

Supplemental Material

Gestational Weight Gain and Exposure of Newborns to Persistent Organic Pollutants

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Table of Contents	Page
Analytical methods	2
Table S1. Distribution of gestational ages (weeks) at the time of the last weight measurements	3
Table S2. Observed descriptive statistics of weekly GWG in the INMA cohorts	4
Table S3. Cord serum concentrations of OCPs, PCBs, and PBDE congeners (ng/mL and ng/g lipid) in the study population	5
Table S4. Maternal concentrations of OCPs, PCBs and PBDE congeners (ng/mL [ng/g lipid]) in the study population	6
Table S5. GWG during pregnancy (using a measure >37.5 weeks of gestation, n=1476) vs estimated GWG using a previous measure	7
Table S6. Adjusted associations of GWG β (95% CI) with ln-transformed POPs concentrations (ng/g lipids) based on alternative models	8
Table S7. Adjusted associations β (95% CI) for ln-transformed individual PCB and BDE congeners (ng/g lipid) according to GWG category	9
Figure S1. Conceptual hypothesis of the study	10
Figure S2. Multivariate Tobit regression coefficients of individual ln-transformed PBDE congeners and GWG	11
Figure S3. Multivariate linear regression coefficients of ln-transformed individual PCBs congeners and GWG	12
Reference	13

Analytical methods

Cord serum concentrations of α -HCH, β -HCH, γ -HCH, δ -HCH, HCB, PeCB, 2,4'-DDT, 4,4'-DDT, 2,4'-DDE, 4,4'-DDE, 2,4'-DDD, 4,4'-DDD, 7 PCB congeners (CB28, CB52, CB101, CB118, CB153, CB138 and CB180) and 14 PBDE congeners (BDE17, BDE28, BDE47, BDE66, BDE71, BDE85, BDE99, BDE100, BDE153, BDE154, BDE138, BDE183, and BDE190 and BDE209) were analyzed.

Briefly, 1 mL of serum was spiked with the surrogate standards tetrabromobenzene (TBB) and decachlorobiphenyl (CB209) and vortex stirred for 30 sec at 2,000 rpm. n-Hexane (3 mL) was added, followed by concentrated sulfuric acid (2 mL). After reaction, the mixture was stirred for 30 sec and the supernatant n-hexane phase was separated by centrifugation. The remaining sulfuric acid solution was re-extracted twice with 2 mL of n-hexane (each by 30 sec stirring and centrifugation). The combined n-hexane extracts (7 mL) were additionally cleaned with sulfuric acid (2 mL, stirring 30 sec). Then, the n-hexane phase was separated by centrifugation and reduced to a small volume under a gentle nitrogen stream. The extract was transferred to gas chromatography (GC) vials using four 25 μ L rinses of isooctane. CB142, BDE118 (20 μ L) and [13 C]-BDE209 (10 μ L) were added as internal standards before injection. Organochlorine compounds (OCs) were determined by GC with electron capture detection. BDE congeners were analyzed by GC coupled to mass spectrometry in chemical ionization and negative ion recording.

Total cholesterol and triglycerides were determined by enzymatic methods in maternal and cord serum samples and total serum lipid concentrations were calculated as described elsewhere (Phillips et al. 1989).

Table S1. Distribution of gestational ages (weeks) at the time of the last weight measurements.

Cohort, week k	Gestational age (weeks)	N^a	%^b
Reference (n = 2413)			
18	16.5 - 19.5	4	0.2
21	19.5 - 22.5	22	0.9
24	22.5 - 25.5	7	0.3
27	25.5 - 28.5	17	0.7
30	28.5 - 31.5	47	1.9
33	31.5 - 34.5	225	9.3
36	34.5 - 37.5	615	25.5
39	37.5 - 42	1476	61.2
Asturias (n = 324)			
18	16.5 - 19.5	1	0.3
21	19.5 - 22.5	1	0.3
24	22.5 - 25.5	1	0.3
27	25.5 - 28.5	4	1.2
30	28.5 - 31.5	12	3.7
33	31.5 - 34.5	52	16.0
36	34.5 - 37.5	123	38.0
39	37.5 - 42	130	40.1

^aNumber of mothers in which the last weight measurement was determined at this gestational

age. ^bPercent of mothers in which the last weight measurement was determined at this gestational age.

Table S2. Observed descriptive statistics of weekly GWG in the INMA cohorts.

Week	N	Mean^a	SD	Min	Max
Reference (n=2413)					
wGWG 18w	915	0.230	0.172	-0.38	0.88
wGWG 21w	1817	0.274	0.159	-0.41	1.16
wGWG 24w	860	0.286	0.140	-0.26	0.83
wGWG 27w	1093	0.311	0.137	-0.19	0.78
wGWG 30w	1069	0.323	0.136	-0.19	0.89
wGWG 33w	1696	0.328	0.130	-0.16	0.96
wGWG 36w	1485	0.332	0.125	-0.16	0.76
wGWG 39w	1476	0.346	0.126	-0.19	0.98
Asturias (n=324)					
wGWG 18w	128	0.259	0.167	-0.17	0.73
wGWG 21w	196	0.293	0.150	-0.26	0.80
wGWG 24w	107	0.346	0.149	-0.06	0.70
wGWG 27w	192	0.328	0.130	-0.02	0.72
wGWG 30w	149	0.342	0.140	-0.13	0.81
wGWG 33w	183	0.356	0.125	0.02	0.84
wGWG 36w	194	0.349	0.133	-0.09	0.73
wGWG 39w	130	0.349	0.127	0.05	0.82

wGWG: weekly gestational weight gain, in kg/week. Only one measurement per interval was taken (the closest to the central value).

^aObserved mean.

Table S3. Cord serum concentrations of OCPs, PCBs, and PBDE congeners (ng/mL and ng/g lipid) in the study population.

Pollutants	1/2LOD	>LOD(%)	GM (95% CI) ng/mL	Median ng/mL	P 90 ng/mL	Range ng/mL	GM (95% CI) ng/g lipid	Median ng/g lipid	P 90 ng/g lipid	Range ng/g lipid
4,4'-DDT	0.012	90	0.12 (0.10, 0.14)	0.08	0.22	nd-1.6	49 (42, 55)	33	97	nd-650
4,4'-DDE	0.003	100	0.69 (0.61, 0.78)	0.47	1.4	nd-6.0	270 (240, 299)	180	580	nd-2000
HCB	0.005	98	0.17 (0.16, 0.19)	0.13	0.37	nd-1.0	67 (62, 73)	50	140	nd-430
β-HCH	0.0006	90	0.067 (0.059, 0.075)	0.045	0.15	nd-0.68	26 (23, 29)	17	56	nd-240
PCB 138	0.012	92	0.11 (0.09, 0.14)	0.08	0.18	nd-4.0	45 (34, 55)	31	68	nd-1600
PCB 153	0.007	98	0.14 (0.13, 0.15)	0.13	0.25	nd-0.87	56 (53, 60)	47	96	nd-320
PCB 180	0.005	97	0.084 (0.079, 0.093)	0.07	0.15	nd-1.1	33 (30, 37)	27	58	nd-400
ΣPCBs	---	99	0.45 (0.41, 0.48)	0.38	0.72	0.068-4.4	175 (161, 190)	150	280	24-1800
BDE 47	0.0013	36	0.0049 (0.003, 0.007)	0.0013	0.009	nd-0.28	1.9 (1.2, 2.8)	0.56	3.4	nd-120
BDE 99	0.0011	31	0.0056 (0.004, 0.007)	0.0011	0.014	nd-0.22	2.2 (1.6, 2.9)	0.5	5.4	nd-90
BDE 153	0.0006	43	0.003 (0.002, 0.05)	0.0006	0.006	nd-0.16	1.5 (0.95, 2.0)	0.29	2.6	nd-66
BDE 209	0.0045	15	0.010 (0.008, 0.012)	0.0045	0.020	nd-0.17	4.1 (3.2, 5.0)	1.9	7.4	nd-61
ΣBDEs	---	61	0.025 (0.019, 0.030)	0.010	0.047	nd-0.67	5.7 (5, 6)	3.9	21	nd-820

CI: confidence interval; GM: geometrical mean; LOD: Limit of detection; nd: not detected. P 90: 90th percentile.

Table S4. Maternal concentrations of OCPs, PCBs and PBDE congeners (ng/mL [ng/g lipid]) in the study population.

Pollutants	1/2LOD	>LOD(%)	GM (95% CI) ng/mL	Median ng/mL	P 90 ng/mL	Range ng/mL	GM (95% CI) ng/g lipid	Median ng/g lipid	P 90 ng/g lipid	Range ng/g lipid
4,4'-DDT	0.012	80	0.12 (0.10, 0.14)	0.10	0.24	nd-2.3	26 (23, 30)	19	45	nd-219
4,4'-DDE	0.003	100	2.0 (1.9, 2.3)	1.4	4.6	0.23-20.6	405 (364, 447)	243	782	35-3945
HCB	0.005	98	0.48 (0.44, 0.52)	0.38	0.90	0.06-4.9	93 (86, 100)	70	181	12.1433
β-HCH	0.0006	90	0.21 (0.19, 0.23)	0.15	0.39	nd-3.8	40 (36, 45)	25	72	nd-326
PCB 138	0.012	100	0.23 (0.21, 0.25)	0.20	0.38	0.05-1.6	45 (41, 48)	39	75	9.05-322
PCB 153	0.007	100	0.38 (0.35, 0.40)	0.34	0.59	0.08-2.4	73 (69, 78)	66	112	12.9-468
PCB 180	0.005	99	0.28 (0.26, 0.31)	0.25	0.44	nd-3.0	55 (50, 60)	48	86	nd-596
∑PCBs	---	100	0.89 (0.83, 0.96)	0.80	1.4	0.12-7.1	175 (162, 187)	155	265	nd-1387
BDE 47	0.0013	22	0.005 (0.004, 0.007)	0.0013	0.014	nd-0.13	1.9 (1.2, 2.8)	0.25	2.7	nd-25
BDE 99	0.0011	60	0.014 (0.012, 0.016)	0.008	0.035	nd-0.16	1.1 (0.82, 1.4)	1.6	6.9	nd-31
BDE 153	0.0006	94	0.019 (0.016, 0.022)	0.013	0.038	nd-0.45	3.8 (3.2, 4.4)	2.6	7.8	nd-84
BDE 209	0.0045	31	0.015 (0.012, 0.018)	0.0045	0.043	nd-0.16	3.1 (2.5, 3.6)	0.9	8.7	nd-39
∑BDEs	---	98	0.056 (0.051, 0.062)	0.042	0.11	nd-0.56	11 (10, 12)	8.2	21	nd-104

CI: confidence interval; GM: geometrical mean; LOD: limit of detection; nd: not detected; P90: 90th percentile.

Table S5. GWG during pregnancy (using a measure >37.5 weeks of gestation, n=1476) vs estimated GWG using a previous measure.

Variable	Time period	N	R²	Bias^a	Me(abs RPD)^b	RMSE	CV(RMSE)^c
twGWG ₃₆ ⁱ	34.5 - 37.5 weeks	870	0.91	0.18	6.03	1.42	0.10
twGWG ₃₃ ⁱ	31.5 - 34.5 weeks	1099	0.86	0.03	7.91	1.92	0.14
twGWG ₃₀ ⁱ	28.5 - 31.5 weeks	635	0.80	0.02	9.54	2.30	0.17
twGWG ₂₇ ⁱ	25.5 - 28.5 weeks	682	0.75	0.21	12.25	2.58	0.19
twGWG ₂₄ ⁱ	22.5 - 25.5 weeks	564	0.67	0.48	13.41	2.93	0.21
twGWG ₂₁ ⁱ	19.5 - 22.5 weeks	1148	0.59	-0.06	16.00	3.40	0.25
twGWG ₁₈ ⁱ	16.5 - 19.5 weeks	574	0.46	-0.11	19.68	3.94	0.29

R²: R-squared; Me (abs RPD): median absolute relative percent difference; RMSE: root-mean-square error; CV(RMSE): coefficient of variation of the RMSE.

^aBias = mean (estimated GWG – observed GWG). ^bMe(abs RPD) = 100 * median (|estimated twGWG_{k39} – obs twGWG₃₉| / obs

twGWG₃₉). ^cCV(RMSE) = [(1/N) Σ_{k=1}^N (estimated twGWG_{k39} – real twGWG_{k39})²]^{1/2} / mean (real twGWG₃₉).

Table S6. Adjusted^a associations of GWG β (95% CI) with ln-transformed POPs concentrations (ng/g lipids) based on alternative models.

POP	Main model	Women with no weight measurements <28.5 weeks excluded (n=7)	Maternal POPs concentrations excluded	Relative difference in newborn concentrations compared with maternal concentrations (X)
Σ PCBs	-0.01 (-0.02, -0.002)	-0.01 (-0.02, -0.004)	-0.02 (-0.03, -0.008)	-0.008 (-0.02, -0.01)
Σ BDEs	-0.02 (-0.05, 0.008)	-0.02 (-0.04, 0.01)	-0.01 (-0.03, 0.007)	-0.003 (-0.02, 0.01)
4,4'-DDE	-0.016 (-0.03, -0.003)	-0.017 (-0.03, 0.004)	-0.02 (-0.04, -0.002)	-0.008 (-0.01, -0.002)
4,4'-DDT	-0.013 (-0.03, 0.005)	-0.016 (-0.03, 0.001)	-0.018 (-0.04, 0.0008)	-0.007 (-0.05, 0.04)
HCB	-0.012 (-0.03, 0.001)	-0.014 (-0.03, -0.001)	-0.012 (-0.03, 0.004)	-0.009 (-0.02, -0.002)
β -HCH	-0.03 (-0.06, -0.003)	-0.03 (-0.06, -0.004)	-0.06 (-0.1, -0.03)	-0.01 (-0.02, -0.004)

^aUnless otherwise specified, models for each POP included the following covariates. Σ PCBs: pre-pregnancy BMI, age, total fish consumption, maternal Σ PCB concentrations. Σ BDEs: pre-pregnancy BMI, total fish consumption, maternal Σ BDE concentrations. 4,4'-DDE: pre-pregnancy BMI, age, maternal education, maternal 4,4'-DDE concentrations. 4,4'-DDT: pre-pregnancy BMI, age, maternal 4,4'-DDT concentrations. HCB: pre-pregnancy BMI, age, total fish consumption, maternal HCB concentrations. β -HCH: pre-pregnancy BMI, age, total fish consumption, maternal β -HCH concentrations.

Table S7. Adjusted^a associations β (95% CI) for ln-transformed individual PCB and BDE congeners (ng/g lipid) according to GWG category.

POP	Inadequate GWG (n = 81)^b	Excessive GWG (n = 135)^b
BDE47	0.46 (-0.17, 1.1)	-0.23 (-0.82, 0.35)
BDE99	0.45 (-0.48, 1.4)	-0.28 (-1.2, 0.58)
BDE153	0.04 (-0.64, 0.57)	-0.36 (-0.91, 0.18)
BDE209	1.26 (0.006, 2.5)	0.51 (-0.63, 1.66)
PCB138	0.16 (-0.013, 0.33)	0.07 (-0.08, 0.22)
PCB153	0.15 (0.02, 0.30)	-0.02 (-0.15, 0.11)
PCB180	0.13 (-0.02, 0.29)	-0.16 (-0.29, -0.01)

^aModels of PCB congeners adjusted by: pre-pregnancy BMI, age, total fish consumption, maternal PCB congener concentrations. Models of PBDE congeners adjusted by: pre-pregnancy BMI, total fish consumption, maternal PBDE congener concentrations. ^bReference group is Recommended GWG (n=108).

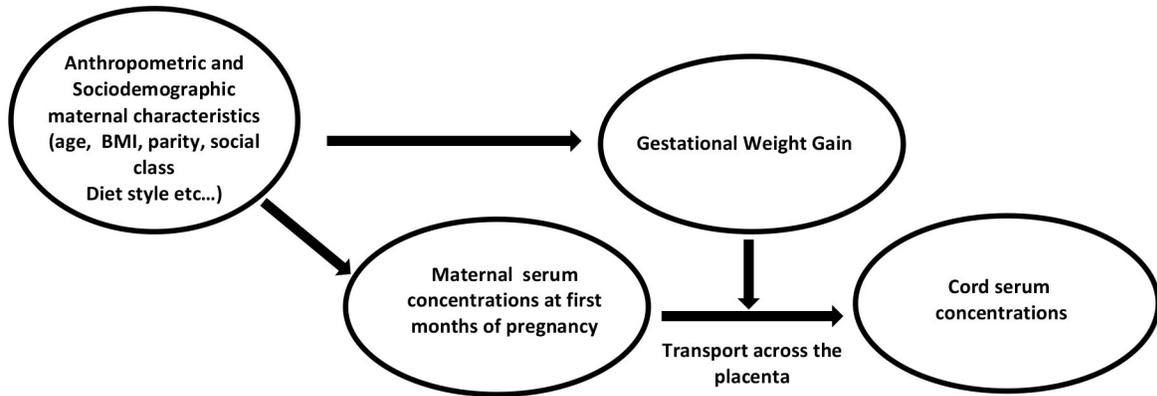


Figure S1. Conceptual hypothesis of the study.

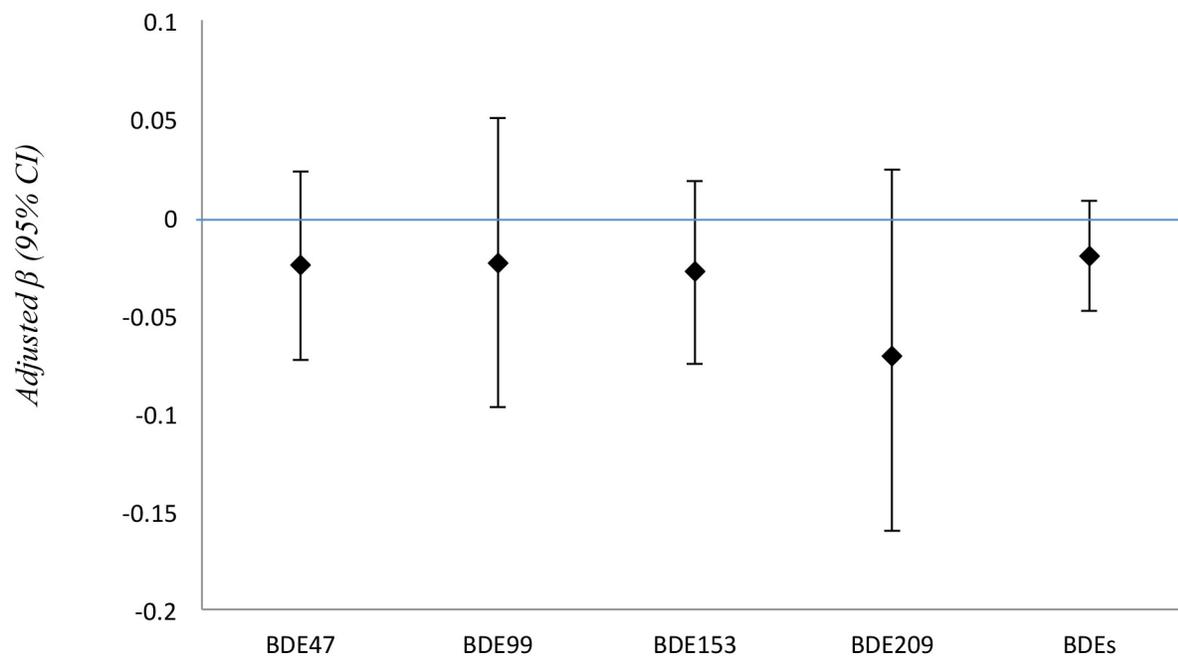


Figure S2. Multivariate Tobit regression coefficients of individual ln-transformed PBDE congeners and GWG.

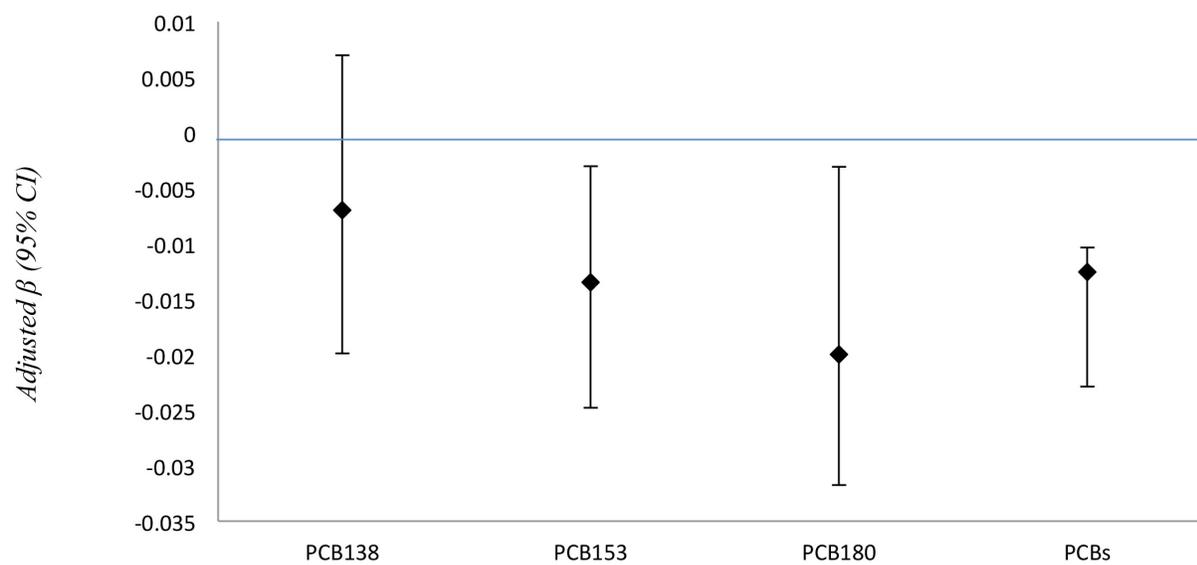


Figure S3. Multivariate linear regression coefficients of ln-transformed individual PCBs congeners and GWG.

Reference

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