

Supporting Information

Longitudinal associations between prenatal exposure to phthalates and steroid hormones in maternal hair samples from the SEPAGES cohort

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Table S1. List of phthalate biomarkers assessed in the SEPAGES cohort.

Parent compounds	Biomarkers
Phthalates	Metabolites measured
Diethyl phthalate (DEP)	Mono-ethyl phthalate (MEP)
Di-isobutyl phthalate (DiBP)	Mono-isobutyl phthalate (MiBP)
Dibutyl phthalate (DBP)	Mono-n-butyl phthalate (MnBP)
Butyl-benzyl phthalate (BBzP)	Mono-benzyl phthalate (MBzP)
Di(2-propylheptyl) phthalate (DPHP)	6-hydroxy-mono-propyl-heptyl phthalate (OH-MPHP)
	Mono(2-ethylhexyl) phthalate (MEHP)
Di(2-ethylhexyl) phthalate (DEHP)	Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)
	Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)
	Mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)
	Mono-hydroxy-isononyl phthalate (OH-MiNP)
Di-isononyl phthalate (DiNP)	Mono-oxo-isononyl phthalate (oxo-MiNP)
	Mono-carboxy-isononyl phthalate (cx-MiNP)
Non-phthalate plasticizers	
Di(isononyl)cyclohexane-1,2-dicarboxylate (DiNCH)	mono-hydroxy isononyl cyclohexane-1,2-dicarboxylate (OH-MiNCH)
	mono-oxo isononyl cyclohexane-1,2-dicarboxylate (oxo-MiNCH)

Table S2. Phthalates and DINCH metabolite standardized concentrations ($\mu\text{g/L}$ or $\mu\text{mol/L}$ for molar sums) and specific gravity per period.

			Second trimester – T2 (N mothers = 382)					Third trimester – T3 (N mothers = 378)				
	LOD ^a	LOQ ^a	%> LOD	%> LOQ	Percentiles			%> LOD	%> LOQ	Percentiles		
					25	50	75			25	50	75
Specific gravity	-	-	-	-	1.015	1.018	1.021	-	-	1.014	1.017	1.020
MEP	0.20	0.50	100	100	11.8	24.1	45.3	100	100	10.6	20.0	41.8
MnBP	0.20	0.50	100	100	8.2	10.8	15.7	100	100	7.87	11.3	16.3
MiBP	0.20	0.50	100	100	11.1	15.4	24.1	100	100	9.59	14.9	23.1
MBzP	0.07	0.20	100	100	3.07	4.46	6.82	100	100	2.70	4.23	6.97
OH-MPHP	0.07	0.20	100	98	0.71	0.85	1.19	100	98	0.64	0.82	1.07
Σ DEHP	/	/	/	/	0.081	0.110	0.156	/	/	0.079	0.110	0.155
MEHP	0.20	0.50	100	99	1.59	2.38	3.78	100	95	1.22	1.96	3.04
MEHHP	0.20	0.50	100	100	5.07	6.96	10.7	100	100	4.78	7.19	10.4
MEOHP	0.20	0.50	100	100	3.51	4.94	7.87	100	100	3.54	5.27	7.94
MECPP	0.70	2.00	100	99	7.62	9.98	13.9	100	100	7.50	10.2	13.9
MMCHP	0.70	2.00	99	99	5.70	7.55	10.4	99	99	5.70	7.61	10.7
Σ DiNP	/	/	/	/	0.027	0.039	0.078	/	/	0.025	0.037	0.065
OH-MiNP	0.10	0.25	100	100	2.71	4.88	11.4	100	100	2.72	4.52	9.59
oxo-MiNP	0.10	0.25	100	99	1.41	2.17	3.90	100	100	1.37	1.99	3.28
cx-MiNP	0.40	1.00	100	100	3.57	4.68	7.19	100	100	3.30	4.45	6.11
Σ DINCH	/	/	/	/	0.007	0.010	0.019	/	/	0.006	0.010	0.017
OH-MiNCH	0.07	0.20	100	100	1.17	1.73	3.26	100	99	1.01	1.58	2.58
oxo-MiNCH	0.07	0.20	99	99	1.01	1.49	2.55	99	99	0.93	1.51	2.62

Abbreviations: (/): not measured; LOD (limit of detection); LOQ (limit of quantification); MnBP (Mono-n-butyl phthalate); MiBP (Mono-iso-butyl phthalate); MBzP (Mono-benzyl phthalate); MEP (Mono-ethyl phthalate); OH-MPHP (Mono-6-hydroxy-propylheptyl phthalate); DEHP (Di (2-ethylhexyl) phthalate); DiNP (Di-isononyl phthalate); DINCH (1,2-Cyclohexane dicarboxylic acid diisononyl ester).

Note: Median molar sum of Σ DEHP concentrations, expressed as ng/mL, were 43.0 at both T2 and T3. Median molar sum of Σ DiNP concentrations were 16.3 ng/mL (T2) and 15.5 ng/mL (T3). Median molar sum of Σ DINCH concentrations were 4.25 ng/mL at both T2 and T3.

Table S3. Adjusted associations between maternal prenatal urinary phthalate metabolite concentrations and hair hormones at delivery.

Biomarker	Cortisol		Cortisone		Dehydrocorticosterone		Testosterone		Progesterone		CC Ratio		TC Ratio	
	PC (95% CI)	p	PC (95% CI)	p	PC (95% CI)	p	PC (95% CI)	p	PC (95% CI)	p	PC (95% CI)	p	PC (95% CI)	p
Maternal second trimester (T2) – N = 382														
MEP	-3.49 (-10.86, 4.50)	0.38	1.57 (-5.78, 9.49)	0.68	2.81 (-8.39, 15.39)	0.64	1.87 (-8.25, 13.10)	0.73	5.70 (-3.70, 16.01)	0.24	-4.98 (-9.05, -0.72)	0.02	5.57 (-5.60, 18.07)	0.34
MnBP	-0.88 (-13.52, 13.61)	0.90	-3.76 (-15.39, 9.46)	0.56	-0.03 (-17.97, 21.84)	1.00	-9.01 (-23.92, 8.82)	0.30	-0.30 (-15.02, 16.97)	0.97	3.00 (-4.50, 11.09)	0.44	-8.21 (-24.23, 11.19)	0.38
MiBP	5.65 (-7.21, 20.29)	0.41	0.97 (-10.68, 14.15)	0.88	4.73 (-13.25, 26.44)	0.63	-4.25 (-19.28, 13.58)	0.62	10.83 (-4.79, 29.00)	0.18	4.63 (-2.63, 12.43)	0.22	-9.45 (-24.57, 8.69)	0.29
MBzP	6.05 (-4.75, 18.07)	0.28	5.63 (-4.55, 16.90)	0.29	16.10 (-0.60, 35.59)	0.06	8.69 (-5.61, 25.17)	0.25	9.40 (-3.52, 24.04)	0.16	0.40 (-5.42, 6.57)	0.90	2.42 (-11.97, 19.16)	0.76
ohMPPH	-3.21 (-15.95, 11.48)	0.65	-1.38 (-13.70, 12.69)	0.84	-0.66 (-19.07, 21.92)	0.95	-7.31 (-23.00, 11.59)	0.42	16.06 (-1.57, 36.86)	0.08	-1.85 (-9.24, 6.15)	0.64	-4.17 (-21.45, 16.92)	0.67
ΣDEHP	-4.16 (-16.14, 9.52)	0.53	-3.69 (-15.09, 9.25)	0.56	-3.34 (-20.36, 17.32)	0.73	0.48 (-15.69, 19.76)	0.96	6.43 (-8.97, 24.45)	0.43	-0.50 (-7.60, 7.16)	0.90	4.82 (-13.14, 26.49)	0.62
ΣDiNP	2.77 (-6.26, 12.67)	0.56	2.81 (-5.74, 12.15)	0.53	-5.65 (-17.43, 7.81)	0.39	-11.61 (-21.63, -0.31)	0.04	-2.54 (-12.50, 8.55)	0.64	-0.04 (-5.02, 5.19)	0.99	-13.96 (-24.35, -2.15)	0.02
ΣDINCH	-2.82 (-10.50, 5.52)	0.49	-0.75 (-8.18, 7.28)	0.85	-4.17 (-14.95, 7.98)	0.48	-4.40 (-14.20, 6.52)	0.41	-4.57 (-13.34, 5.08)	0.34	-2.08 (-6.45, 2.49)	0.36	-1.67 (-12.43, 10.42)	0.78
Maternal third trimester (T3) – N = 378														
MEP	-0.24 (-7.54, 7.64)	0.95	4.11 (-3.07, 11.83)	0.27	4.96 (-6.02, 17.22)	0.39	5.15 (-4.76, 16.11)	0.32	5.05 (-3.89, 14.83)	0.28	-4.18 (-8.12, -0.07)	0.05	5.40 (-5.22, 17.22)	0.33
MnBP	6.56 (-6.05, 20.85)	0.32	4.43 (-7.25, 17.58)	0.47	3.13 (-14.15, 23.88)	0.74	8.21 (-8.18, 27.53)	0.35	5.14 (-9.30, 21.88)	0.50	2.04 (-4.86, 9.43)	0.57	1.55 (-14.87, 21.14)	0.86
MiBP	9.61 (-2.30, 22.97)	0.12	9.06 (-2.14, 21.53)	0.12	11.90 (-5.35, 32.31)	0.19	11.99 (-3.62, 30.13)	0.14	12.69 (-1.51, 28.94)	0.08	0.51 (-5.73, 7.16)	0.88	2.17 (-13.06, 20.08)	0.79
MBzP	13.25 (2.65, 24.94)	0.01	10.00 (0.26, 20.70)	0.04	17.33 (1.67, 35.39)	0.03	16.19 (2.20, 32.11)	0.02	10.50 (-1.57, 24.06)	0.09	2.95 (-2.55, 8.76)	0.30	2.60 (-10.68, 17.86)	0.72
ohMPPH	2.02 (-10.83, 16.72)	0.77	3.45 (-8.86, 17.43)	0.60	0.62 (-17.28, 22.39)	0.95	-5.10 (-20.38, 13.11)	0.56	6.22 (-9.27, 24.37)	0.45	-1.39 (-8.49, 6.26)	0.71	-6.98 (-22.94, 12.28)	0.45
ΣDEHP	0.92 (-12.75, 16.72)	0.90	3.83 (-9.45, 19.07)	0.59	0.12 (-18.98, 23.72)	0.99	11.65 (-7.62, 34.95)	0.25	13.42 (-4.32, 34.45)	0.15	-2.81 (-10.35, 5.36)	0.49	10.64 (-9.72, 35.59)	0.33
ΣDiNP	-3.70 (-13.25, 6.90)	0.48	-1.32 (-10.56, 8.88)	0.79	-9.87 (-22.55, 4.89)	0.18	5.31 (-8.10, 20.68)	0.46	0.38 (-11.19, 13.46)	0.95	-2.41 (-7.91, 3.41)	0.41	9.36 (-5.49, 26.54)	0.23
ΣDINCH	-2.18 (-10.33, 6.72)	0.62	1.09 (-6.87, 9.72)	0.80	-10.71 (-21.29, 1.30)	0.08	-1.53 (-12.11, 10.32)	0.79	-1.29 (-10.87, 9.32)	0.80	-3.23 (-7.79, 1.55)	0.18	0.66 (-10.89, 13.71)	0.92

CC (Cortisol to Cortisone ratio); TC (Testosterone to Cortisol ratio). Significant (p-value <0.05) and borderline (p-value <0.10) results highlighted in bold. Percent change (PC), 95% Confidence Intervals (95% CI) and p-values (p). PC and 95% CIs represent the percent change in hormone levels for each doubling in exposure biomarkers. Models adjusted for: maternal age at conception (continuous), pre-pregnancy body mass index (BMI, normal weight vs. overweight/obese), education level (below vs. Master's degree or above), active smoking during pregnancy (smoker vs. non-smoker), passive tobacco exposure (exposed vs. non-exposed), parity (nulliparous vs. uniparous/multiparous), maternal anxiety/depression score at third trimester (continuous), infant sex and season of hair collection (winter vs. remaining seasons), hair analysis batch (dichotomous) and time elapsed (continuous) between hair sampling and hormone measurement.

Table S4. Sex-specific adjusted associations between maternal prenatal urinary phthalate metabolite concentrations and hair hormones at delivery (only associations with a p-value for exposure-sex interaction <0.10 are displayed).

Biomarker	Cortisol			Cortisone			Dehydrocorticosterone			Testosterone			Progesterone			CC Ratio			TC Ratio		
	PC (95% CI)	p	p-int	PC (95% CI)	p	p-int	PC (95% CI)	p	p-int	PC (95% CI)	p	p-int	PC (95% CI)	p	p-int	PC (95% CI)	p	p-int	PC (95% CI)	p	p-int
Maternal second trimester (T2) – N = 382																					
MEP	-	-	0.86	-	-	0.53	-	-	0.68	-	-	0.11	-	-	0.85	-	-	0.44	-	-	0.17
MnBP	-	-	0.05	-	-	0.08	-	-	0.00	-	-	0.64	-	-	0.39	-	-	0.55	-	-	0.33
MnBP Boys	14.9 (-7.30, 41.4)	0.21	-	8.67 (-9.88, 32.0)	0.18	-	40.4 (3.53, 91.9)	0.03	-	-	-	-	-	-	-	-	-	-	-	-	-
MnBP Girls	-9.88 (-24.7, 7.92)	0.23	-	-11.7 (-26.3, 6.44)	0.38	-	-22.6 (-40.5, 0.00)	0.05	-	-	-	-	-	-	-	-	-	-	-	-	-
MiBP	-	-	0.61	-	-	0.60	-	-	0.27	-	-	0.74	-	-	0.09	-	-	0.98	-	-	0.97
MiBP Boys	-	-	-	-	-	-	-	-	-	-	-	-	24.0 (-1.38, 54.8)	0.07	-	-	-	-	-	-	-
MiBP Girls	-	-	-	-	-	-	-	-	-	-	-	-	-0.05 (-18.5, 22.6)	0.99	-	-	-	-	-	-	-
MBzP	-	-	0.39	-	-	0.53	-	-	0.38	-	-	0.53	-	-	0.97	-	-	0.64	-	-	0.23
ohMPHP	-	-	0.72	-	-	0.92	-	-	0.72	-	-	0.67	-	-	0.28	-	-	0.63	-	-	0.52
ΣDEHP	-	-	0.91	-	-	0.89	-	-	0.57	-	-	0.62	-	-	0.66	-	-	0.97	-	-	0.71
ΣDiNP	-	-	0.56	-	-	0.87	-	-	0.90	-	-	0.09	-	-	0.42	-	-	0.19	-	-	0.25
ΣDiNP Boys	-	-	-	-	-	-	-	-	-	-21.9 (-34.3, -7.15)	0.00	-	-	-	-	-	-	-	-	-	-
ΣDiNP Girls	-	-	-	-	-	-	-	-	-	0.28 (-15.7, 19.3)	0.98	-	-	-	-	-	-	-	-	-	-
ΣDINCH	-	-	0.92	-	-	0.91	-	-	0.24	-	-	0.65	-	-	0.20	-	-	0.99	-	-	0.63
Maternal third trimester (T3) – N = 378																					
MEP	-	-	0.45	-	-	0.74	-	-	0.53	-	-	0.70	-	-	0.38	-	-	0.43	-	-	0.86
MnBP	-	-	0.27	-	-	0.15	-	-	0.07	-	-	0.29	-	-	0.28	-	-	0.66	-	-	0.84
MnBP Boys	-	-	-	-	-	-	20.1 (-0.93, 45.7)	0.14	-	-	-	-	-	-	-	-	-	-	-	-	-
MnBP Girls	-	-	-	-	-	-	-11.1 (-31.7, 14.9)	0.36	-	-	-	-	-	-	-	-	-	-	-	-	-
MiBP	-	-	0.48	-	-	0.18	-	-	0.65	-	-	0.35	-	-	0.24	-	-	0.32	-	-	0.72
MBzP	-	-	0.50	-	-	0.50	-	-	0.36	-	-	0.59	-	-	0.79	-	-	0.94	-	-	0.98
ohMPHP	-	-	0.74	-	-	0.40	-	-	0.93	-	-	0.08	-	-	0.13	-	-	0.40	-	-	0.16
ohMPHP Boys	-	-	-	-	-	-	-	-	-	16.5 (-5.39, 42.4)	0.15	-	-	-	-	-	-	-	-	-	-
ohMPHP Girls	-	-	-	-	-	-	-	-	-	0.07 (-22.6, 29.2)	0.99	-	-	-	-	-	-	-	-	-	-
ΣDEHP	-	-	0.30	-	-	0.88	-	-	0.48	-	-	0.16	-	-	0.89	-	-	0.11	-	-	0.57
ΣDiNP	-	-	0.39	-	-	0.60	-	-	0.63	-	-	0.16	-	-	0.31	-	-	0.50	-	-	0.50
ΣDINCH	-	-	0.86	-	-	0.83	-	-	0.27	-	-	0.30	-	-	0.16	-	-	0.96	-	-	0.40

Significant (p-value <0.05) and borderline (p-value <0.10) results highlighted in bold. Percent change (PC), 95% Confidence Intervals (95% CI), p-values (p) and exposure-sex interaction p-value (p-int). Associations were sex-stratified only if p-int was below 0.10. PC and 95% CIs represent the percent change in hormone levels for each doubling in exposure biomarkers. Models adjusted for the same set of covariates as main models.

Table S5. Associations between maternal prenatal urinary phthalate metabolite concentrations and hair hormones at delivery, additionally adjusted for

Biomarker	Cortisol		Cortisone		Dehydrocorticosterone		Testosterone		Progesterone		CC Ratio		TC Ratio	
	PC (95% CI)	p	PC (95% CI)	p	PC (95% CI)	p	PC (95% CI)	p	PC (95% CI)	p	PC (95% CI)	p	PC (95% CI)	p
Maternal second trimester (T2) – N = 382														
MEP	-4.38 (-11.90, 3.79)	0.28	0.53 (-6.96, 8.63)	0.89	0.15 (-11.05, 12.76)	0.98	0.53 (-9.74, 11.98)	0.92	5.12 (-4.51, 15.72)	0.31	-4.88 (-9.09, -0.48)	0.03	5.15 (-6.32, 18.02)	0.39
MnBP	-3.32 (-16.77, 12.30)	0.66	-7.99 (-20.09, 5.96)	0.25	-8.85 (-26.58, 13.15)	0.40	-14.90 (-30.05, 3.53)	0.11	-3.26 (-18.83, 15.30)	0.71	5.07 (-3.30, 14.16)	0.24	-12.01 (-28.72, 8.61)	0.23
MiBP	4.55 (-9.17, 20.34)	0.53	-2.02 (-14.20, 11.89)	0.76	-2.49 (-20.42, 19.49)	0.81	-8.79 (-24.18, 9.72)	0.33	9.98 (-6.71, 29.65)	0.26	6.70 (-1.28, 15.33)	0.10	-12.88 (-28.52, 6.19)	0.17
MBzP	5.38 (-5.86, 17.97)	0.36	4.15 (-6.37, 15.86)	0.45	12.23 (-4.63, 32.07)	0.16	6.88 (-7.85, 23.98)	0.38	8.69 (-4.76, 24.03)	0.22	1.18 (-4.96, 7.72)	0.71	1.33 (-13.59, 18.82)	0.87
ohMPPH	-4.62 (-17.55, 10.35)	0.52	-3.43 (-15.84, 10.80)	0.62	-5.57 (-23.50, 16.56)	0.59	-10.10 (-25.74, 8.85)	0.27	15.31 (-2.73, 36.71)	0.10	-1.23 (-8.90, 7.09)	0.76	-5.69 (-23.19, 15.79)	0.57
ΣDEHP	-6.22 (-18.58, 8.00)	0.37	-6.63 (-18.27, 6.67)	0.31	-9.91 (-26.53, 10.47)	0.32	-2.84 (-19.30, 16.98)	0.76	4.98 (-11.04, 23.88)	0.56	0.44 (-7.14, 8.63)	0.91	3.56 (-15.14, 26.37)	0.73
ΣDiNP	2.09 (-7.18, 12.29)	0.67	1.62 (-7.11, 11.17)	0.73	-9.18 (-20.82, 4.18)	0.17	-13.87 (-23.93, -2.49)	0.02	-3.77 (-13.92, 7.58)	0.50	0.47 (-4.70, 5.92)	0.86	-15.61 (-26.12, -3.61)	0.01
ΣDINCH	-3.53 (-11.32, 4.94)	0.40	-1.74 (-9.25, 6.38)	0.66	-6.64 (-17.32, 5.42)	0.27	-5.78 (-15.64, 5.23)	0.29	-5.50 (-14.37, 4.28)	0.26	-1.82 (-6.30, 2.87)	0.44	-2.38 (-13.30, 9.92)	0.69
Maternal third trimester (T3) – N = 378														
MEP	-2.75 (-10.62, 5.81)	0.52	2.78 (-5.07, 11.28)	0.50	3.15 (-8.78, 16.63)	0.62	0.95 (-9.55, 12.66)	0.87	6.37 (-3.65, 17.43)	0.22	-5.38 (-9.69, -0.87)	0.02	3.81 (-7.76, 16.82)	0.53
MnBP	2.88 (-11.84, 20.06)	0.72	0.53 (-13.08, 16.27)	0.94	-3.04 (-22.57, 21.41)	0.79	-2.22 (-20.02, 19.53)	0.83	8.09 (-9.83, 29.58)	0.40	2.34 (-6.08, 11.52)	0.60	-4.96 (-23.44, 17.98)	0.64
MiBP	7.86 (-5.90, 23.62)	0.28	7.70 (-5.28, 22.46)	0.26	10.16 (-9.69, 34.37)	0.34	4.83 (-12.23, 25.20)	0.60	18.49 (1.02, 38.99)	0.04	0.14 (-7.19, 8.05)	0.97	-2.81 (-19.73, 17.68)	0.77
MBzP	12.98 (1.17, 26.18)	0.03	9.33 (-1.50, 21.35)	0.09	17.53 (0.05, 38.06)	0.05	12.31 (-2.76, 29.72)	0.11	13.56 (-0.28, 29.32)	0.06	3.35 (-2.84, 9.93)	0.30	-0.59 (-14.93, 16.16)	0.94
ohMPPH	-1.08 (-14.35, 14.25)	0.88	0.88 (-11.92, 15.54)	0.90	-3.13 (-21.45, 19.48)	0.77	-12.29 (-27.24, 5.73)	0.17	7.30 (-9.39, 27.07)	0.41	-1.95 (-9.49, 6.23)	0.63	-11.33 (-27.50, 8.45)	0.24
ΣDEHP	-3.66 (-18.06, 13.27)	0.65	0.33 (-13.86, 16.86)	0.97	-5.30 (-25.19, 19.87)	0.65	3.51 (-16.15, 27.77)	0.75	17.11 (-3.11, 41.56)	0.10	-3.98 (-12.24, 5.06)	0.38	7.44 (-14.35, 34.78)	0.53
ΣDiNP	-6.12 (-15.85, 4.74)	0.26	-3.33 (-12.81, 7.18)	0.52	-13.01 (-25.80, 1.99)	0.09	1.18 (-12.27, 16.69)	0.87	0.50 (-11.65, 14.32)	0.94	-2.88 (-8.62, 3.22)	0.35	7.77 (-7.55, 25.64)	0.34
ΣDINCH	-4.58 (-13.06, 4.71)	0.32	-0.70 (-9.04, 8.40)	0.88	-14.33 (-25.12, -1.98)	0.02	-6.14 (-16.83, 5.93)	0.30	-1.40 (-11.62, 10.00)	0.80	-3.91 (-8.75, 1.18)	0.13	-1.63 (-13.66, 12.07)	0.80

urinary specific gravity.

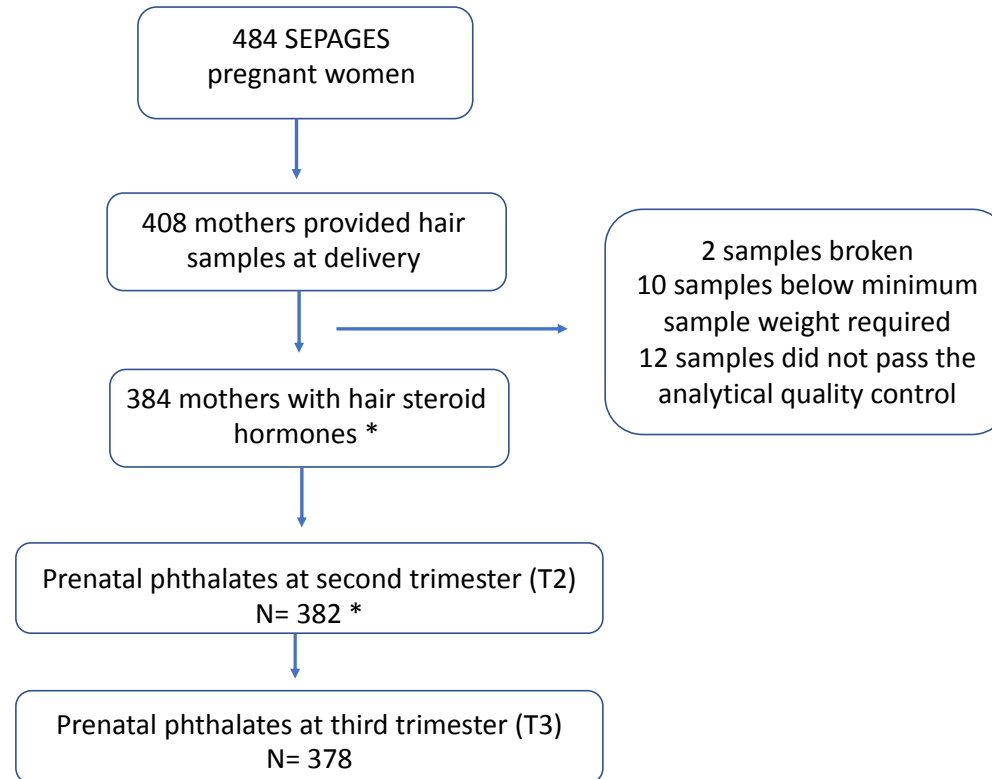
CC (Cortisol to Cortisone ratio); TC (Testosterone to Cortisol ratio). Significant (p-value <0.05) and borderline (p-value <0.10) results highlighted in bold. Percent change (PC), 95% Confidence Intervals (95% CI) and p-values (p). PC and 95% CIs represent the percent change in hormone levels for each doubling in exposure biomarkers. Models adjusted for: maternal age at conception (continuous), pre-pregnancy body mass index (BMI, normal weight vs. overweight/obese), education level (below vs. Master's degree or above), active smoking during pregnancy (smoker vs.

non-smoker), passive tobacco exposure (exposed vs. non-exposed), parity (nulliparous vs. uniparous/multiparous), maternal anxiety/depression score at third trimester (continuous), infant sex and season of hair collection (winter vs. remaining seasons), hair analysis batch (dichotomous) and time elapsed (continuous) between hair sampling and hormone measurement.

Table S6. Adjusted associations between maternal prenatal urinary phthalate metabolite concentrations and hair hormones at delivery after removing exposure and hormone outliers (n=14) corresponding to percentiles 1 and 99 of their respective distributions.

Biomarker	Cortisol		Cortisone		Dehydrocorticosterone		Testosterone		Progesterone		CC Ratio		TC Ratio	
	PC (95% CI)	p	PC (95% CI)	p	PC (95% CI)	p	PC (95% CI)	p	PC (95% CI)	p	PC (95% CI)	p	PC (95% CI)	p
Maternal second trimester (T2) – N = 368														
MEP	-2.84 (-10.41, 5.36)	0.48	-1.61 (-8.83, 6.18)	0.68	-1.21 (-12.32, 11.31)	0.84	-1.81 (-11.67, 9.15)	0.73	5.77 (-3.88, 16.38)	0.25	-2.31 (-5.69, 1.18)	0.19	5.95 (-5.21, 18.41)	0.31
MnBP	1.58 (-11.75, 16.93)	0.83	8.15 (-5.31, 23.52)	0.25	16.64 (-4.91, 43.08)	0.14	-6.60 (-22.12, 12.00)	0.46	2.67 (-13.05, 21.23)	0.76	2.36 (-3.68, 8.77)	0.45	0.50 (-17.16, 21.91)	0.96
MiBP	2.15 (-10.56, 16.66)	0.75	0.48 (-11.30, 13.82)	0.94	4.73 (-13.74, 27.14)	0.64	1.19 (-15.20, 20.74)	0.90	7.54 (-7.96, 25.65)	0.36	3.44 (-2.32, 9.54)	0.25	-4.95 (-20.92, 14.24)	0.59
MBzP	4.95 (-6.20, 17.42)	0.40	6.60 (-4.08, 18.46)	0.23	14.25 (-3.06, 34.65)	0.11	11.41 (-3.93, 29.20)	0.15	7.98 (-5.39, 23.23)	0.25	0.93 (-3.76, 5.85)	0.70	2.67 (-11.98, 19.77)	0.74
ohMPPH	4.54 (-10.39, 21.96)	0.57	7.09 (-7.50, 23.99)	0.36	6.62 (-15.00, 33.74)	0.58	-0.01 (-18.56, 22.77)	1.00	11.74 (-7.04, 34.31)	0.24	6.09 (-0.93, 13.61)	0.09	-10.08 (-27.46, 11.46)	0.33
ΣDEHP	-9.37 (-21.50, 4.64)	0.18	-4.43 (-16.60, 9.51)	0.51	-6.87 (-24.77, 15.30)	0.51	-1.44 (-18.58, 19.30)	0.88	5.32 (-11.23, 24.95)	0.55	0.87 (-5.25, 7.38)	0.79	9.37 (-10.24, 33.28)	0.37
ΣDiNP	6.18 (-3.31, 16.61)	0.21	7.18 (-1.88, 17.08)	0.12	-7.27 (-19.11, 6.30)	0.28	-9.31 (-19.84, 2.60)	0.12	-2.89 (-13.02, 8.41)	0.60	2.95 (-1.16, 7.24)	0.16	-13.83 (-24.06, -2.22)	0.02
ΣDINCH	-0.07 (-8.06, 8.62)	0.99	3.13 (-4.69, 11.59)	0.44	-6.32 (-17.23, 6.03)	0.30	-8.49 (-18.05, 2.18)	0.11	-2.91 (-12.04, 7.16)	0.56	0.81 (-2.77, 4.51)	0.66	-4.57 (-14.82, 6.90)	0.42
Maternal third trimester (T3) – N = 364														
MEP	1.80 (-5.87, 10.11)	0.65	2.42 (-4.84, 10.23)	0.52	5.75 (-5.78, 18.69)	0.34	1.75 (-8.13, 12.70)	0.74	8.26 (-1.22, 18.65)	0.09	-2.60 (-5.76, 0.66)	0.12	3.70 (-6.80, 15.38)	0.50
MnBP	9.39 (-3.90, 24.52)	0.17	13.19 (0.27, 27.78)	0.05	12.77 (-6.84, 36.51)	0.22	2.45 (-13.38, 21.17)	0.78	5.55 (-9.43, 23.01)	0.49	1.93 (-3.51, 7.68)	0.49	-0.05 (-16.07, 19.02)	1.00
MiBP	13.63 (0.92, 27.95)	0.03	14.95 (2.71, 28.65)	0.02	26.05 (6.34, 49.42)	0.01	16.93 (0.45, 36.12)	0.04	15.90 (0.81, 33.24)	0.04	-0.42 (-5.36, 4.77)	0.87	3.46 (-11.67, 21.17)	0.67
MBzP	12.49 (1.62, 24.52)	0.02	10.48 (0.47, 21.49)	0.04	19.58 (2.67, 39.28)	0.02	15.72 (1.17, 32.36)	0.03	9.16 (-3.40, 23.35)	0.16	1.97 (-2.44, 6.57)	0.39	3.44 (-9.97, 18.84)	0.63
ohMPPH	-8.08 (-21.20, 7.21)	0.28	-4.54 (-17.48, 10.43)	0.53	-7.64 (-26.60, 16.23)	0.50	1.15 (-17.62, 24.21)	0.91	-4.43 (-20.61, 15.04)	0.63	-2.02 (-8.31, 4.70)	0.55	3.48 (-16.03, 27.53)	0.75
ΣDEHP	0.83 (-12.86, 16.66)	0.91	1.24 (-11.73, 16.12)	0.86	1.58 (-18.40, 26.47)	0.89	8.45 (-10.66, 31.64)	0.41	10.97 (-6.57, 31.80)	0.23	-2.11 (-8.00, 4.16)	0.50	13.32 (-7.02, 38.10)	0.21
ΣDiNP	-1.80 (-11.61, 9.10)	0.73	1.28 (-8.32, 11.87)	0.80	-9.16 (-22.44, 6.41)	0.23	6.81 (-6.98, 22.64)	0.35	-3.44 (-14.90, 9.56)	0.59	-0.22 (-4.63, 4.39)	0.92	9.31 (-5.12, 25.94)	0.22
ΣDINCH	1.32 (-7.38, 10.85)	0.77	2.50 (-5.74, 11.45)	0.56	-8.56 (-19.83, 4.31)	0.18	-6.62 (-17.00, 5.05)	0.25	0.46 (-9.60, 11.64)	0.93	0.81 (-2.99, 4.76)	0.68	3.54 (-8.38, 17.01)	0.58

CC (Cortisol to Cortisone ratio); TC (Testosterone to Cortisol ratio). Significant (p-value <0.05) and borderline (p-value <0.10) results highlighted in bold. Percent change (PC), 95% Confidence Intervals (95% CI) and p-values (p). PC and 95% CIs represent the percent change in hormone levels for each doubling in exposure biomarkers. Models adjusted for: maternal age at conception (continuous), pre-pregnancy body mass index (BMI, normal weight vs. overweight/obese), education level (below vs. Master's degree or above), active smoking during pregnancy (smoker vs. non-smoker), passive tobacco exposure (exposed vs. non-exposed), parity (nulliparous vs. uniparous/multiparous), maternal anxiety/depression score at third trimester (continuous), infant sex and season of hair collection (winter vs. remaining seasons), hair analysis batch (dichotomous) and time elapsed (continuous) between hair sampling and hormone measurement.

Figure S1. Flow-chart of the study population.

* A woman presented an implausible value for testosterone that was not included in the analyses. This only affected analyses at T2, since the woman did not have phthalate measurements at T3.

Figure S2. SEPAGES within-subject urine pooling design and follow-up according to the current analysis.

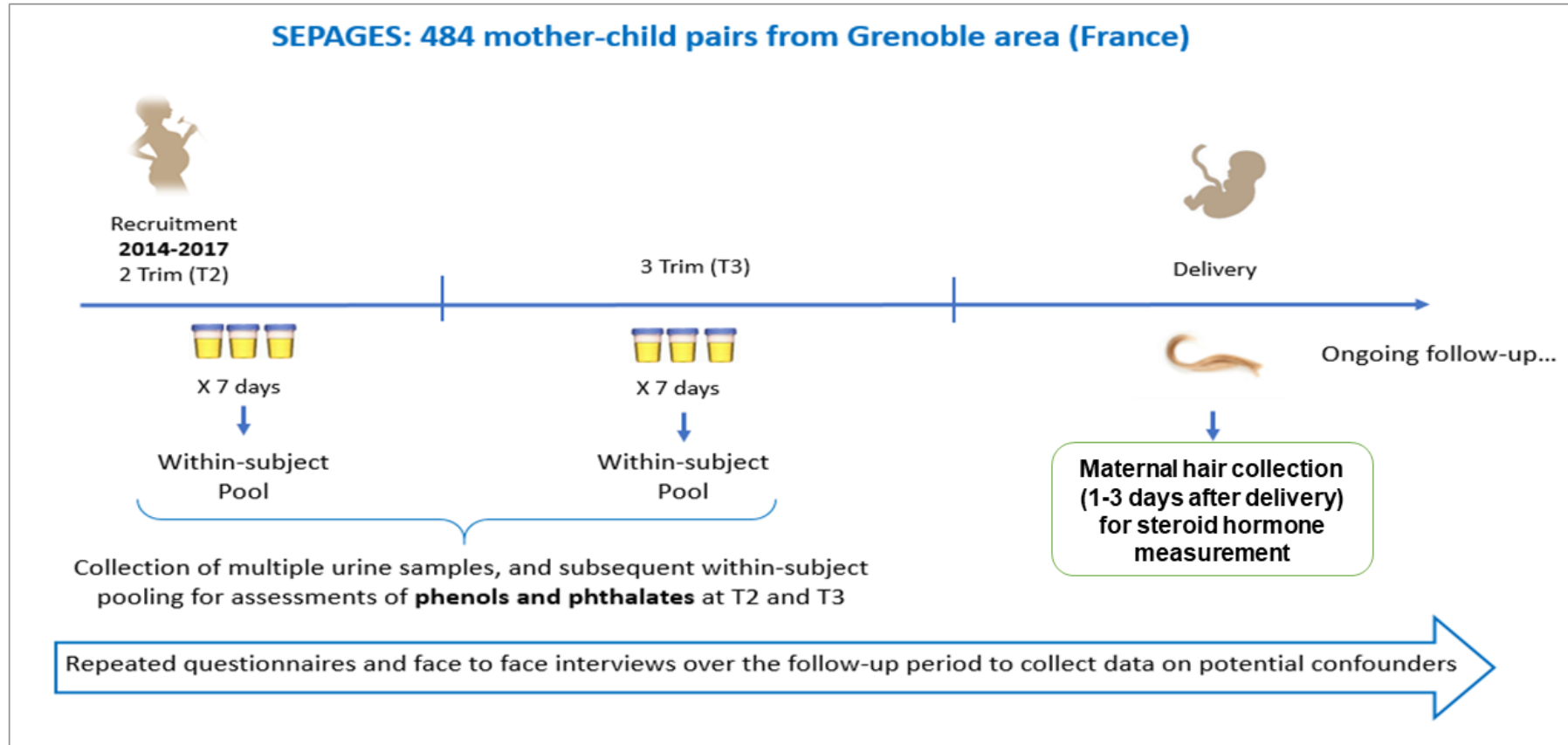
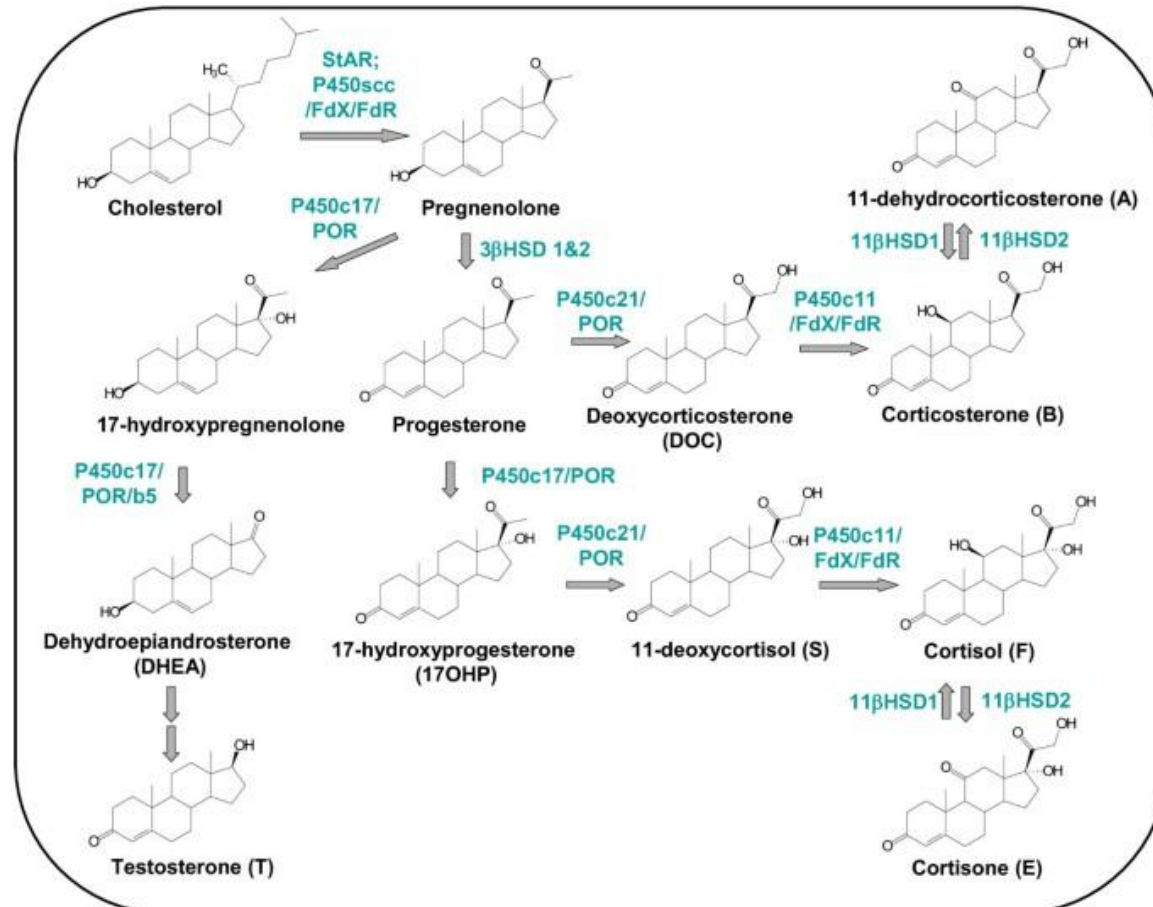
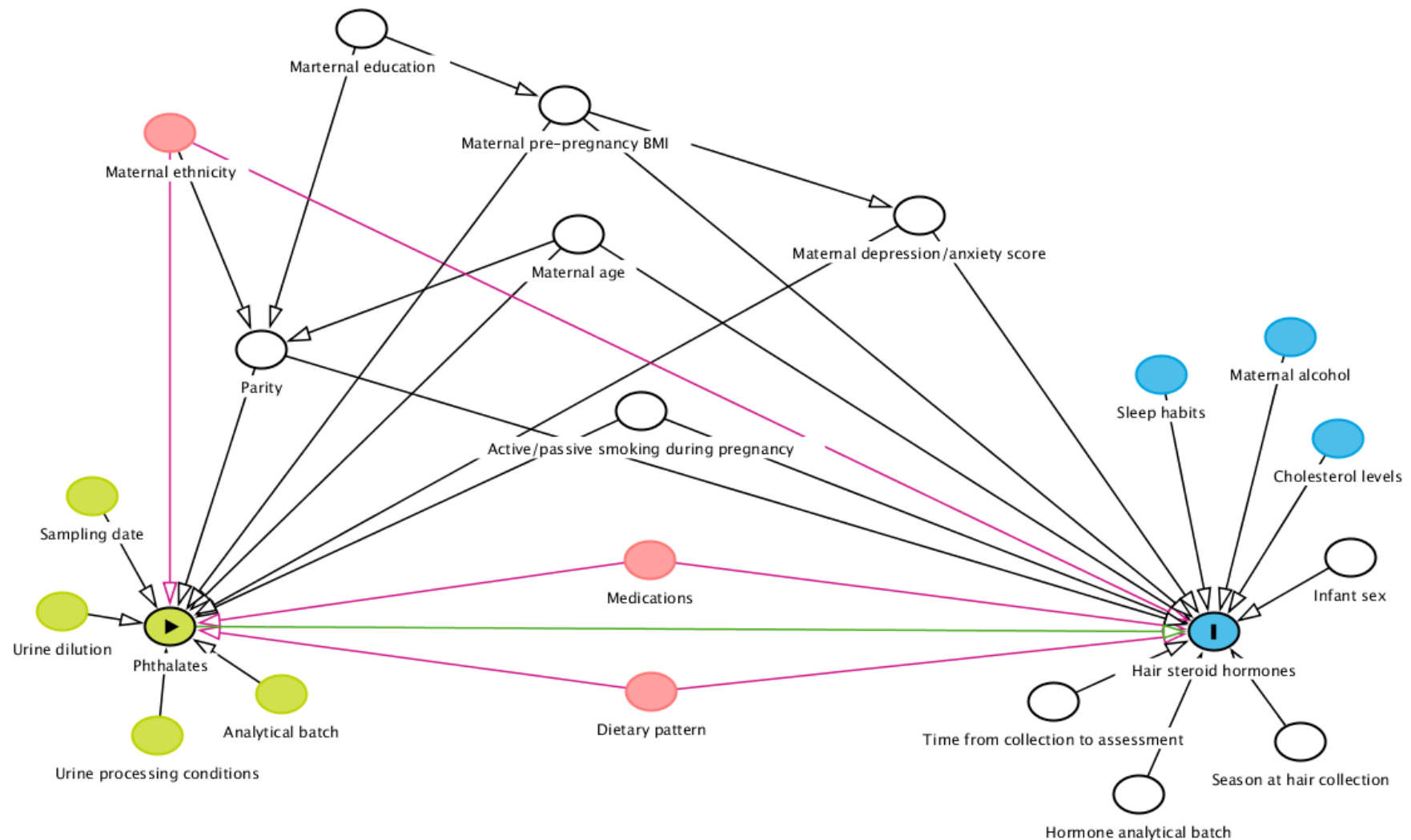


Figure S3. Biosynthesis of steroid hormone assessed and the enzymes involved. Reproduced with permission from Pozo et al., (2014).



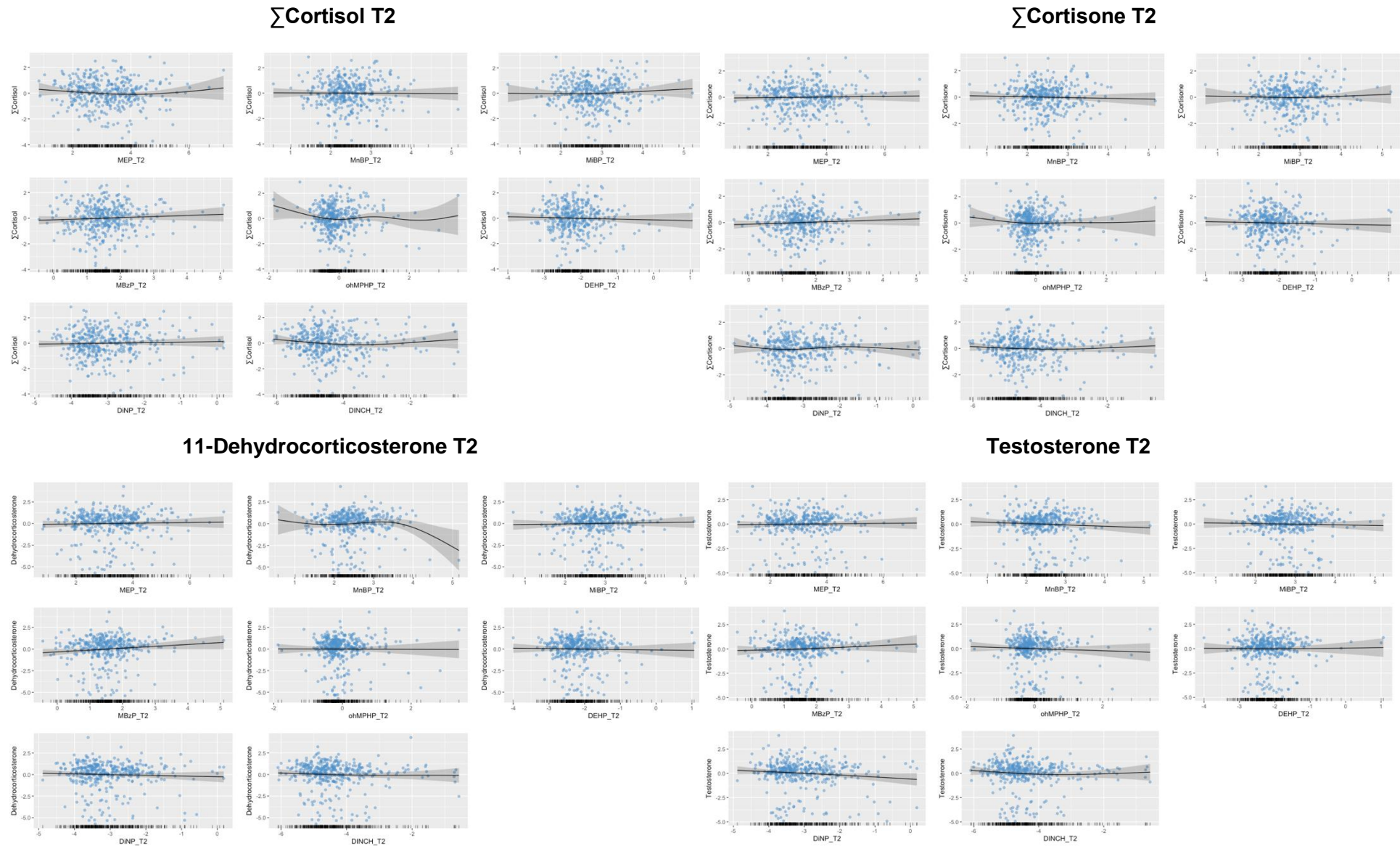
Pozo OJ, Marcos J, Fabregat A, Ventura R, Casals G, Aguilera P, Segura J, To-Figueras J. Adrenal hormonal imbalance in acute intermittent porphyria patients: results of a case control study. *Orphanet J Rare Dis.* 2014 Apr 16;9:54. doi: 10.1186/1750-1172-9-54.

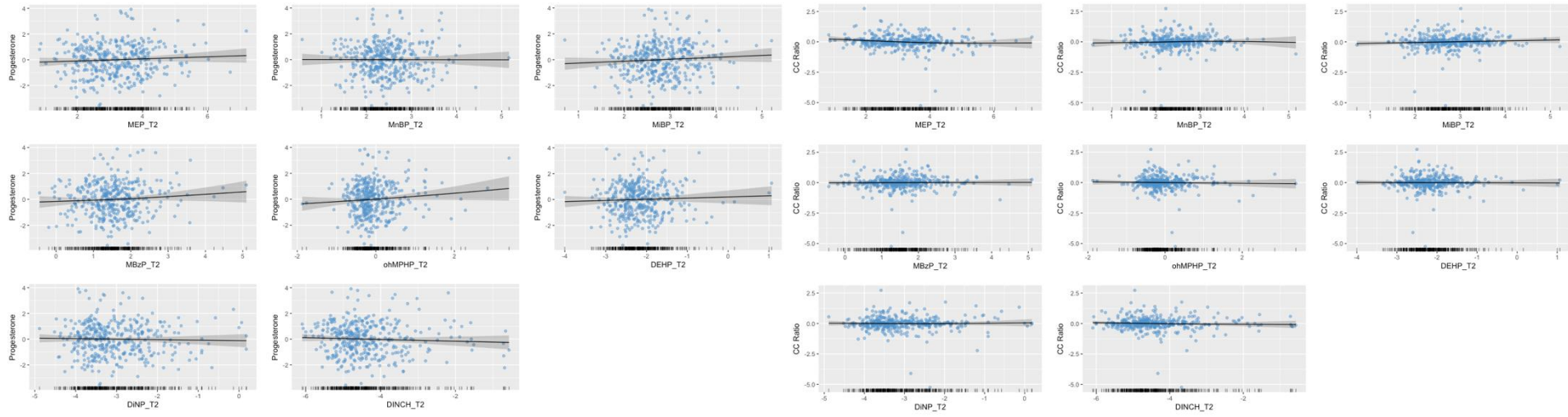
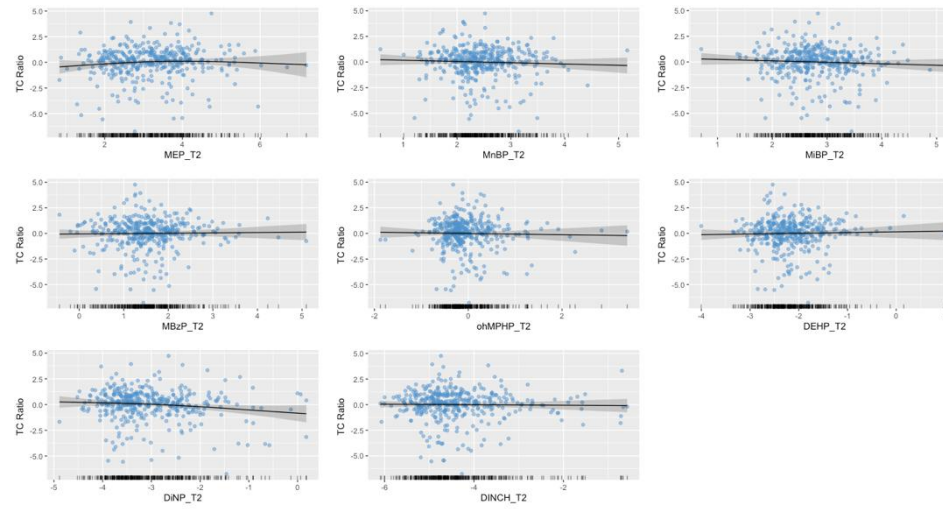
Figure S4. Directed acyclic graph (DAG) for prenatal main models.

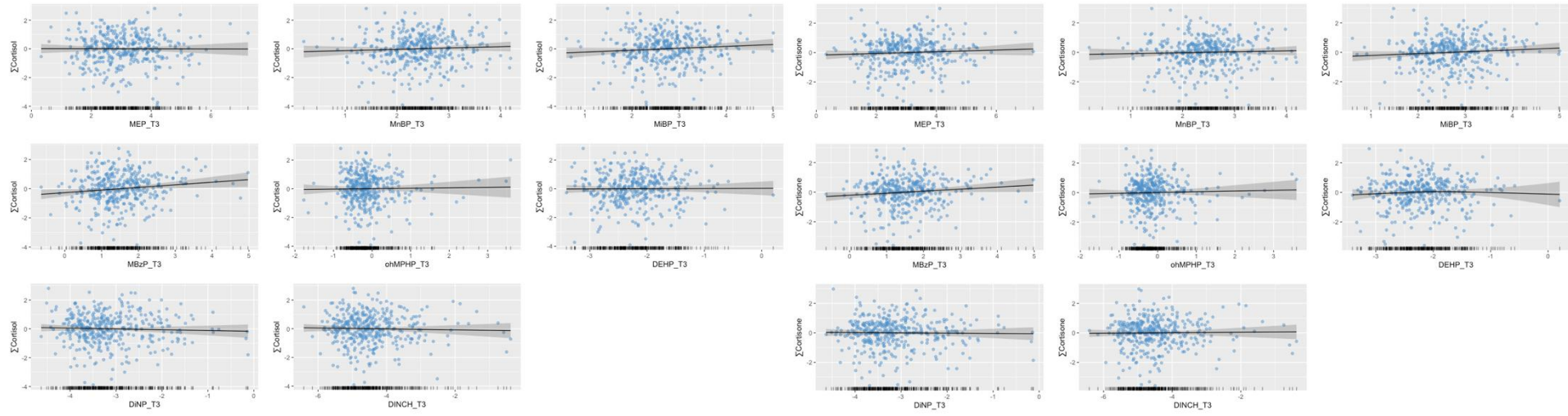


Note: White color represents variables adjusted in the main analysis. Red color represents variables not included in the main analysis that could behave as confounders. Covariates considered but excluded from analyses due to low variability or no relation with the outcome: maternal ethnicity (95.5% Caucasian) and maternal alcohol (very low intake). Data on medications and dietary patterns have not been cured and processed at the moment of this submission.

Figure S5. Generalized additive models (GAMs) depicting the functional shape of the adjusted exposure-hormone associations investigated during the second (T2) and third (T3) trimesters. Dots represent residuals.

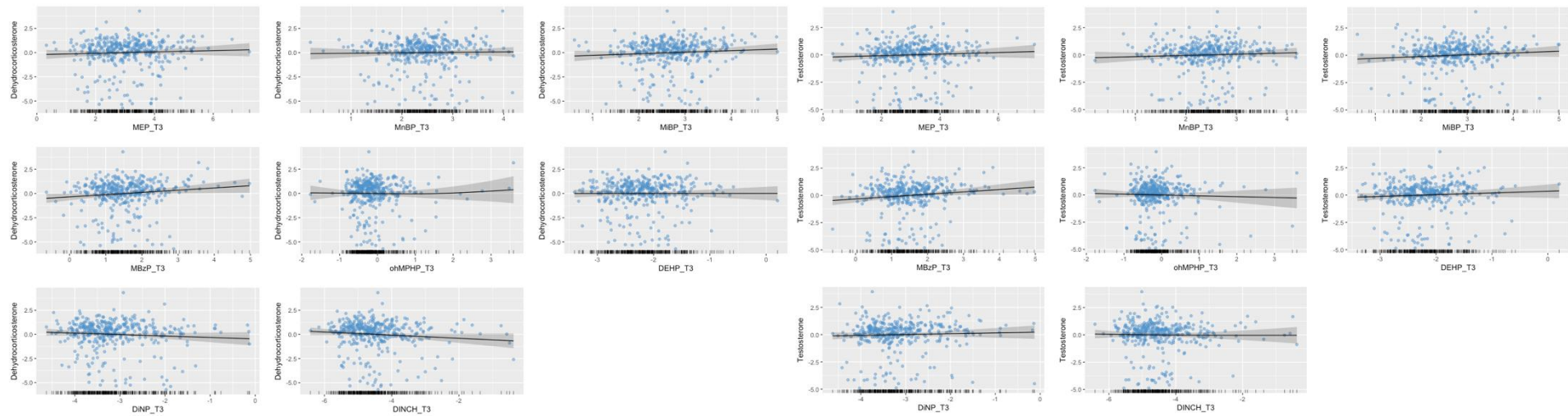


Progesterone T2**CC Ratio T2****TC Ratio T2**

Σ Cortisol T3 Σ Cortisone T3

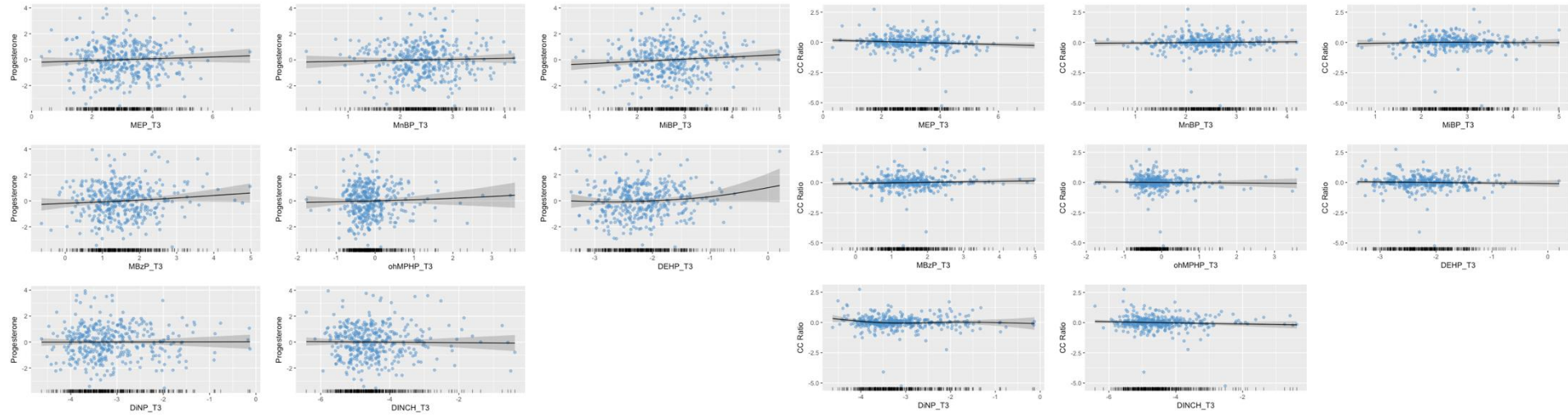
11-Dehydrocorticosterone T3

Testosterone T3



Progesterone T3

CC Ratio T3



TC Ratio T3

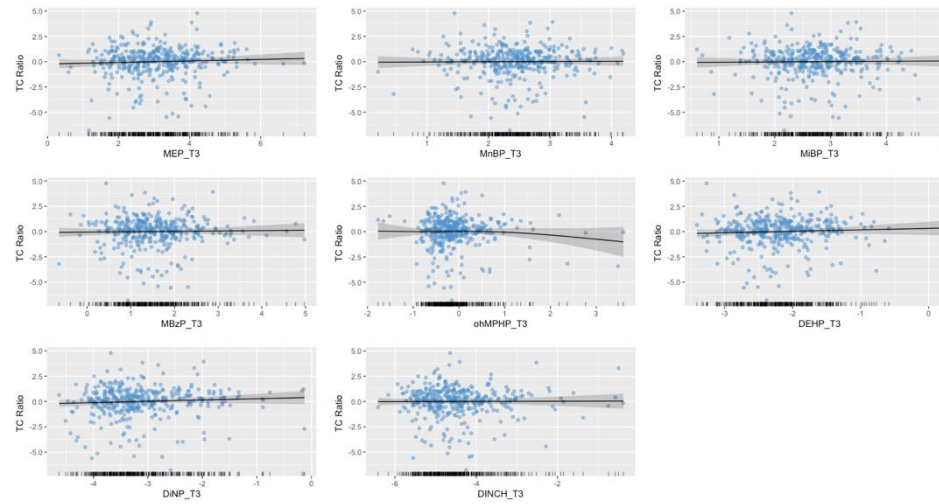


Figure S6. Spearman correlation heatmap of standardized phthalate metabolite concentrations measured at second (T2) and third (T3) trimesters of gestation (N=376).

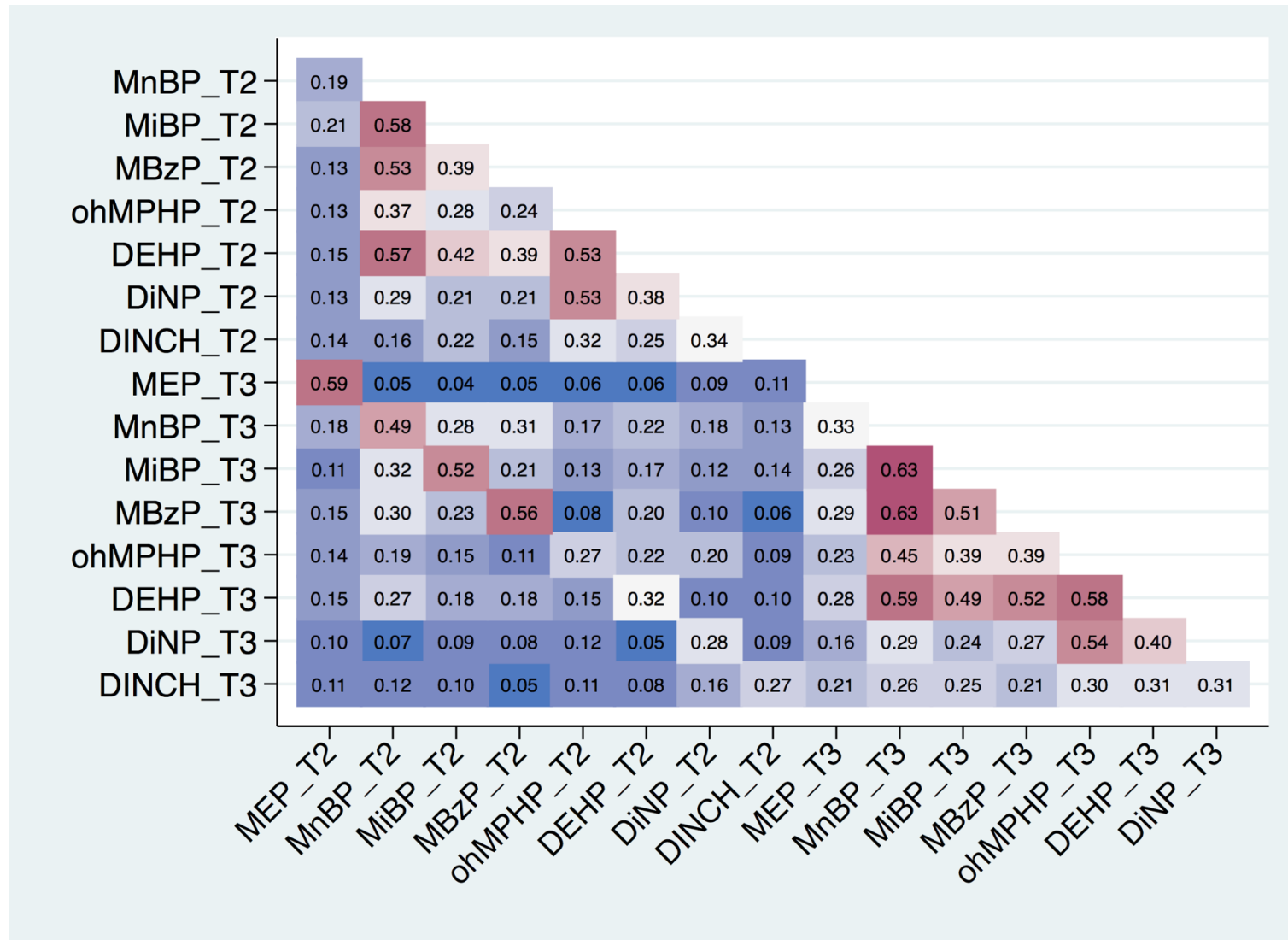


Figure S7. Spearman correlation heatmap of hormone levels measured in maternal hair samples at delivery (N=382).

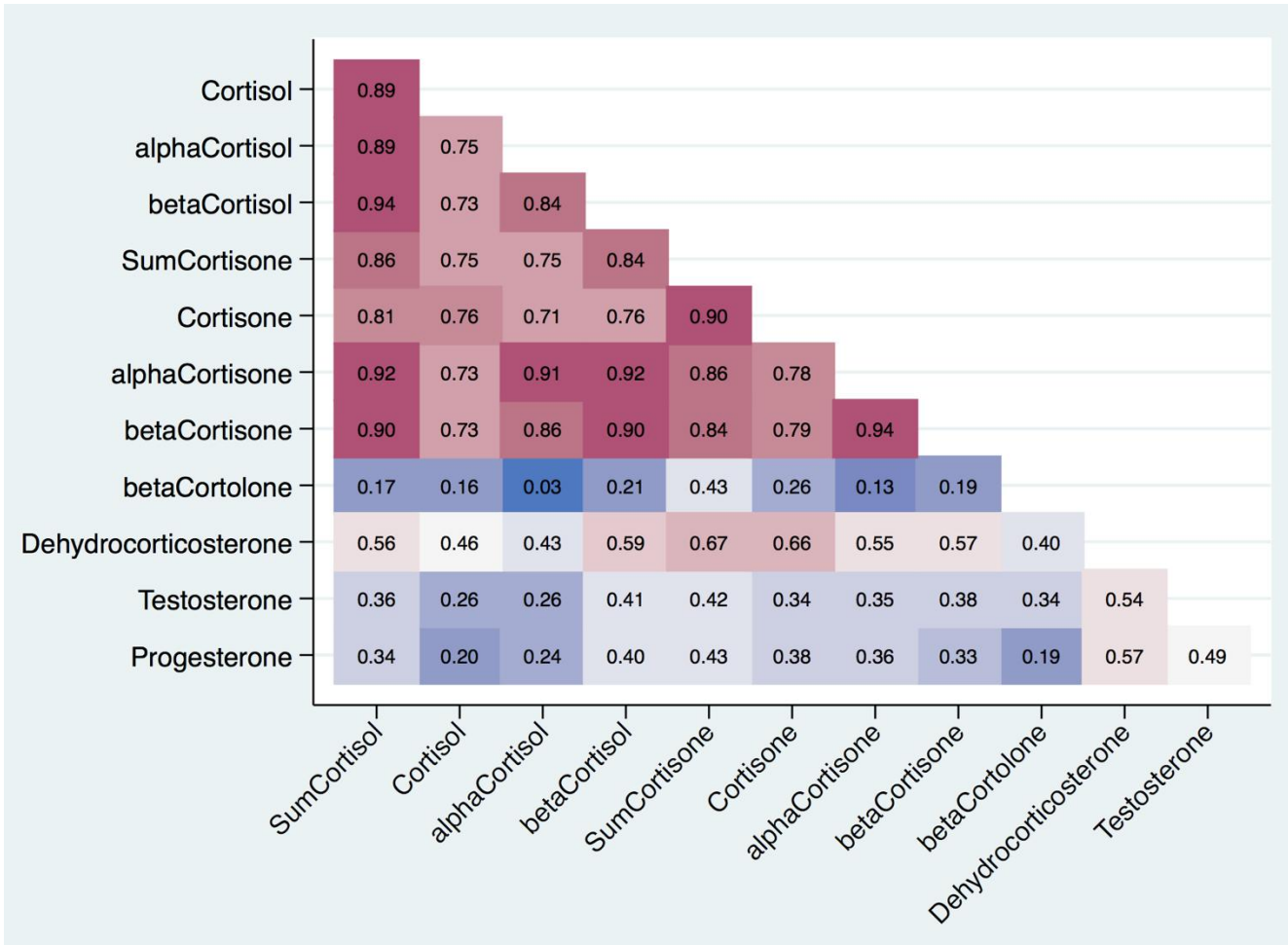
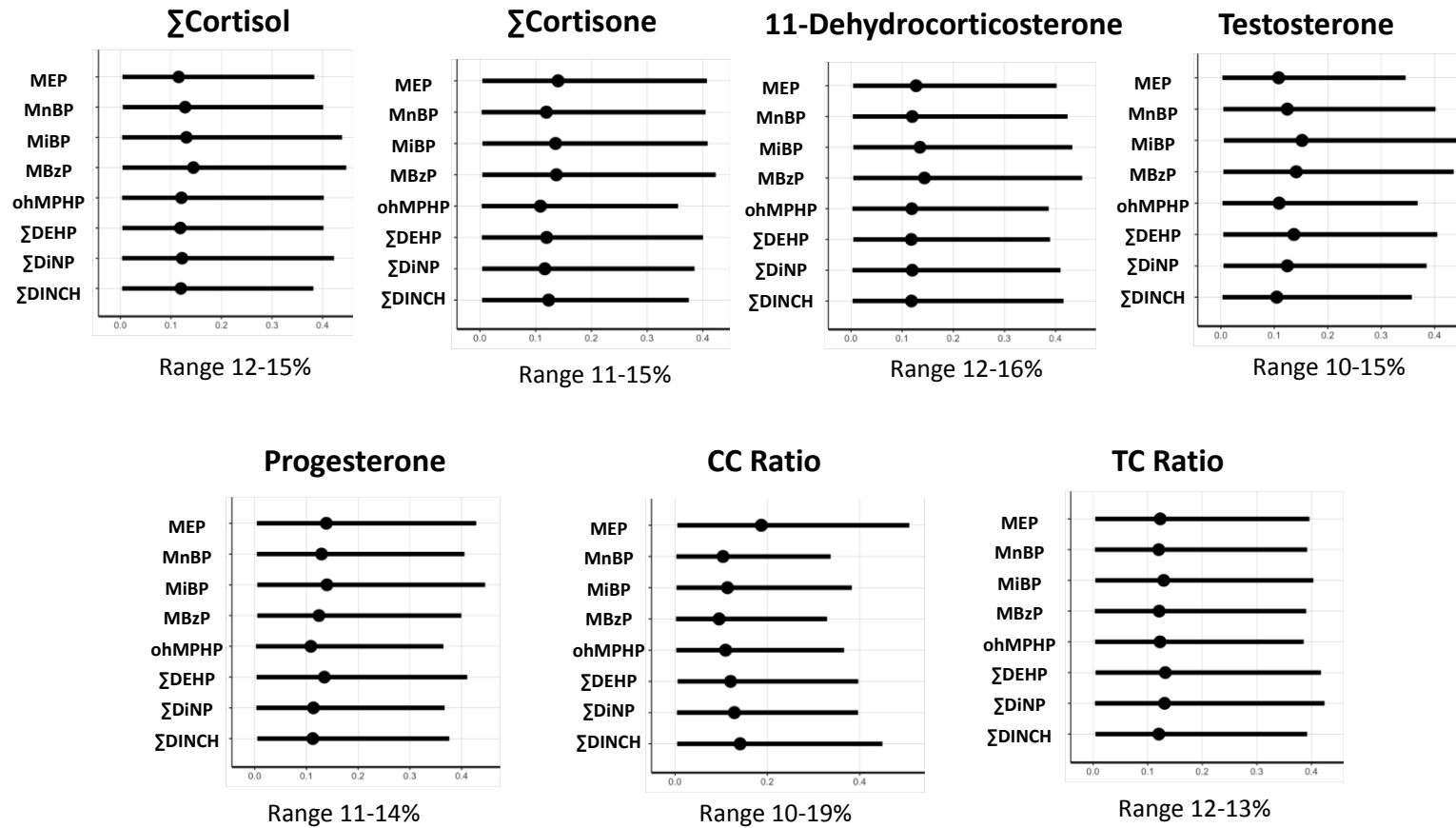


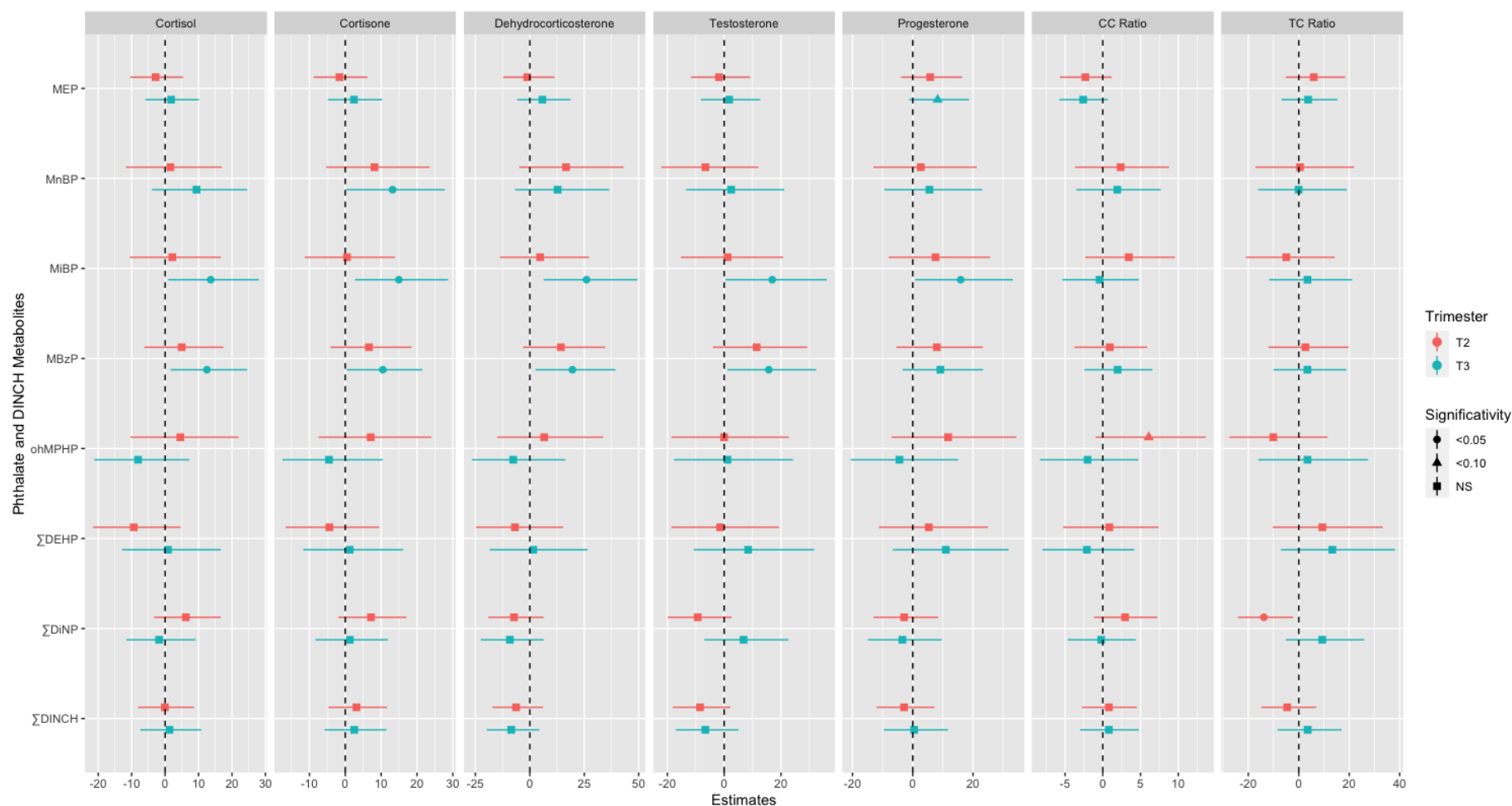
Figure S8. Relative weights of phthalates for the mixture associations during the third trimester of pregnancy.



Abbreviations: MEP (Monoethyl phthalate), MnBP (Mono-n-butyl phthalate), MiBP (Mono-isobutyl phthalate), MBzP (Mono-benzyl phthalate), ohMPHP (6-hydroxy-mono-propyl-heptyl phthalate), DEHP (Di-2-ethylhexyl phthalate), DiNP (Di-isononyl phthalate), DINCH (Di(isononyl)cyclohexane-1,2-dicarboxylate).

Note: In general, a similar contribution of the compounds to the mixture associations was observed. Notwithstanding, the contribution of MBzP and MiBP was higher in general.

Figure S9. Adjusted associations between maternal prenatal urinary phthalate metabolite concentrations and hair hormones at delivery after removing exposure and hormone outliers (n=14) corresponding to percentiles 1 and 99 of their respective distributions.



Note: Geometric figures (circles, triangles and squares) and error bars represent linear regression effect estimates with their corresponding 95% confidence intervals expressed as percent change (PC) in hormone levels for each doubling in exposure biomarkers. Models adjusted for: maternal age at conception (continuous), pre-pregnancy body mass index (BMI, normal weight vs. overweight/obese), education level (below vs. Master's degree or above), active smoking during pregnancy (smoker vs. non-smoker), passive tobacco exposure (exposed vs. non-exposed), parity (nulliparous vs. uniparous/multiparous), maternal anxiety/depression score at third trimester (continuous), infant sex and season of hair collection (winter vs. remaining seasons), hair analysis batch (dichotomous) and time elapsed (continuous) between hair sampling and hormone measurement. . CC (Cortisol to Cortisone ratio); TC (Testosterone to Cortisol ratio).