabolites: MEHP, MnOP, MEOHP, MiNP, and MEHHP.

[f] Molar sum of medium-chain phthalate metabolites: MBzP, MnBP, MEHP, MEOHP, MEHHP, MCHP, MNP.

Table 5
Association between anopenile and anoscrotal distances in male infants at birth and ln-transformed chemical metabolites in 1st trimester maternal urine, expressed as a beta-coefficient (change in mm) with 95% CI from an adjusted linear regression model.

	Anopenile distance[a]				Anoscrotal distance[b]			
Chemical	Coefficient	Lower 95% CI	Upper 95% CI	*P* value	Coefficient	Lower 95% CI	Upper 95% CI	*P* value
Total TCS	0.1638	−0.2164	0.5439	0.39	−0.1734	−0.5332	0.1864	0.34
Free TCS	0.0374	−0.3436	0.4184	0.85	0.0148	−0.3430	0.3725	0.94
Total BPA	−0.2137	−1.0887	0.6612	0.63	0.3266	−0.4320	1.0852	0.40
MEP	0.6023	−0.0355	1.2400	0.06	0.0505	−0.5185	0.6195	0.86
MnBP	**1.1689**	**0.0207**	**2.317**	**0.05**	−0.3757	−1.3718	0.6204	0.46
MBzP	0.7959	−0.2003	1.7922	0.12	−0.1318	−1.0004	0.7368	0.76
MEHHP	0.3400	−0.743	1.4231	0.53	0.6692	−0.3501	1.6886	0.20
MCPP	−0.4644	−1.3952	0.4665	0.32	−0.0568	−0.8243	0.7106	0.88
MEOHP	0.4156	−0.7412	1.5724	0.48	0.7740	−0.3331	1.8811	0.17
MEHP	0.4314	−0.6456	1.5083	0.43	0.7481	−0.2615	1.7576	0.14
Σ DEHP[c]	0.4596	−0.713	1.6322	0.44	0.8670	−0.2426	1.9766	0.12
Σ LMW[d]	**1.1048**	**0.1313**	**2.0782**	**0.03**	−0.1865	−1.0224	0.6495	0.66
Σ HMW[e]	0.2750	−0.9555	1.5054	0.66	0.7927	−0.3735	1.9589	0.18
Σ MC[f]	1.1518	−0.2092	2.5128	0.10	0.2590	−0.9471	1.4651	0.67

The numerical figures in bold are statistically significant.
[a] Phenols controlled for: specific gravity (SG), site, education, smoking status, gestational age, weight-for-length *z*-score; Phthalates controlled for: SG, site, smoking status, BMI, maternal race, gestational age, weight-for-length *z*-score.
[b] Phenols controlled for: SG, site, passive smoke, smoking status, gestational age, infant age; Phthalates controlled for: SG, site, education, smoking status, BMI, gestational age.
[c] Molar sum of DEHP metabolites: MEHP, MEOHP and MEHHP.
[d] Molar sum of low molecular weight phthalate metabolites: MMP, MEP, MnBP, MCHP, MCPP and MBzP.
[e] Molar sum of high molecular weight phthalate metabolites: MEHP, MnOP, MEOHP, MiNP, and MEHHP.
[f] Molar sum of medium-chain phthalate metabolites: MBzP, MnBP, MEHP, MEOHP, MEHHP, MCHP, MNP.

Table 6
Summary statistics for 6-month assessments.

Outcome	N	Min	Max	Mean	Standard deviation	25th percentile	50th percentile	75th percentile	Coefficient of variation[a]
2D:4D digit ratio, left hand (females)	214	0.7971	1.1167	0.9444	0.0480	0.9130	0.9429	0.9714	0.9444
2D:4D digit ratio, right hand (females)	214	0.8000	1.2400	0.9568	0.0587	0.9194	0.9524	0.9853	0.9568
2D:4D digit ratio, left hand (males)	204	0.7714	1.0962	0.9409	0.0535	0.9023	0.9412	0.9695	0.9409
2D:4D digit ratio, right hand (males)	206	0.7206	1.1379	0.9539	0.0564	0.9194	0.9583	0.9853	0.9539

[a] Coefficient of variation across repeated measures.

Table 7
Association between 2D:4D digit ratios of the left and right hands in female infants at six months of age and ln-transformed chemical metabolites in 1st trimester maternal urine, expressed as a beta-coefficient with 95% CI from an adjusted linear regression model.

	2D:4D digit ratio (left hand)[a]				2D: 4D digit ratio (right hand)[b]			
Chemical	Coefficient	Lower 95% CI	Upper 95% CI	*P* value	Coefficient	Lower 95% CI	Upper 95% CI	*P* value
Total TCS	−0.0018	−0.0055	0.0019	0.34	0.0014	−0.0029	0.0056	0.52
Free TCS	−0.0026	−0.0062	0.0011	0.16	0.0018	−0.0023	0.0058	0.39
Total BPA	−0.0023	−0.0108	0.0062	0.59	**−0.0111**	**−0.0201**	**−0.0021**	**0.02**
MEP	0.0003	−0.0055	0.0062	0.91	0.0016	−0.0049	0.0081	0.62
MnBP	0.0006	−0.0089	0.0102	0.90	**0.0122**	**0.0018**	**0.0227**	**0.02**
MBzP	−0.0025	−0.0113	0.0063	0.58	0.0030	−0.0067	0.0127	0.55
MEHHP	−0.0041	−0.0145	0.0062	0.43	−0.0024	−0.0138	0.0091	0.68
MCPP	−0.0013	−0.0080	0.0054	0.71	−0.0019	−0.0091	0.0054	0.61
MEOHP	−0.0056	−0.0168	0.0056	0.33	−0.0023	−0.0149	0.0103	0.72
MEHP	−0.0054	−0.0156	0.0048	0.30	−0.0017	−0.0129	0.0096	0.77
Σ DEHP[c]	−0.0047	−0.0159	0.0066	0.41	−0.0017	−0.0142	0.0107	0.79
Σ LMW[d]	−0.0008	−0.0095	0.0080	0.87	0.0058	−0.0039	0.0154	0.24
Σ HMW[e]	−0.0041	−0.0159	0.0077	0.49	−0.0012	−0.0140	0.0116	0.85
Σ MC[f]	−0.0016	−0.0136	0.0104	0.80	0.0116	−0.0013	0.0245	0.08

The numerical figures in bold are statistically significant.
[a] Phenols controlled for: specific gravity (SG), time of urine collection, time since last void, infant age, weight-for-length *z*-score; Phthalates controlled for: SG, time of urine collection, time since last void, infant age, weight-for-length *z*-score.
[b] Phenols controlled for: SG, BMI, infant age, maternal age, site, mother born in Canada, weight-for-length *z*-score; Phthalates controlled for: SG, site, mother born in Canada, infant age.
[c] Molar sum of DEHP metabolites: MEHP, MEOHP and MEHHP.
[d] Molar sum of low molecular weight phthalate metabolites: MMP, MEP, MnBP, MCHP, MCPP and MBzP.
[e] Molar sum of high molecular weight phthalate metabolites: MEHP, MnOP, MEOHP, MiNP, and MEHHP.
[f] Molar sum of medium-chain phthalate metabolites: MBzP, MnBP, MEHP, MEOHP, MEHHP, MCHP, MNP.

Table 8
Association between 2D:4D digit ratios of the left and right hands in male infants at six months of age and ln-transformed chemical metabolites in 1st trimester maternal urine, expressed as a beta-coefficient with 95% CI from an adjusted linear regression model.

	2D:4D digit ratio (left hand)[a]				2D: 4D digit ratio (right hand)[b]			
Chemical	Coefficient	Lower 95% CI	Upper 95% CI	*P* value	Coefficient	Lower 95% CI	Upper 95% CI	*P* value
Total TCS	0.0004	−0.0038	0.0045	0.87	−0.0011	−0.0048	0.0026	0.56
Free TCS	−0.0011	−0.0054	0.0032	0.61	−0.0022	−0.0061	0.0016	0.25
Total BPA	−0.0012	−0.0094	0.0071	0.78	0.0044	−0.0032	0.0120	0.26
MEP	−0.0036	−0.0096	0.0025	0.24	0.0037	−0.0015	0.0089	0.16
MnBP	0.0042	−0.0064	0.0149	0.43	−0.0074	−0.0177	0.0028	0.15
MBzP	−0.0012	−0.0104	0.0080	0.79	0.0007	−0.0076	0.0091	0.86
MEHHP	−0.0035	−0.0152	0.0083	0.56	−0.0063	−0.0173	0.0047	0.26
MCPP	−0.0029	−0.0110	0.0053	0.49	−0.0049	−0.0125	0.0028	0.21
MEOHP	−0.0024	−0.0150	0.0102	0.70	−0.0031	−0.0149	0.0087	0.61
MEHP	−0.0021	−0.0147	0.0105	0.74	−0.0001	−0.0119	0.0116	0.98
Σ DEHP[c]	−0.0040	−0.0167	0.0087	0.53	−0.0054	−0.0172	0.0065	0.37
Σ LMW[d]	−0.0025	−0.0106	0.0055	0.54	0.0043	−0.0026	0.0112	0.22
Σ HMW[e]	−0.0060	−0.0200	0.0081	0.40	−0.0073	−0.0204	0.0058	0.27
Σ MC[f]	0.0002	−0.0123	0.0128	0.97	−0.0086	−0.0204	0.0032	0.15

[a] Phenols controlled for: specific gravity (SG), BMI, infant age, weight-for-length *z*-score; Phthalates controlled for: SG, BMI, infant age, weight-for-length *z*-score.
[b] Phenols controlled for: SG, smoking status, season of urine collection, infant age; Phthalates controlled for: SG, smoking status, season of urine collection, infant age.
[c] Molar sum of DEHP metabolites: MEHP, MEOHP and MEHHP.
[d] Molar sum of low molecular weight phthalate metabolites: MMP, MEP, MnBP, MCHP, MCPP and MBzP.
[e] Molar sum of high molecular weight phthalate metabolites: MEHP, MnOP, MEOHP, MiNP, and MEHHP.
[f] Molar sum of medium-chain phthalate metabolites: MBzP, MnBP, MEHP, MEOHP, MEHHP, MCHP, MNP.

digit ratios is known and prenatal BPA exposure at levels of possible human exposure (i.e., 5 μg/kg/day), has been shown to significantly feminize 2D:4D digit ratios in male rats, primarily in the right paw (Auger et al. 2013). It should be noted that while the mean 2D:4D digit ratios in the males were lower than in females in our analysis, the differences were slight and not significantly smaller, similar to those reported in another study where sexual dimorphism at birth was only significant for the left hand (Ventura et al. 2013). These authors suggested that postnatal androgen exposure may be more important in accentuating the sex differences and stabilizing 2D:4D ratios (Ventura et al. 2013). Our previous analysis of 2D:4D ratios in MIREC mothers and young children (Vélez et al. 2016, 2017) suggests that this metric may not be useful as an indicator of prenatal androgen or anti-androgen exposure in humans. However, limitations of this analysis include: potential inaccuracy of the 2D:4D measurements; no consideration of potential post-natal factors such as infant feeding (i.e., breast, cow or soy milk) and hormone fluctuations in infant boys between birth and 6 months of age; and limited generalizability of results to populations with lower education and income and from diverse racial and ethnic groups.

The strengths of our study include the collection of urine and detailed potential confounder data within the critical genital reproductive programming window of the 1st trimester, as well as measurement of infant outcomes by trained staff. The question arises however, about how well a single urinary measure of chemicals with short elimination half-lives represents exposure during the window of interest. Intraclass correlation coefficients for these chemicals in urine of pregnant women range from low for many phthalates and BPA to moderately high for triclosan (Fisher et al. 2015; Braun et al. 2012; Adibi et al. 2008; Weiss et al. 2015; Bertelsen et al. 2014; Philippat et al. 2013; Meeker et al. 2013). However, the sensitivity to correctly classify a participant into a 'high' exposure category using a randomly chosen sample in studies of pregnant women has tended to be good for BPA (0.65–0.70) (Fisher et al. 2015; Braun et al. 2012), MnBP (0.62–0.73) (Fisher et al. 2015; Braun et al. 2012; Adibi et al., 2008), MBzP (0.62–0.74) (Fisher et al. 2015; Braun et al. 2012; Adibi et al. 2008), MEP (0.62–0.81) (Fisher et al. 2015; Braun et al. 2012; Adibi et al. 2008), MCPP (0.71–0.74) (Fisher et al. 2015; Adibi et al. 2008), the DEHP metabolites (0.60–0.75) (Fisher et al. 2015; Adibi et al. 2008) and TCS (0.83) (Weiss et al. 2015). However, the potential for misclassification of exposure remains a concern. In addition, we did not exclude women with adrenal insufficiency from the study; however, as this disease is very rare (Anand and Beuschlein 2018), it is not expected to have affected our results.

In the present study, we investigated the association between individual target chemicals of concern and endocrine sensitive outcomes. While we acknowledge that the chemicals studied may act together in complex interactions that cannot be ignored, the effects of chemical mixtures remain poorly understood. Regardless, we note that the anti-androgenic effects of phthalates are well-documented in animal studies of males (Kay et al. 2014) and linked with decreased circulating testosterone concentrations in boys and men (Jurewicz et al. 2013; Mieritz et al. 2012; Meeker and Ferguson 2014; Mendiola et al. 2012), effects that are relevant to the central thesis of our study. However, it is possible that the phthalate levels in MIREC were so low as to not affect circulating testosterone.

5. Conclusions

In this prospective cohort study and in contrast to similar American studies, we found no significant association between maternal phthalate exposure and reduced AGD in male infants; however, we did find significant positive associations with maternal urinary MnBP and the molar sum of LMW phthalate metabolites. In female infants, significant associations between phthalates and anoclitoris distance were observed: negative for MBzP and positive for MEP. Furthermore, no significant associations were observed between prenatal BPA or TCS exposure and AGD in male or female infants. While some significant associations were observed between prenatal exposure to MnBP or total BPA and 2D:4D digit ratios in girls, there was no consistency between

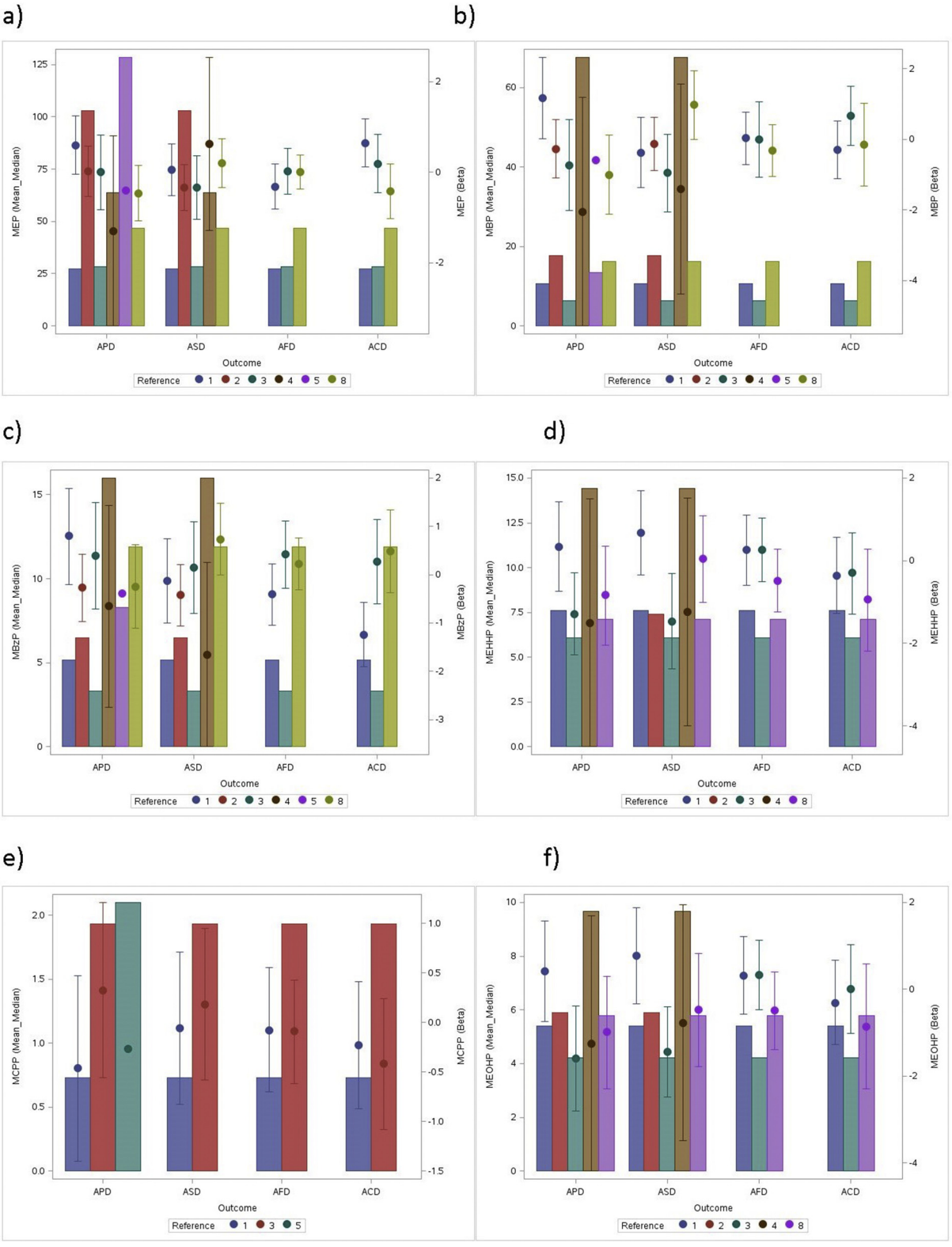

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Fig. 1. The following plots compare the results of several reference AGD studies in male and female infants. The Bar-Dot Plots display the geometric mean or median concentrations (mg/ml) reported by the study as a bar plot (Left Y Axis) and the dot plots show the beta coefficients with the 95% confidence intervals (Right Y Axis). a) mono-ethyl phthalate (MEP); b) mono-*n*-butyl phthalate (MBP); c) mono-benzyl phthalate (MBzP); d) mono-(2-ethyl-5-hydroxy-hexyl) phthalate (MEHHP); e) mono-(3-carboxypropyl) phthalate (MCPP); f) mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP); g) mono-(2-ethylhexyl) phthalate (MEHP); h) sum of di-2-ethylhexyl phthalate (DEHP) metabolites; i) triclosan (TCS); j) bisphenol A (BPA).
Reference 1: MIREC Canada
Reference 2: Jensen et al. 2016, Denmark
Reference 3: Barrett et al. 2016, USA
Reference 4: Bornehag et al. 2015, Sweden
Reference 5: Swan et al. 2005, USA
Reference 6: Barrett et al. 2017, USA
Reference 7: Lassen et al. 2016, Denmark
Reference 8: Wenzel et al. 2018, USA.

the left and right hand.

A major limitation of this work is that the exposure is based on a single urinary measure, albeit during the 1st trimester. Furthermore, our results may not be generalizable to populations with a higher preponderance of non-Caucasians and of lower socio-economic status.

The inconsistencies in results among studies of phthalates and AGD suggest that this issue is yet to be resolved. Our results for 2D:4D should be interpreted with caution as preliminary rather than confirmatory due to the novelty of this measure for identifying potential androgenic or anti-androgenic exposures in utero.

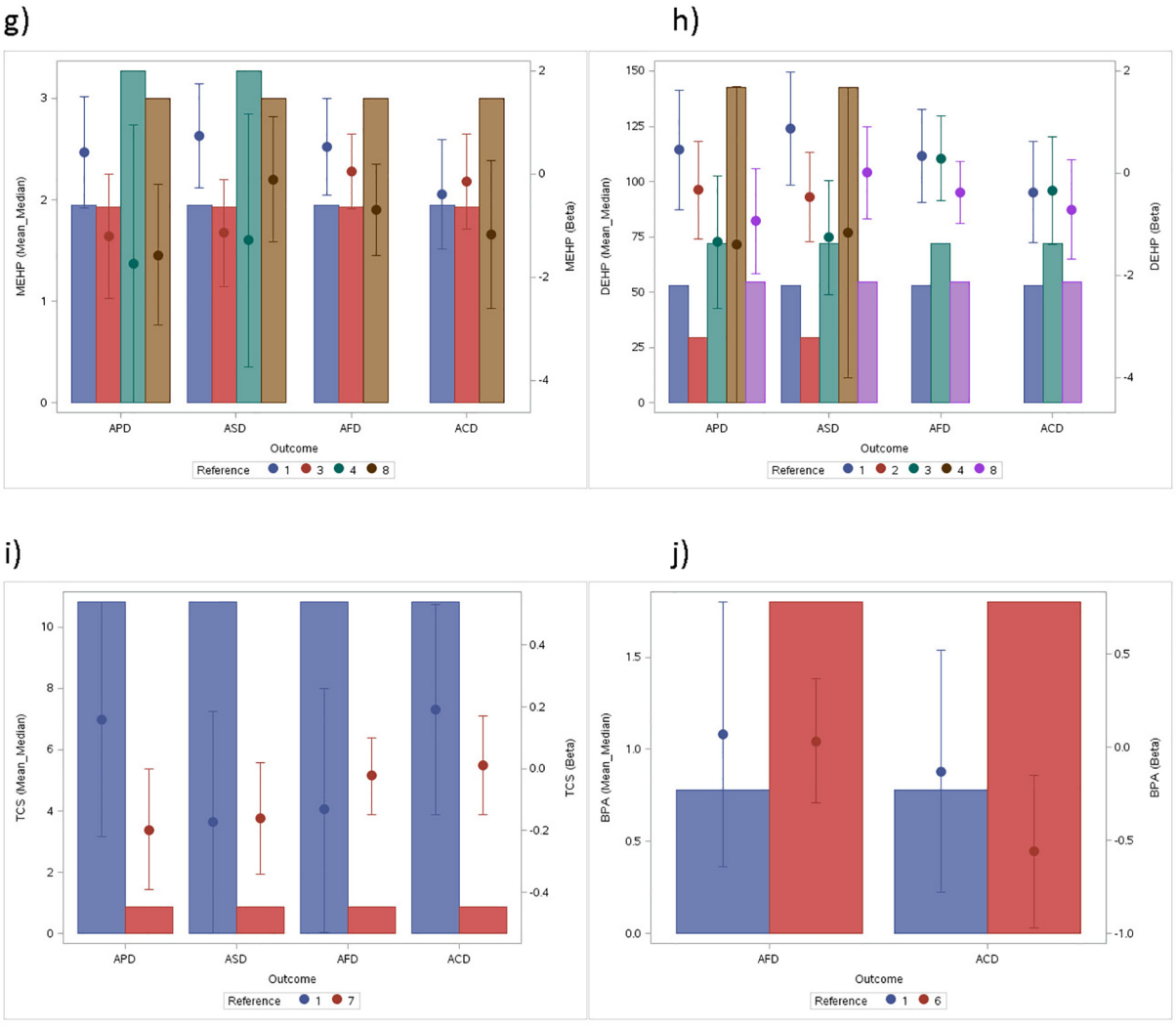

Fig. 1. (*continued*)

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Competing financial interests

All authors declare they have no potential conflicts of interest including any relevant financial interests, activities, relationships or affiliations.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.08.034.

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