Supplemental Information

Maternal Blood Metal levels in Association with Birth Outcomes in Northern Puerto Rico

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Table S1. Demographic Characteristics of n = 810 Pregnant Women from Puerto Rico (2010− 2017)

variable	**Mean (SD)**	
maternal age	26.7 (5.5)	
parity (# live births)	0.7 (0.8)	
characteristic	**category**	**count (percent)**
Insurance type	private	477 (56.7%)
	public (mi salud)	303 (36.0%)
	missing	30 (3.6%)
maternal education	<=high school/ged	174 (20.7%)
	some college or technical school	284 (33.7%)
	college degree	245 (29.1%)
	master's degree or higher	95 (11.3%)
	missing	12 (1.4%)
household income	<$10,000	225 (26.7%)
	≥$10,000 to <$30,000	229 (27.2%)
	≥$30,000 to <$50,000	156 (18.5%)
	≥$50,000	93 (11.0%)
	missing	107 (12.7%)
marital status	single	162 (19.2%)
	married or living together	639 (75.9%)
	missing	9 (1.1%)
gravidity (# pregnancies)	0	337 (40.0%)
	1	290 (34.4%)
	>1	175 (20.8%)
	missing	8 (1.0%)
prepregnancy BMI (kg m−2)	≤25	412 (48.9%)
	>25 to ≤30	247 (29.3%)
	>30	145 (17.2%)

	missing	6 (0.7%)
employment status	employed	488 (58.0%)
	unemployed	309 (36.7%)
	missing	13 (1.5%)
smoking	never	676 (80.3%)
	ever	115 (13.7%)
	current	11 (1.3%)
	missing	8 (1.0%)
exposure to secondhand smoking	none	694 (82.4%)
	up to 1 hour	25 (3.0%)
	more than 1 hour	51 (6.1%)
	missing	40 (4.8%)
alcohol consumption	none	387 (46.0%)
	before pregnancy	354 (42.0%)
	within the last few months	57 (6.8%)
	missing	12 (1.4%)

Table S2. Blood biomarker concentrations (ng/ml) in n = 810 pregnant women from Puerto Rico[a] in 2010− 2017 (stratified by preterm birth status) and comparison with U.S. population-based samples of women ages 18− 40 from NHANES.[b,c]

Metals	LOD	% >LOD	Preterm Birth				Term Birth				NHANES		
			N	GM	GSD	Median	N	GM	GSD	Median	GM	GSD	Median
Co	0.2	98.3	112	0.3	1.4	0.3	1031	0.3	1.4	0.3			
Cs	0.04	99.9	112	1.2	1.4	1.2	1031	1.1	1.4	1.2			
Cu	9	99.9	112	1623.1	1.2	1620.2	1031	1543.2	1.3	1555.4			
Mn	2	99.9	112	11.9	1.4	12.0	1031	11.2	1.4	11.2	10.7	1.4	10.6
Ni	0.5	96.4	112	1.0	1.7	1.0	1031	1.0	1.6	1.0			
Zn	24	99.9	112	5003.6	1.1	5030.1	1031	4640.5	1.3	4712.1			
As	0.3	49.3	112	0.3	1.7	0.2	1031	0.3	1.8	0.2			
Cd	0.1	60.8	112	0.1	1.6	0.1	1031	0.1	1.7	0.1	0.3	2.2	0.3
Hg	0.2	99.9	112	1.3	1.7	1.3	1031	1.2	1.7	1.2	0.7	2.5	0.7
Pb	0.02	99.9	112	3.9	1.6	3.6	1031	3.2	1.5	3.2	6.4	1.8	6.1

[a] Includes biomarker concentrations for up to 2 repeated samples per woman (n = 1,143 samples); [b] Females 18−40 years of age; n = 3,585 for biomarkers measured in 2009-2010, 2011-2012, 2013-2014, and 2015-2016 NHANES; [c] NHANES, National Health and Nutrition Examination Survey; LOD, limit of detection; GM, geometric mean, GSD, geometric standard deviation;

Table S3. Change in birth outcomes associated with exposure biomarker concentration at each visit during pregnancy. Effect estimates presented as changes or odds ratio (OR) for IQR increase in exposure biomarker concentration. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking.

Metals	**Gestational age**						**Birthweight z-score**					
	Visit 1			**Visit 3**			**Visit 1**			**Visit 3**		
	N	Change in days (95% CI)	P value	N	Change in days (95% CI)	P value	N	Change in z-score (95% CI)	P value	N	Change in z-score (95% CI)	P value
Co	610	-0.6 (-1.8, 0.7)	0.38	472	-0.7 (-2.1, 0.6)	0.30	568	-0.01 (-0.1, 0.08)	0.82	440	**0.13 (0.02, 0.23)**	**0.02****
Cs	610	0.2 (-1.4, 1.7)	0.84	472	-1.3 (-3.0, 0.4)	0.14	568	0.00 (-0.11, 0.11)	0.97	440	**0.16 (0.02, 0.30)**	**0.02***
Cu	610	-0.3 (-1.3, 0.6)	0.47	472	-0.6 (-1.9, 0.8)	0.43	568	0.00 (-0.07, 0.06)	0.89	440	0.09 (-0.02, 0.21)	0.10
Mn	610	-1.0 (-2.6, 0.5)	0.19	472	-1.0 (-2.6, 0.6)	0.21	568	0.02 (-0.09, 0.14)	0.71	440	0.11 (-0.02, 0.24)	0.10
Ni	610	0.5 (-0.6, 1.7)	0.37	472	0.2 (-1.1, 1.4)	0.76	568	0.00 (-0.09, 0.09)	1.00	440	**0.13 (0.03, 0.24)**	**0.01***
Zn	610	-0.7 (-1.6, 0.2)	0.15	472	**-1.4 (-2.8, 0.0)**	**0.05***	568	0.01 (-0.06, 0.07)	0.84	440	0.00 (-0.12, 0.12)	0.99
As	610	1.2 (-0.5, 2.9)	0.17	472	0.2 (-1.4, 1.9)	0.77	568	0.08 (-0.04, 0.21)	0.21	440	0.06 (-0.08, 0.20)	0.38
Cd	610	-1.2 (-3.3, 0.8)	0.25	472	0.2 (-1.8, 2.2)	0.86	568	-0.04 (-0.20, 0.11)	0.56	440	-0.04 (-0.20, 0.12)	0.63
Hg	610	0.6 (-1.0, 2.2)	0.47	472	-0.5 (-2.1, 1.0)	0.51	568	0.01 (-0.11, 0.13)	0.88	440	0.07 (-0.06, 0.19)	0.32
Pb	610	-1.9 (-3.3, -0.4)	**0.01****	472	**-1.6 (-3.2, 0.0)**	**0.05****	568	0.04 (-0.07, 0.14)	0.50	440	0.08 (-0.05, 0.21)	0.25

	Preterm birth (overall)						**Preterm birth (spontaneous)**					
	Visit 1			**Visit 3**			**Visit 1**			**Visit 3**		
	N	OR (95% CI)	P value	N	OR (95% CI)	P value	N	OR (95% CI)	P value	N	OR (95% CI)	P value
Co	610	1.08 (0.82, 1.42)	0.57	472	1.03 (0.73, 1.44)	0.88	587	1.23 (0.90, 1.70)	0.20	449	1.15 (0.73, 1.82)	0.54
Cs	610	1.11 (0.77, 1.61)	0.59	472	1.39 (0.89, 2.18)	0.15	587	1.09 (0.69, 1.71)	0.72	449	1.25 (0.70, 2.25)	0.45
Cu	610	1.23 (0.87, 1.74)	0.23	472	1.28 (0.90, 1.82)	0.17	587	1.19 (0.78, 1.82)	0.43	449	1.14 (0.71, 1.82)	0.59
Mn	610	1.29 (0.92, 1.80)	0.14	472	1.23 (0.82, 1.85)	0.31	587	1.38 (0.92, 2.06)	0.12	449	1.20 (0.71, 2.05)	0.50
Ni	610	0.88 (0.67, 1.16)	0.37	472	1.03 (0.75, 1.41)	0.87	587	0.89 (0.64, 1.25)	0.51	449	1.10 (0.73, 1.66)	0.63
Zn	610	**1.80 (1.23, 2.64)**	**0.002****	472	**1.59 (1.07, 2.36)**	**0.02***	587	**1.81 (1.13, 2.90)**	**0.01****	449	1.32 (0.79, 2.20)	0.29
As	610	0.79 (0.52, 1.20)	0.27	472	0.95 (0.61, 1.48)	0.83	587	0.92 (0.57, 1.49)	0.73	449	0.87 (0.46, 1.62)	0.65
Cd	610	1.08 (0.69, 1.70)	0.74	472	0.78 (0.47, 1.29)	0.34	587	1.49 (0.87, 2.54)	0.15	449	0.80 (0.41, 1.55)	0.51
Hg	610	1.09 (0.75, 1.57)	0.65	472	**1.47 (0.99, 2.19)**	**0.06***	587	1.23 (0.79, 1.91)	0.36	449	**2.18 (1.28, 3.72)**	**0.004***
Pb	610	**1.70 (1.20, 2.43)**	**0.003***	472	**1.49 (1.00, 2.20)**	**0.05***	587	**1.82 (1.19, 2.77)**	**0.01****	449	1.34 (0.78, 2.28)	0.29

	SGA						**LGA**					
	Visit 1			**Visit 3**			**Visit 1**			**Visit 3**		
	N	OR (95% CI)	P value	N	OR (95% CI)	P value	N	OR (95% CI)	P value	N	OR (95% CI)	P value

Co	505	0.99 (0.72, 1.37)	0.97	403	0.75 (0.52, 1.09)	0.14	521	0.95 (0.72, 1.26)	0.72	0.72	1.30 (0.92, 1.84)	0.14
Cs	505	0.93 (0.66, 1.31)	0.68	403	0.68 (0.45, 1.03)	0.07	521	1.06 (0.75, 1.51)	0.73	0.73	1.47 (0.9, 2.41)	0.12
Cu	505	1.00 (0.80, 1.26)	0.99	403	0.98 (0.71, 1.35)	0.89	521	0.96 (0.81, 1.14)	0.65	0.65	1.22 (0.82, 1.82)	0.33
Mn	505	0.87 (0.59, 1.27)	0.46	403	**0.65 (0.43, 0.97)**	**0.03****	521	0.98 (0.69, 1.38)	0.91	0.91	1.12 (0.70, 1.77)	0.64
Ni	505	**0.71 (0.51, 1.00)**	**0.05***	403	**0.63 (0.45, 0.88)**	**0.01****	521	0.81 (0.61, 1.07)	0.14	0.14	1.23 (0.89, 1.70)	0.20
Zn	505	1.02 (0.79, 1.30)	0.90	403	1.04 (0.72, 1.50)	0.83	521	1.04 (0.80, 1.34)	0.77	0.77	0.95 (0.64, 1.42)	0.80
As	505	**0.63 (0.37, 1.05)**	**0.08***	403	0.75 (0.47, 1.22)	0.25	521	0.97 (0.65, 1.44)	0.87	0.87	1.01 (0.63, 1.60)	0.98
Cd	505	0.78 (0.45, 1.34)	0.36	403	1.08 (0.66, 1.75)	0.77	521	0.77 (0.48, 1.23)	0.27	0.27	1.21 (0.70, 2.07)	0.49
Hg	505	0.93 (0.62, 1.39)	0.73	403	0.73 (0.49, 1.09)	0.13	521	1.12 (0.79, 1.59)	0.53	0.53	1.14 (0.74, 1.77)	0.56
Pb	505	0.92 (0.66, 1.27)	0.60	403	0.76 (0.50, 1.17)	0.21	521	0.97 (0.71, 1.32)	0.84	0.84	0.82 (0.52, 1.31)	0.42

Table S4. Change in birth outcomes associated with tertiles of average exposure[abcdef]. Effect estimates presented as changes or odds ratio (OR) for IQR increase in exposure biomarker concentration. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking.

Metals	Gestational age					Birthweight z-score				
	N	Change in days (95% CI)	P value	Change in days (95% CI)	P value	N	Change in z-score (95% CI)	P value	Change in z-score (95% CI)	P value
Essential metals		**Tertile 1 vs 2**		**Tertile 3 vs 2**			**Tertile 1 vs 2**		**Tertile 3 vs 2**	
Co[a]	764	-0.8 (-3.4, 1.8)	0.54	-1.7 (4.2, 0.9)	0.21	712	-0.01 (-0.20, 0.19)	0.95	0.01 (-0.18, 0.21)	0.88
Cs[b]	764	-0.6 (-3.1, 2.0)	0.67	-0.5 (-3.1,2.1)	0.71	712	-0.05 (-0.24, 0.15)	0.63	0.08 (-0.12, 0.28)	0.44
Cu[a]	764	-1.2 (-3.8, 1.3)	0.35	**-2.5 (-5.1,0.1)**	**0.06***	712	**-0.20 (-0.39, 0.00)**	**0.05***	**-0.23 (-0.43, -0.03)**	**0.02****
Mn[a]	764	-1.4 (-4.0, 1.2)	0.28	**-2.9 (-5.5, 0.3)**	**0.03****	712	0.05 (-0.14, 0.25)	0.60	**0.18 (-0.01, 0.38)**	**0.07***
Ni[a]	764	0.4 (-2.2, 3.0)	0.75	2.0 (-0.6, 4.5)	0.13	712	-0.03 (-0.23, 0.17)	0.78	0.04 (-0.15, 0.23)	0.69
Zn[a]	764	1.0 (-1.6, 3.5)	0.46	-1.1 (-3.7, 1.5)	0.40	712	0.01 (-0.19, 0.20)	0.95	-0.05 (-0.24, 0.15)	0.64
Non-essential metals		**Tertile 2 vs 1**		**Tertile 3 vs 1**			**Tertile 2 vs 1**		**Tertile 3 vs 1**	
As[c]	764	1.5 (-0.6, 3.6)	0.17	-	-	712	0.04 (-0.12, 0.20)	0.61	-	-
Cd[c]	764	-1.4 (-3.6, 0.7)	0.19	-	-	712	0.01 (-0.15, 0.17)	0.91	-	-
Hg[d]	764	-1.5 (-4.1, 1.0)	0.24	0.8 (-1.8, 3.4)	0.56	712	0.13 (-0.06, 0.33)	0.18	0.03 (-0.16, 0.23)	0.74
Pb[d]	764	0.2 (-2.4, 2.7)	0.90	**-2.9 (-5.5, 0.3)**	**0.03****	712	-0.08 (-0.28, 0.11)	0.42	0.10 (-0.09, 0.30)	0.30

Metals	Preterm birth (overall)					Preterm birth (spontaneous)				
	N	OR (95% CI)	P value	OR (95% CI)	P value	N	OR (95% CI)	P value	OR (95% CI)	P value
Essential metals		**Tertile 1 vs 2**		**Tertile 3 vs 2**			**Tertile 1 vs 2**		**Tertile 3 vs 2**	
Co[a]	764	0.79 (0.43, 1.43)	0.43	1.12 (0.64, 1.97)	0.69	732	0.51 (0.22, 1.17)	0.11	1.23 (0.62, 2.42)	0.55
Cs[b]	764	1.09 (0.60, 1.95)	0.78	1.20 (0.66, 2.18)	0.54	732	1.16 (0.55, 2.43)	0.70	1.13 (0.53, 2.43)	0.75
Cu[a]	764	1.01 (0.53, 1.93)	0.97	**1.70 (0.95, 3.05)**	**0.07***	732	1.14 (0.50, 2.57)	0.76	1.79 (0.85, 3.79)	0.13
Mn[a]	764	1.24 (0.66, 2.32)	0.50	**1.84 (1.02, 3.29)**	**0.04****	732	**2.24 (0.93, 5.38)**	**0.07***	**3.17 (1.37, 7.33)**	**0.01****
Ni[a]	764	1.00 (0.58, 1.73)	1.00	**0.58 (0.31, 1.07)**	**0.08***	732	1.10 (0.55, 2.18)	0.79	0.53 (0.23, 1.18)	0.12
Zn[a]	764	0.56 (0.29, 1.10)	0.09	1.44 (0.84, 2.47)	0.19	732	0.67 (0.30, 1.48)	0.32	1.21 (0.60, 2.43)	0.59
Non-essential metals		**Tertile 2 vs 1**		**Tertile 3 vs 1**			**Tertile 2 vs 1**		**Tertile 3 vs 1**	
As[c]	764	0.75 (0.46, 1.20)	0.23	-	-	732	0.73 (0.40, 1.34)	0.31	-	-
Cd[c]	764	1.14 (0.70, 1.86)	0.60	-	-	732	1.50 (0.78, 2.87)	0.22	-	-
Hg[d]	764	**1.90 (1.06, 3.42)**	**0.03****	1.36 (0.72, 2.57)	0.34	732	**3.04 (1.32, 7.00)**	**0.01****	**2.20 (0.91, 5.32)**	**0.08***
Pb[d]	764	1.11 (0.58, 2.11)	0.76	**1.88 (1.03, 3.43)**	**0.04****	732	0.74 (0.32, 1.74)	0.49	1.75 (0.85, 3.61)	0.13

		SGA					LGA			
	N	OR (95% CI)	P value	OR (95% CI)	P value	N	OR (95% CI)	P value	OR (95% CI)	P value
Essential metals		**Tertile 1 vs 2**		**Tertile 3 vs 2**			**Tertile 1 vs 2**		**Tertile 3 vs 2**	
Co[a]	639	1.21 (0.65, 2.28)	0.55	1.09 (0.58, 2.05)	0.80	645	0.74 (0.41, 1.34)	0.32	0.75 (0.42, 1.36)	0.35
Cs[b]	639	1.56 (0.86, 2.85)	0.14	0.81 (0.41, 1.61)	0.55	645	1.04 (0.55, 1.95)	0.90	1.31 (0.72, 2.39)	0.37
Cu[a]	639	**2.05 (1.04, 4.04)**	**0.04****	**1.86 (0.92, 3.75)**	**0.08***	645	1.25 (0.69, 2.24)	0.46	0.76 (0.40, 1.43)	0.40
Mn[a]	639	0.96 (0.53, 1.74)	0.89	0.66 (0.35, 1.27)	0.22	645	1.09 (0.59, 2.01)	0.79	1.15 (0.63, 2.11)	0.64
Ni[a]	639	0.88 (0.50, 1.56)	0.67	**0.36 (0.18, 0.73)**	**0.004****	645	0.78 (0.42, 1.43)	0.42	0.80 (0.44, 1.43)	0.45
Zn[a]	639	1.05 (0.56, 1.96)	0.88	1.05 (0.56, 1.98)	0.88	645	0.71 (0.39, 1.27)	0.24	**0.58 (0.31, 1.06)**	**0.07***
Non-essential metals		**Tertile 2 vs 1**		**Tertile 3 vs 1**			**Tertile 2 vs 1**		**Tertile 3 vs 1**	
As[c]	639	0.79 (0.47, 1.32)	0.37	-	-	645	0.95 (0.58, 1.55)	0.83	-	-
Cd[c]	639	0.73 (0.44, 1.23)	0.24	-	-	645	0.79 (0.48, 1.30)	0.36	-	-
Hg[d]	639	0.70 (0.37, 1.32)	0.27	0.88 (0.48, 1.61)	0.68	645	1.66 (0.90, 3.05)	0.10	1.17 (0.61, 2.24)	0.64
Pb[d]	639	1.37 (0.76, 2.46)	0.29	0.56 (0.28, 1.13)	0.11	645	1.15 (0.64, 2.08)	0.64	0.79 (0.42, 1.47)	0.45

[a] Referent levels were set at tertile 2 for essential metals (Co, Cu, Mn, Ni, Zn).

[b] Cs is not regarded as essential to the health of plants or animals, nor does it present a hazard to them. For this analysis, Cs was considered as essential metal, therefore, referent levels were set at tertile 2.

[c] As, Cd were categorized as below LOD or above LOD as they have 50 and 40 % of samples <LOD

[d] Referent levels were set at tertile 1 for non-essential metals (Hg, Pb).

** p value < 0.01.

* p value from 0.05 to 0.01.

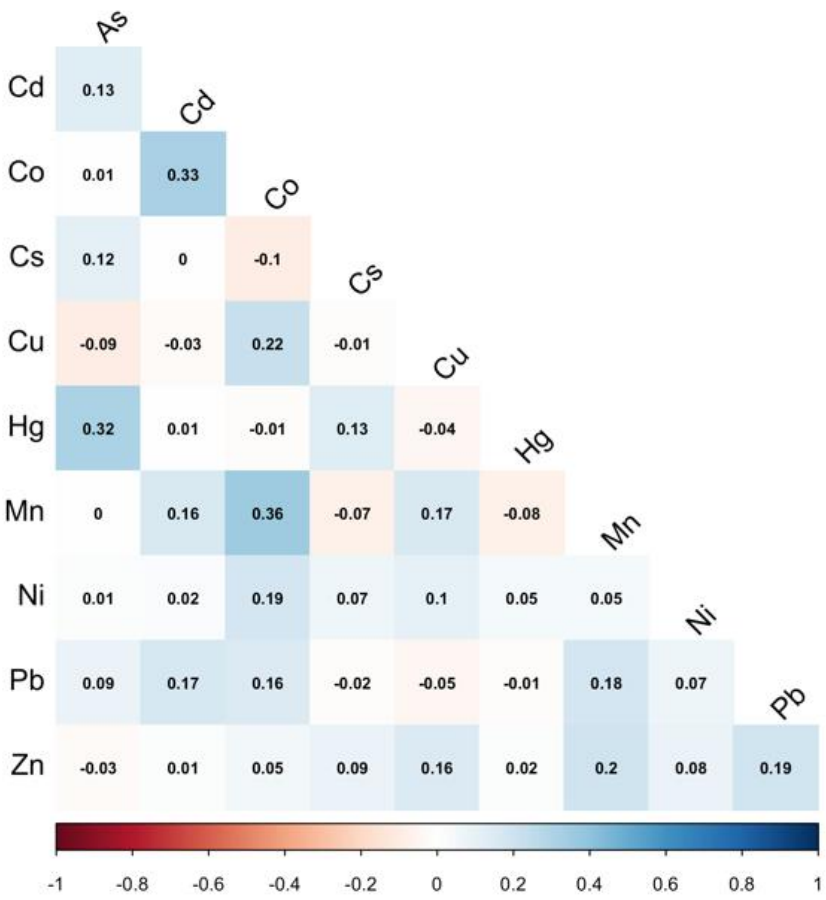
Table S5. Estimated environmental risk score (ERS) weights (regression coefficient) for metals selected for each birth outcome. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking.

	Weights					
Metal	Gestational Age	Birthweight z-score	Preterm birth (overall)	Preterm birth (spontaneous)	SGA	LGA
Co						
Cs						
Cu						
Mn						
Ni						
Zn			0.005			
As						
Cd						
Hg						
Pb	-0.090		0.070	0.120		

Table S6. Posterior inclusion probabilities (PIPs) for group inclusion and conditional inclusion into birth outcome models, using Bayesian kernel machine regression (BKMR) model. Models were adjusted for maternal age, maternal education, pre-pregnancy BMI, and exposure to secondhand smoking.

Metal	Group	Gestational Age		Birthweight z-score		Preterm Birth (overall)		Preterm Birth (spontaneous)		SGA		LGA	
		groupPIP	condPIP	groupPIP	condPIP	groupPIP	condPIP	groupPIP	condPIP	groupPIP	condPIP	groupPIP	condPIP
As	1	0.62	0.06	0.63	0.26	0.71	0.04	0.64	0.09	0.51	0.32	0.37	0.20
Cd	1	0.62	0.35	0.63	0.24	0.71	0.04	0.64	0.07	0.51	0.43	0.37	0.34
Hg	1	0.62	0.03	0.63	0.28	0.71	0.02	0.64	0.20	0.51	0.11	0.37	0.21
Pb	1	**0.62**	**0.57**	0.63	0.21	**0.71**	**0.90**	**0.64**	**0.64**	0.51	0.14	0.37	0.25
Co	2	0.35	0.65	**0.62**	**0.52**	0.51	0.49	0.68	0.49	0.47	0.51	0.47	0.60
Mn	2	0.35	0.35	0.62	0.48	**0.51**	**0.51**	**0.68**	**0.51**	0.47	0.49	0.47	0.40
Cs	3	0.26	0.19	0.62	0.23	0.90	0.03	0.59	0.08	0.85	0.08	0.41	0.30
Cu	3	0.26	0.29	0.62	0.25	0.90	0.06	0.59	0.19	0.85	0.05	0.41	0.28
Ni	3	0.26	0.09	0.62	0.28	0.90	0.05	0.59	0.24	**0.85**	**0.83**	0.41	0.18
Zn	3	0.26	0.43	0.62	0.24	**0.90**	**0.85**	0.59	0.49	0.85	0.05	0.41	0.24

Figure S1. Heatmap of pairwise correlations between blood GM concentrations among pregnant women in the PROTECT study[a].



[a] The correlation heatmap was created using natural log transformed blood metal concentrations.