Sex-Specific Associations between Prenatal Exposure to Di(2-ethylhexyl) Phthalate, Epigenetic Age Acceleration, and Susceptibility to Early Childhood Upper Respiratory Infections

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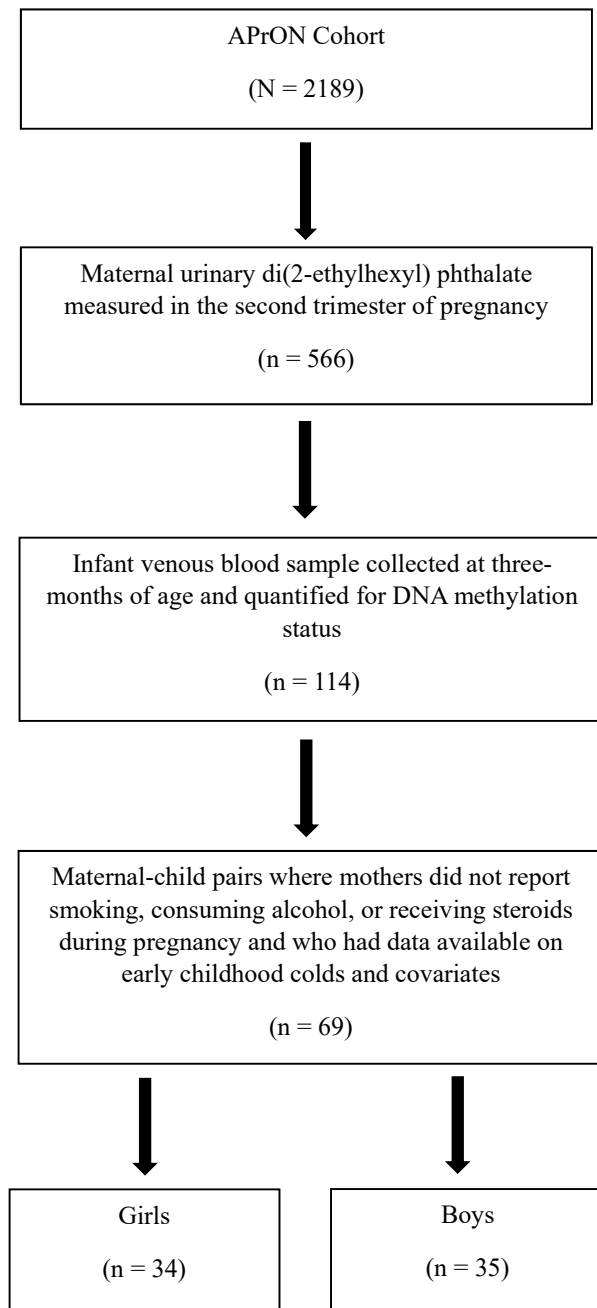[15] University of Calgary, Calgary, AB T2N 1N4, Canada
[16] University of Alberta, Edmonton, AB T6G 2R3, Canada
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[†] Membership of the Group is provided in the Acknowledgments.

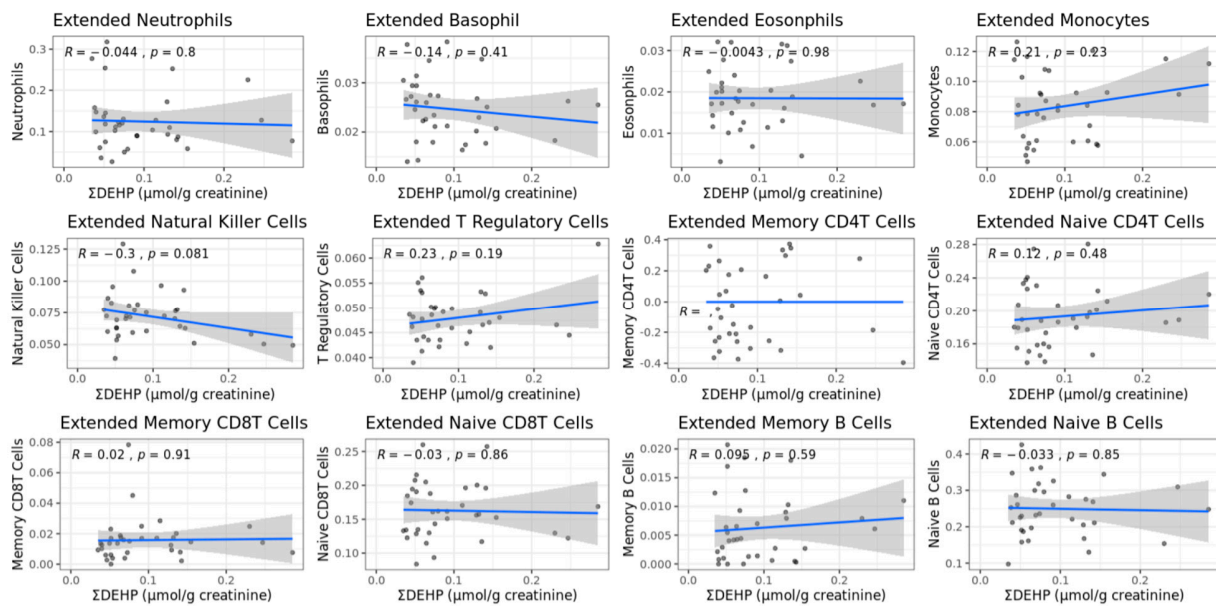**Figure S1.** Flowchart of participants included in the present study.

Figure S2 Estimated cell type proportions in three-month-old infant venous blood were not associated with prenatal DEHP exposure across the entire cohort (n=69). Each graph represents the association between each of the 12 estimated cell type proportions (on the y-axis) and DEHP exposure (on the x-axis). On each graph is the Pearson's correlation (*R*) and *p*-value of the correlation between estimated cell type proportion and DEHP exposure.
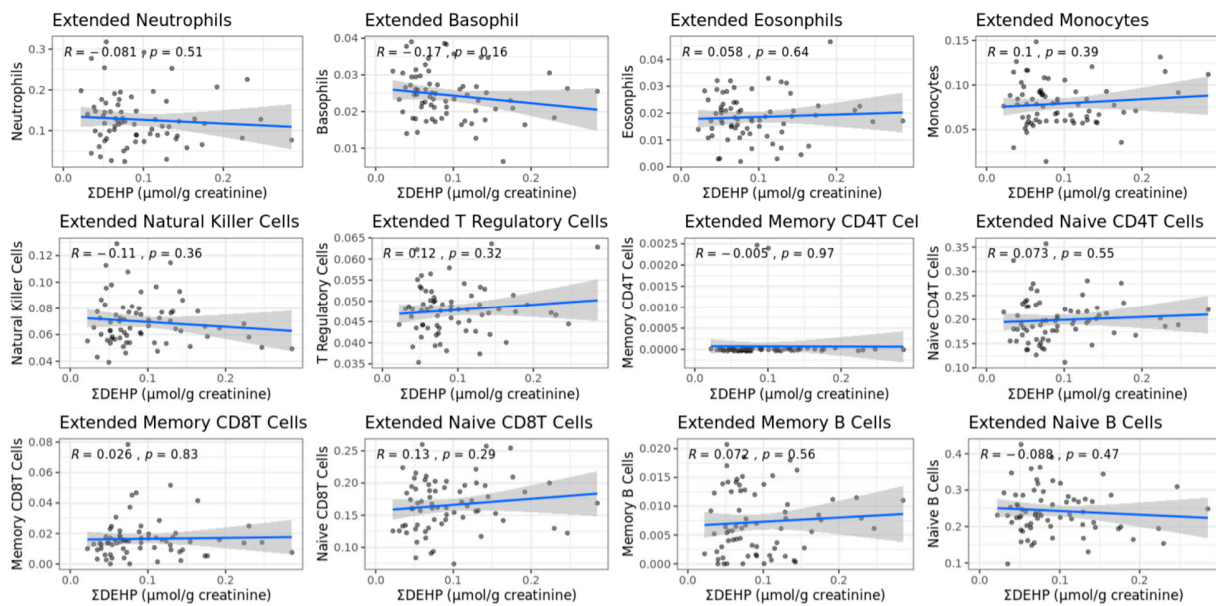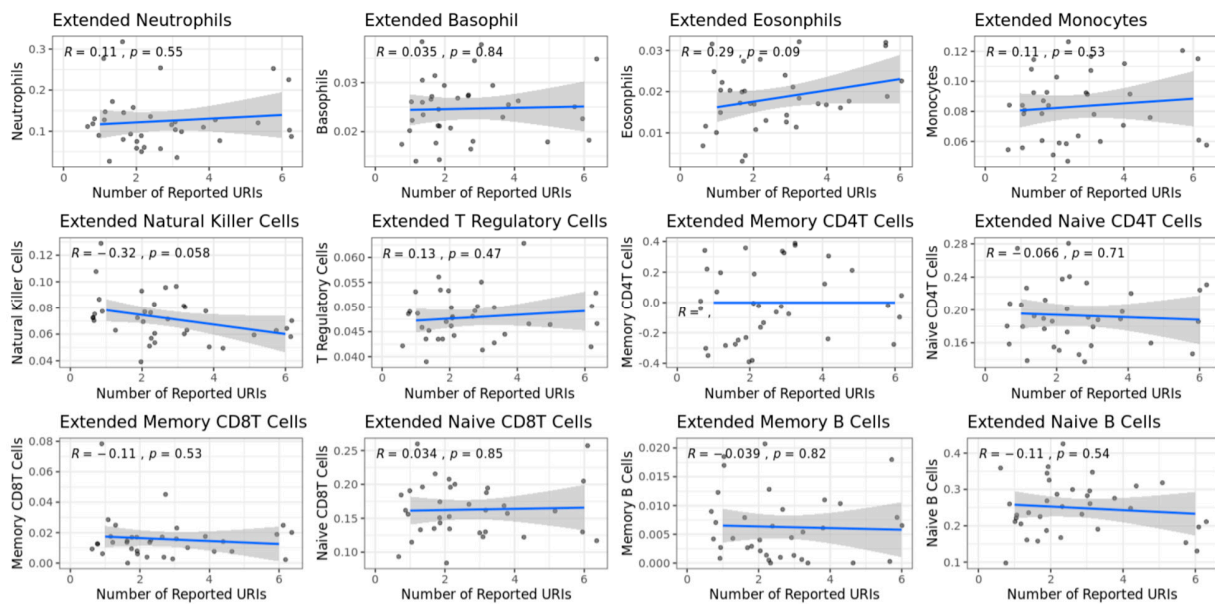
Figure S3 Estimated cell type proportions in three-month-old infant venous blood were not associated with childhood upper respiratory infections (URIs) across the entire cohort (n=69). Each graph represents the association between each of the 12 estimated cell type proportions (on the y-axis) and childhood URIs (on the x-axis). On each graph is the Pearson's correlation (R) and p-value of the correlation between estimated cell type proportion and childhood URIs.
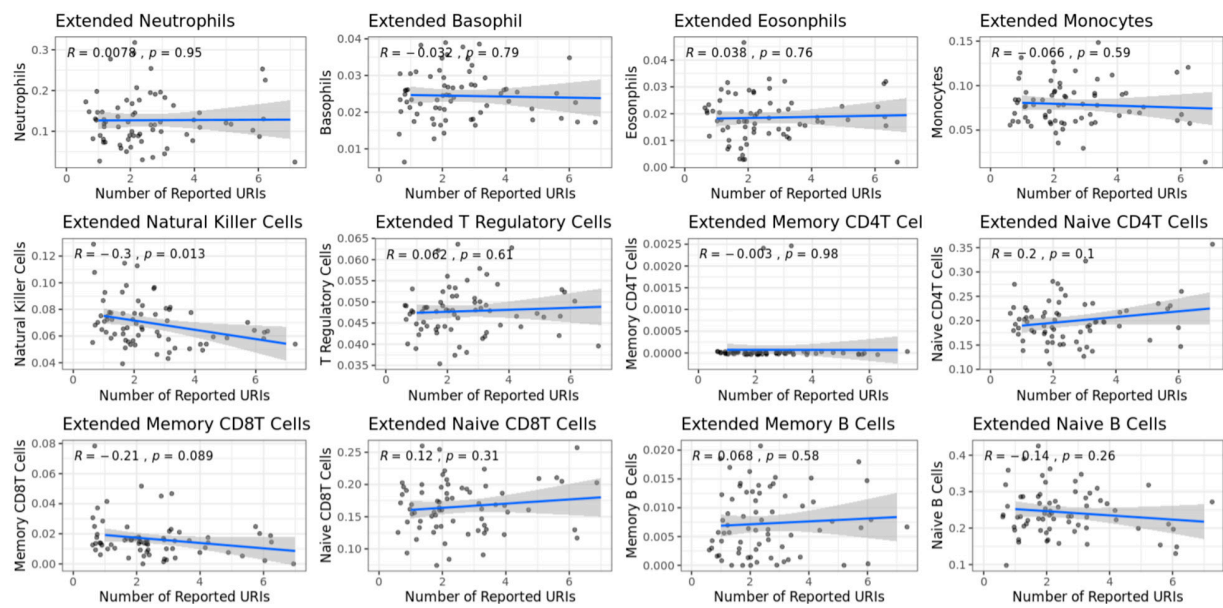
Figure S4 Estimated cell type proportions in three-month-old infant girls' venous blood were not associated with prenatal DEHP exposure (n=34). Each graph represents the association between each of the 12 estimated cell type proportions (on the y-axis) and DEHP exposure (on the x-axis). On each graph is the Pearson's correlation (*R*) and *p*-value of the correlation between estimated cell type proportion and DEHP exposure.

Figure S5 Estimated cell type proportions in three-month-old infant girls' venous blood were not associated with childhood upper respiratory infections (URIs) (n=34). Each graph represents the association between each of the 12 estimated cell type proportions (on the y-axis) and childhood URIs (on the x-axis). On each graph is the Pearson's correlation (*R*) and *p*-value of the correlation between estimated cell type proportion and childhood URIs.
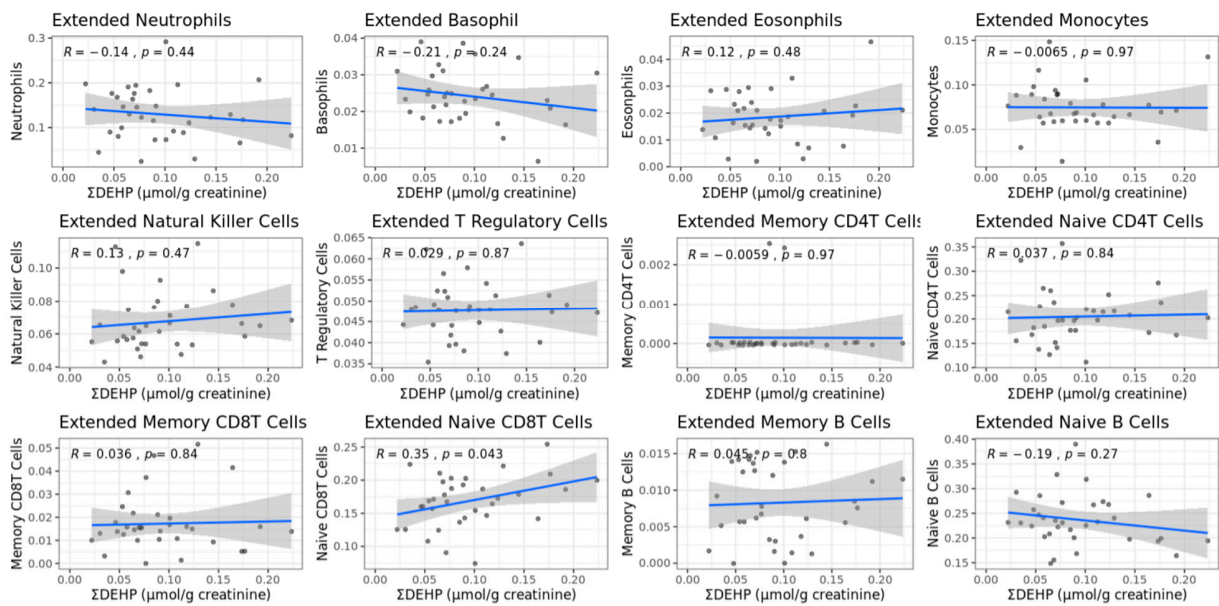
Figure S6 Estimated cell type proportions in three-month-old infant boys' venous blood were not associated with prenatal DEHP exposure (n=35). Each graph represents the association between each of the 12 estimated cell type proportions (on the y-axis) and DEHP exposure (on the x-axis). On each graph is the Pearson's correlation (*R*) and *p*-value of the correlation between estimated cell type proportion and DEHP exposure.

Figure S7 Estimated cell type proportions in three-month-old infant boys' venous blood were not associated with childhood upper respiratory infections (URIs) (n=35). Each graph represents the association between each of the 12 estimated cell type proportions (on the y-axis) and childhood URIs (on the x-axis). On each graph is the Pearson's correlation (R) and p-value of the correlation between estimated cell type proportion and childhood URIs.
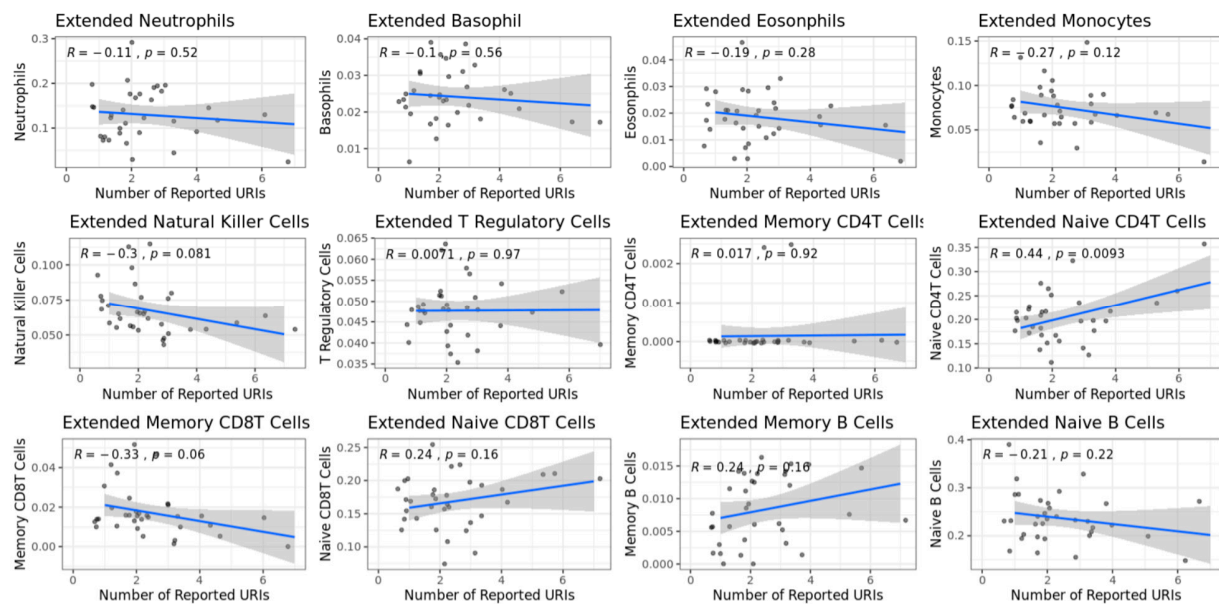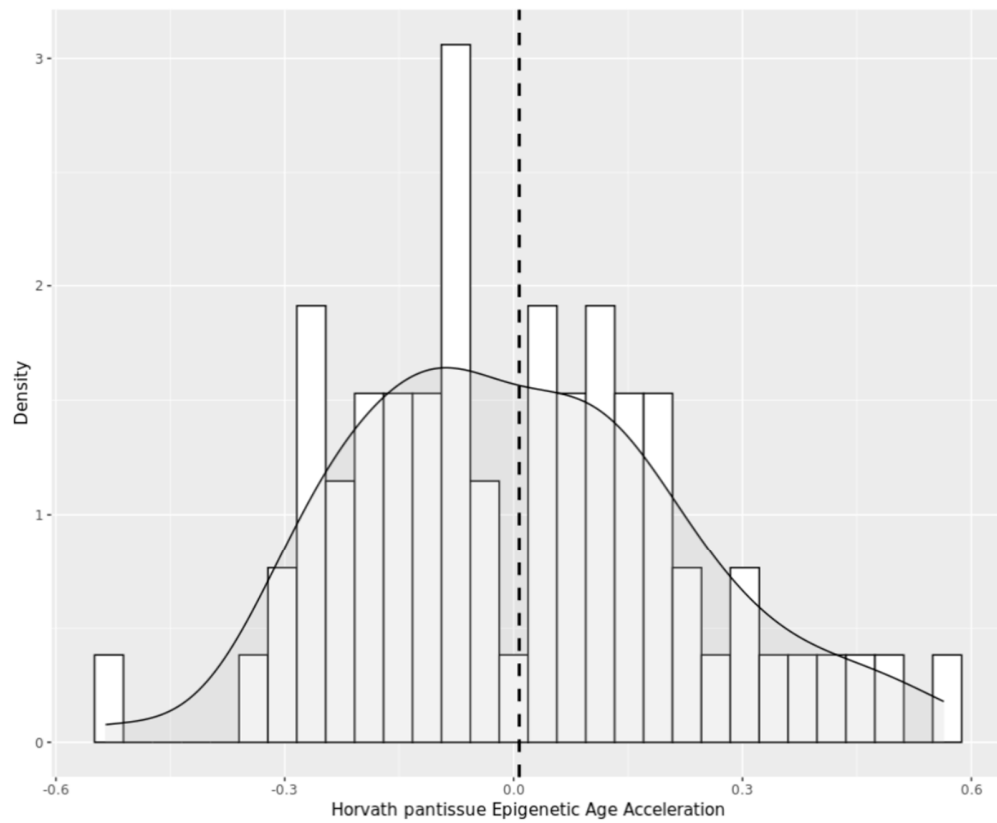
Figure S8. Histogram with kernel density plot of epigenetic age acceleration (EAA) values estimated from the Horvath pan-tissue clock in these samples. The dashed line indicates the mean, which is centered at approximately zero, and the data are approximately normally distributed.

Figure S9. Estimated epigenetic age acceleration (EAA) and epigenetic age difference from the Horvath pan-tissue clock were the same in this sample due to the narrow age range during blood sample collection. The Horvath pan-tissue EAA was derived from residuals of a regression of chronological age on predicted biological age is on the x-axis. While Horvath pan-tissue epigenetic age difference was derived from the difference of chronological age minus predicted biological age. As the correlation is so high, these measures of EAA are synonymous in this sample.
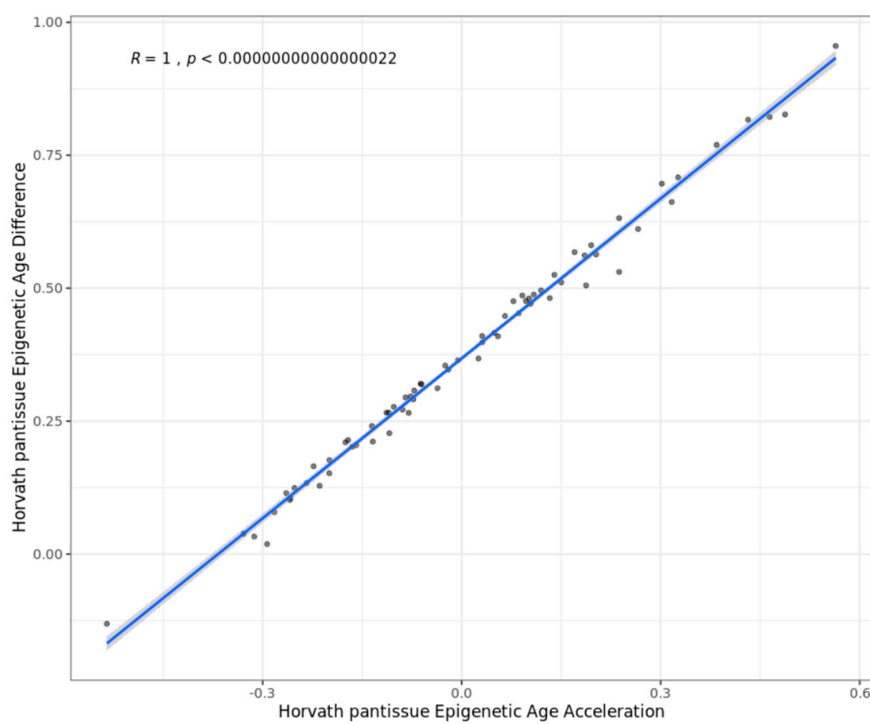
Table S1. Maternal and child characteristics for the sub-sample stratified by child sex (n = 34 girls).

	n (%)	Mean (SD)
Maternal Characteristics		
Age (years)	-	30.42 (4.01)
White	31 (91.18%)	-
Married/Cohabiting	34 (100.00%)	-
Household Income > $CAD 70k[1]	27 (79.41%)	-
Child Characteristics		
Birthweight (g)	-	3356.29 (389.66)
Gestational age at birth (weeks)	-	39.52 (1.35)
Age at blood draw (weeks)	-	12.52 (1.02)
Number of colds	-	2.42 (1.44)

[1] CAD (Canadian dollars).

Table S2. Maternal and child characteristics for the sub-sample stratified by child sex (n = 35 boys).

	n (%)	Mean (SD)
Maternal Characteristics		
Age (years)	-	32.03 (3.81)
White	33 (94.29)	-
Married/Cohabiting	35 (100.00%)	-
Household Income > $CAD 70k[1]	29 (82.86)	-
Child Characteristics		
Birthweight (g)	-	3599.03 (578.47)
Gestational age at birth (weeks)	-	39.86 (1.47)
Age at blood draw (weeks)	-	12.52 (0.92)
Number of colds	-	2.66 (1.59)

[1] CAD (Canadian dollars).

Table S3. Creatinine-adjusted prenatal phthalate metabolite concentrations (µg/g creatinine) and DEHP (µmol/g creatinine) in maternal second trimester urine for the sex-stratified groups.

Metabolite	% > LOD	Minimum	Maximum	GM	25th Percentile	50th Percentile	75th Percentile
Girls (n = 34)							
MEHP [1]	100%	0.712	13.1	3.34	1.93	3.78	6.27
MEHHP [1]	100%	2.32	33.5	11.0	8.17	10.9	15.0
MEOHP [1]	100%	2.59	22.3	9.27	6.72	9.26	12.8
MECPP [1]	100%	6.41	41.5	16.7	12.0	16.5	18.7
DEHP [2]	-	0.0222	0.224	0.0824	0.0586	0.0810	0.119
Boys (n = 35)							
MEHP [1]	100%	1.77	31.0	3.72	2.31	3.03	4.48
MEHHP [1]	100%	3.79	30.7	10.3	6.60	9.42	16.6
MEOHP [1]	100%	4.08	25.7	8.99	5.65	8.28	13.7
MECPP [1]	100%	7.45	62.4	16.6	11.9	15.3	23.4
DEHP [2]	-	0.0350	0.285	0.0805	0.0516	0.0728	0.130

[1] LOD = 0.10 µg/L. [2] molar sum of individual DEHP metabolites.

Note. LOD, limit of detection; GM, geometric mean; MEHP, mono(2-ethyl-hexyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxy-hexyl) phthalate; MEOHP, mono(2-ethyl-5-oxyohexyl) phthalate; MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; ΣDEHPs, sum of low di(2-ethylhexyl) phthalate.

Table S4. Sensitivity analysis adjusting for prenatal exposure to bisphenol A (BPA).

	B (95% CI)
Overall (n = 69)	
Total Effect (path c)	6.20[†] (1.01, 11.39)
Path a	-0.23 (-1.23, 0.77)
Path b	3.08[†] (1.80, 4.36)
Direct effect (path c′)	7.09[†] (1.86, 12.31)
Indirect effect (path ab)	-1.40 (-5.00, 1.61)
Girls (n = 34)	
Total Effect (path c)	-5.79 (-14.56, 2.99)
Path a	-2.32[†] (-3.54, -1.10)
Path b	1.45 (-1.45, 4.35)
Direct effect (path c′)	-4.03 (-16.09, 8.03)
Indirect effect (path ab)	-3.32 (-8.82, 2.28)
Boys (n = 35)	
Total Effect (path c)	9.01[†] (1.64, 16.39)
Path a	0.90 (-0.56, 2.35)
Path b	3.66[†] (1.74, 5.78)
Direct effect (path c′)	7.13 (-0.23, 14.49)
Indirect effect (path ab)	3.36 (-0.63, 8.21)

[†] $p < 0.05$.