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Supplemental information

Associations of internal persistent organic pollutant levels with sex hormones: An analysis by sex and menopausal status in a Spanish cohort

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Supplementary Material

Figure S1. Directed acyclic graph of the hypothesized association between PCBs/OCPs exposure and sex hormone disruption

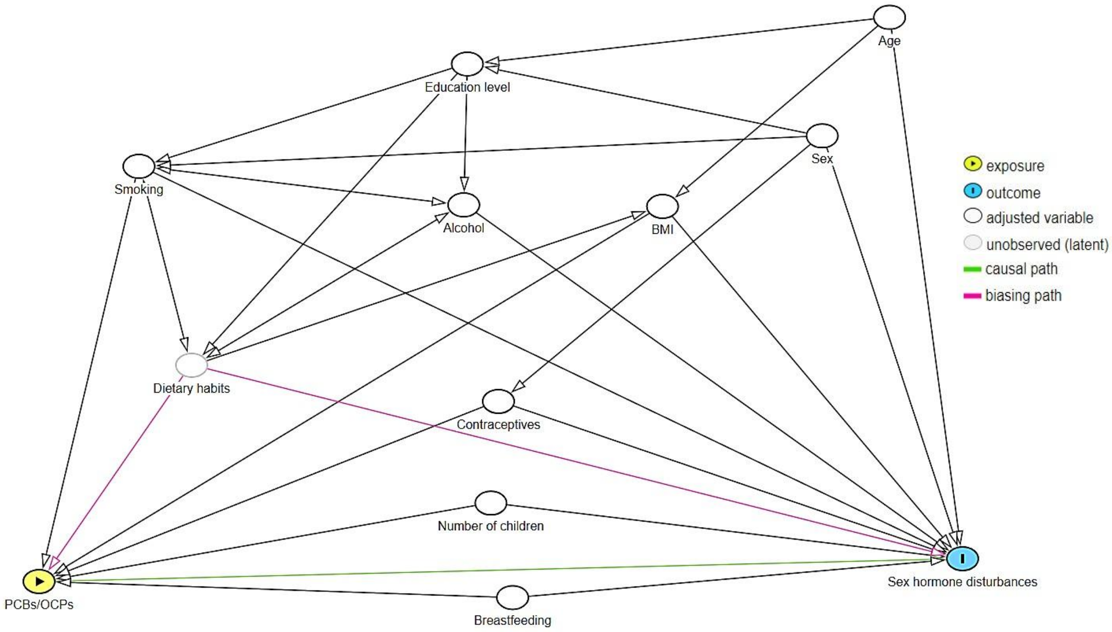
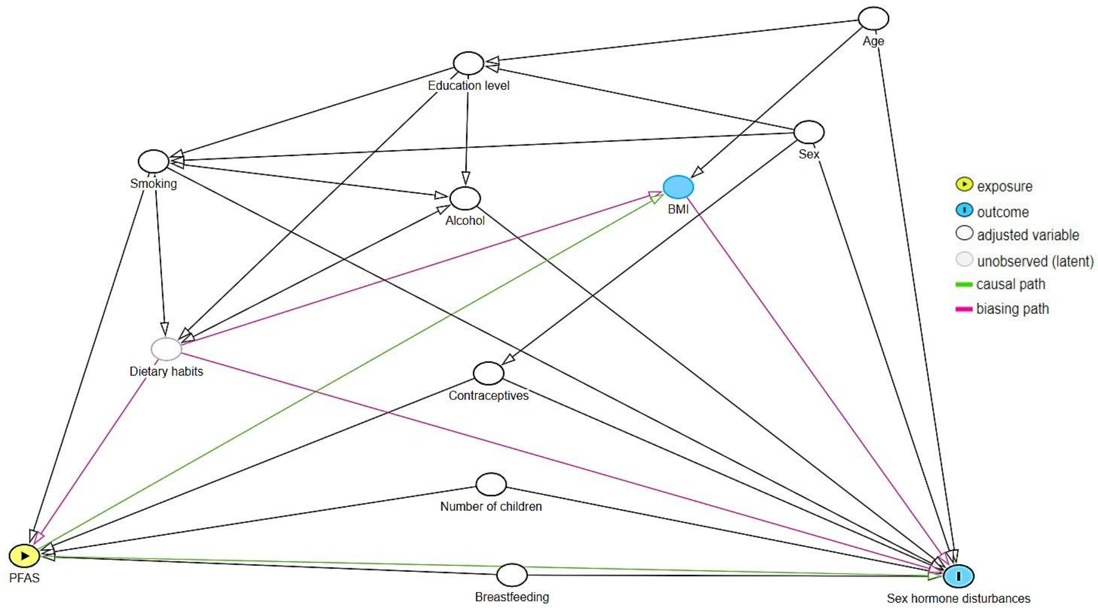


Figure S2. Directed acyclic graph of the hypothesized association between PFAS exposure and sex hormone disruption



PCBs: polychlorinated biphenyl; OCPs: organochlorine pesticides; PFAS: per- and polyfluoroalkyl substances; BMI: body mass index.

Table S1. Characteristics of study population with measures of polychlorinated biphenyls (PCBs)/organochlorine pesticides (OCPs) and sex hormone analyses (Subgroup 1) (n=234)

Characteristics [n (%)]	**Men** 113 (48.3)	**Women** 121 (51.7)	**Premenopausal women** 63 (26.9)	**Postmenopausal women** 58 (24.8)
Sociodemographic characteristics				
Age [[median (IQR)]	51.0 (35.0-63.0)	48.0 (36.0-60.0)	37.0 (29.0-44.0)	60.5 (54.3-68.8)
BMI [median (IQR)]	26.6 (25.0-29.1)	26.2 (23.4-29.5)	23.88 (22.1-27.7)	27.62 (25.4-30.9)
Education level [n (%)]				
No formal education	36 (31.9)	36 (29.8)	7 (11.1)	29 (50.0)
Primary education	42 (37.2)	58 (47.9)	33 (52.4)	25 (43.1)
≥ Secondary education	35 (31.0)	27 (22.3)	23 (36.5)	4 (6.9)
Occupation				
Manual worker	25 (22.1)	23 (19.0)	18 (28.6)	5 (8.6)
Non-manual worker	79 (69.9)	98 (81.0)	45 (71.4)	53 (91.4)
Retired	9 (8.0)	0 (0.00)	0 (0.00)	0 (0.00)
Lifestyle				
Smoking [n (%)]				
Non-smoker	27 (23.9)	72 (59.5)	25 (39.7)	47 (81.0)
Current smoker	35 (31.0)	31 (25.6)	23 (36.5)	8 (13.8)
Former smoker	51 (45.1)	18 (14.9)	15 (23.8)	3 (5.2)
Alcohol consumer [n (%)]	87 (77.0)	36 (29.8)	27 (42.9)	9 (15.5)
Women reproductive health				
Oral contraceptives [n (%)]		48 (39.7)	36 (57.1)	12 (20.7)
Number of children [n (%)]				
No children		18 (14.9)	14 (22.2)	4 (6.9)
1-2		52 (43.0)	37 (58.7)	15 (25.9)
3-9		51 (42.1)	12 (19.0)	49 (67.2)
Ever breastfed [n (%)]		86 (71.1)	39 (61.9)	47 (81.0)

BMI: Body mass index. Age is expressed in years and BMI in kg/m^2.

Table S2. Characteristics of study population with measures of o per- and polyfluoroalkyl substances (PFAS) and sex hormone analyses (Subgroup 2) (n=99)

Characteristics [n (%)]	**Men** 48 (48.5)	**Women** 51 (51.5)	**Premenopausal women** 24 (24.2)	**Postmenopausal women** 27 (24.3)
Sociodemographic characteristics				
Age [median (IQR)]	47.0 (34.5-56.3)	49.0 (31.0-61.0)	29.0 (23.8-42.0)	60.0 (55.5-67.5)
BMI [median (IQR)]	27.4 (25.3-30.0)	26.70 (24.1-29.4)	24.1 (22.4-27.4)	27.7 (26.0-30.0)
Education level [n (%)]				
No formal education	11 (22.9)	16 (31.4)	2 (8.3)	14 (51.9)
Primary education	24 (50.0)	23 (45.1)	11 (45.8)	12 (44.4)
≥ Secondary education	13 (27.1)	12 (23.5)	11 (45.8)	1 (3.7)
Occupation				
Manual worker	9 (18.8)	10 (19.6)	8 (33.3)	2 (7.4)
Non-manual worker	37 (77.1)	41 (80.4)	16 (66.7)	25 (92.6)
Retired	2 (4.2)	0 (0.00)	0 (0.00)	0 (0.00)
Lifestyle				
Smoking [n (%)]				
Non-smoker	7 (14.9)	35 (68.6)	10 (41.7)	25 (92.6)
Current smoker	22 (39.6)	10 (19.6)	8 (33.3)	2 (7.4)
Former smoker	19 (39.6)	6 (11.8)	6 (25.0)	0 (0.0)
Alcohol consumer [n (%)]	39 (81.3)	15 (29.4)	10 (41.7)	5 (18.5)
Women reproductive health				
Oral contraceptives [n (%)]		14 (27.5)	11 (45.8)	3 (11.1)
Number of children [n (%)]				
No children		13 (25.5)	10 (41.7)	3 (11.1)
1-2		16 (31.4)	13 (54.2)	3 (11.1)
3-9		22 (43.1)	1 (4.2)	21 (77.8)
Ever breastfed [n (%)]		32 (62.7)	9 (37.5)	23 (85.2)

BMI: Body mass index. Age is expressed in years and BMI in kg/m^2.

Table S3. Persistent Organic Pollutants (POPs) analysed, limit and frequency of detection

	Limit of detection (LOD) (ng/g lipid)	n < LOD	Detected percentage
Organochlorine pesticides (OCPs) and polychlorinated (PCBs) (n = 234)			
PCB-138	0.01	3	98.72
PCB-153	0.03	0	100
PCB-180	0.03	0	100
HCB	0.04	4	98.29
β-HCH	0.04	5	97.86
***p,p'*-DDE**	0.04	0	100
Per- and polyfluoroalkyl substances (PFAS) (n = 99)			
PFOA	0.16	0	100
PFNA	0.16	9	90.91
PFHxS	0.25	17	82.83
PFOS	0.33	0	100
PFDA	0.20	54	45.45
PFUDA	0.20	60	39.39

PCBs: polychlorinated biphenyls; OCPs: organochlorine pesticides; HCB: hexachlorobenzene; β-HCH: beta-hexachlorocyclohexane; p,p'-DDE p,p'-dichlorodiphenyldichloroethylene; PFOA: perfluorooctanoic acid; PFNA: perfluorononanoic acid; PFHxS: perfluorohexane sulfonic acid; PFOS: perfluorooctane sulfonic acid; PFDA: perfluorodecanoic acid; PFUDA: perfluoroundecanoic acid.

Table S4. Adipose tissue concentrations of organochlorine pesticides (OCPs) and polychlorinated (PCBs) (Subgroup 1) (n=234)

PCBs, OCPS concentrations (ng/g lipid) [median (IQR)]	**Men** 113 (48.3)	**Women** 121 (51.7)	**Premenopausal women** 63 (26.9)	**Postmenopausal women** 58 (24.8)
PCB-138	71.93 (24.54-125.50)	100.98 (33.19-154.80)	82.16 (16.28-123.92)	119.86 (44.82-181.46)
PCB-153	209.00 (125.26-330.29)	49.56 (146.03-394.57)	201.92 (115.66-339.10)	282.23 (176.91-487.40)
PCB-180	179.60 (99.69-290.19)	190.56 (109.58-300.73)	170.91 (74.03-257.38)	234.77 (139.96-353.85)
HCB	8.61 (3.51-16.87)	26.00 (8.39-49.90)	13.10 (4.86-35.05)	42.95 (20.39-63.00)
β-HCH	7.33 (1.94-13.48)	16.54 (6.90-27.63)	9.33 (1.10-18.06)	25.94 (16.59-35.62)
***p,p'*-DDE**	63.31 (28.28-171.94)	130.93 (40.31-282.00)	78.86 (29.88-147.63)	177.50 (89.91-367.20)

SD: standard deviation; PCBs: polychlorinated biphenyls; OCPs: organochlorine pesticides; HCB: hexachlorobenzene; β-HCH: beta-hexachlorocyclohexane; p,p'-DDE p,p'-dichlorodiphenyldichloroethylene.

Table S5. Serum concentrations of per- and polyfluoroalkyl substances (PFAS) (Subgroup 2) (n=99)

PFAS concentrations (µg/L) [median (IQR)]	**Men** 48 (48.5)	**Women** 51 (51.5)	**Premenopausal women** 24 (24.2)	**Postmenopausal women** 27 (27.3)
PFOA	1.68 (1.41-2.24)	1.09 (0.81-1.54)	1.07 (0.75-1.29)	1.18 (0.84-1.75)
PFNA	0.39 (0.29-0.49)	0.27 (0.18-0.39)	0.22 (0.15-0.32)	0.38 (0.24-0.48)
PFHxS	0.92 (0.64-1.24)	0.55 (0.17-0.75)	0.27 (0.15-0.57)	0.66 (0.35-1.05)
PFOS	13.76 (9.53-27.16)	9.68 (6.04-16.87)	7.63 (4.63-12.16)	13.80 (8.39-17.55)

SD: standard deviation; PFOA: perfluorooctanoic acid; PFNA: perfluorononanoic acid; PFHxS: perfluorohexane sulfonic acid; PFOS: perfluorooctane sulfonic acid.

Table S6. Associations of PCBs/OCPs (n = 126) and PFAS (n = 48) with sex hormones levels in men. Multivariable robust linear regression analyses.

	Estradiol			FSH		
	β	**SE**	**p-value**	**β**	**SE**	**p-value**
PCB-138	0.0338	0.024	0.161	0.0042	0.0056	0.4517
PCB-153	0.0159	0.0096	0.1100	**0.0042**	**0.0022**	**0.0599***
PCB-180	0.0105	0.0127	0.4109	0.0027	0.0029	0.3510
HCB	-0.0143	0.0783	0.855	-0.133	0.0179	0.4574
β-HCH	-0.0034	0.0941	0.9711	**0.0373**	**0.0212**	**0.0808***
***p,p'*-DDE**	-0.0002	0.0077	0.9829	0.0023	0.0017	0.1805
PFOA	0.4512	2.9224	0.8781	0.1938	0.7926	0.8081
PFNA	11.1179	8.8200	0.2148	3.7531	2.3176	0.1132
PFHxS	2.0623	3.6591	0.5762	**2.1042**	**0.9149**	**0.0263****
PFOS	-0.0787	0.2143	0.7154	0.0687	0.0568	0.2331
	LH			**SHGB**		
PCB-138	-0.0031	0.0082	0.7084	-0.0168	0.0285	0.5558
PCB-153	-0.0003	0.0032	0.9229	0.0053	0.0115	0.6444
PCB-180	-0.0012	0.0043	0.7866	-0.0091	0.0151	0.5492
HCB	0.0147	0.0262	0.5764	0.0165	0.0931	0.8594
β-HCH	-0.0078	0.0315	0.8047	-0.085	0.1114	0.4468
***p,p'*-DDE**	-0.0017	0.0025	0.5166	-0.0122	0.0090	0.1805
PFOA	-1.2788	1.2546	0.3142	4.8417	3.9125	0.2231
PFNA	-4.0380	3.7905	0.2931	18.9983	11.6987	0.1122
PFHxS	-0.8764	1.5874	0.5840	-0.3681	4.9402	0.941
PFOS	-0.1355	0.0900	0.1403	0.3486	0.2793	0.2192
	TT					
PCB-138	**-0.0057**	**0.0032**	**0.0794***			
PCB-153	-0.0016	0.0013	0.2341			
PCB-180	-0.0013	0.0017	0.4544			
HCB	-0.0021	0.0107	0.8419			
β-HCH	0.0059	0.0128	0.6436			
***p,p'*-DDE**	0.0005	0.0010	0.6475			
PFOA	0.2955	0.3146	0.3532			
PFNA	**2.2856**	**0.880**	**0.0131****			
PFHxS	0.4112	0.4026	0.3132			
PFOS	0.0311	0.0224	0.1737			

PCB: polychlorinated biphenyl; OCPs: organochlorine pesticides; PFAS: per- and polyfluoroalkyl substances. SE: standard error; HCB: hexachlorobenzene; β-HCH: beta-hexachlorocyclohexane; p,p'-DDE p,p'-dichlorodiphenyldichloroethylene; PFOA: perfluorooctanoic acid; PFNA: perfluorononanoic acid; PFHxS: perfluorohexane sulfonic acid*; PFOS: perfluorooctane sulfonic acid; FSH: follicle stimulating hormone; LH: luteinizing hormone; SHBG: sex hormone binding globulin; TT: total testosterone. Significant and marginally significant associations are in bold.*

Models were adjusted for age, BMI, smoking habit, alcohol consumption and education level

Continuous variables were entered in the models without any transformation.

**Marginally significant association (<0.1)*

*** Significant association (<0.05)*

Table S7. Associations of PCBs/OCPs (n = 127) and PFAS (n = 51) with sex hormones levels in women. Multivariable robust linear regression analyses.

	Estradiol			FSH		
	β	**SE**	**p-value**	**β**	**SE**	**p-value**
PCB-138	0.0265	0.0476	0.5792	0.0213	0.0254	0.4035
PCB-153	0.0102	0.0194	0.6000	0.0039	0.0102	0.7057
PCB-180	0.0113	0.0262	0.6672	0.0042	0.0138	0.7641
HCB	-0.0700	0.1318	0.5962	-0.058	0.0714	0.4180
β-HCH	0.0171	0.1652	0.9178	0.0399	0.0887	0.6536
***p,p'*-DDE**	0.0237	0.0181	0.1917	0.0014	0.0098	0.8835
PFOA	-6.2366	7.8243	0.4302	1.9360	4.1956	0.6471
PFNA	-28.4110	36.6395	0.4428	4.9932	14.1617	0.7263
PFHxS	-12.7395	9.8260	0.2024	0.5939	3.8210	0.8773
PFOS	-0.2284	0.4126	0.5830	-0.0021	0.1586	0.9896
	LH			**SHGB**		
PCB-138	-0.0034	0.0164	0.8369	-0.0035	0.04	0.9309
PCB-153	-0.0003	0.0066	0.9694	-0.0134	0.0161	0.4075
PCB-180	-0.0075	0.0087	0.3915	-0.0192	0.022	0.3846
HCB	-0.0215	0.0461	0.6413	-0.1054	0.1114	0.3462
β-HCH	0.0373	0.0567	0.5117	-0.1544	0.1399	0.2721
***p,p'*-DDE**	0.0073	0.0061	0.2384	0.0094	0.0150	0.5311
PFOA	-3.4866	2.9174	0.2393	-6.1931	8.3281	0.4615
PFNA	0.6064	14.2839	0.9664	-54.2303	39.9386	0.1823
PFHxS	-4.0154	3.8490	0.3033	-9.8354	11.0592	0.3793
PFOS	0.1234	0.1564	0.4349	0.1707	0.4509	0.7070
	TT					
PCB-138	0.0001	0.0002	0.7392			
PCB-153	<0.0001	0.0001	0.9784			
PCB-180	<0.0001	0.0001	0.7117			
HCB	-0.0002	0.0006	0.7989			
β-HCH	-0.0002	0.0008	0.8390			
***p,p'*-DDE**	-0.0001	0.0001	0.1676			
PFOA	-0.0168	0.0419	0.6904			
PFNA	0.1922	0.1915	0.3218			
PFHxS	0.0182	0.0551	0.7423			
PFOS	-0.0007	0.0023	0.7614			

PCB: polychlorinated biphenyl; OCPs: organochlorine pesticides; PFAS: per- and polyfluoroalkyl substances. SE: standard error; HCB: hexachlorobenzene; β-HCH: beta-hexachlorocyclohexane; p,p'-DDE p,p'-dichlorodiphenyldichloroethylene; PFOA: perfluorooctanoic acid; PFNA: perfluorononanoic acid; PFHxS: perfluorohexane sulfonic acid*; PFOS: perfluorooctane sulfonic acid; FSH: follicle stimulating hormone; LH: luteinizing hormone; SHBG: sex hormone binding globulin; TT: total testosterone.*

Models were adjusted for age, BMI, smoking habit, alcohol consumption, education level, number of children, breastfeeding and use of contraceptives.

Continuous variables were entered in the models without any transformation.

Table S8. Associations PCBs/OCPs (n = 68) and PFAS (n = 24) with sex hormones levels in premenopausal women. Multivariable robust linear regression analyses.

	Estradiol			FSH		
	β	**SE**	**p-value**	**β**	**SE**	**p-value**
PCB-138	**-0.2140**	**0.1169**	**0.0724***	-0.0084	0.0129	0.518
PCB-153	-0.0648	0.0484	0.1863	-0.006	0.0053	0.2558
PCB-180	**-0.1047**	**0.0589**	**0.0810***	-0.0074	0.0065	0.2634
HCB	**-1.8512**	**0.2786**	**<0.0001****	-0.0215	0.0368	0.5619
β-HCH	**-1.7495**	**0.7237**	**0.0190***	0.0030	0.0878	0.9727
***p,p'*-DDE**	0.0068	0.0770	0.9302	0.0029	0.0082	0.7293
PFOA	14.8819	12.6172	0.2611	-1.8656	1.2521	0.162
PFNA	9.3518	56.8588	0.8721	-4.2240	5.3633	0.4462
PFHxS	13.3920	21.3762	0.5427	-2.3210	2.0861	0.2877
PFOS	**1.4298**	**0.6639**	**0.0523***	-0.0009	0.0488	0.9860
	LH			**SHGB**		
PCB-138	-0.0035	0.0143	0.8076	-0.1402	0.0864	0.1102
PCB-153	-0.0034	0.0058	0.5675	-0.0488	0.0355	0.1748
PCB-180	-0.0113	0.0070	0.1111	**-0.0725**	**0.0432**	**0.0991***
HCB	-0.0190	0.0402	0.6396	-0.2784	0.2398	0.2506
β-HCH	-0.0325	0.0947	0.7326	**-1.2459**	**0.5607**	**0.0304****
***p,p'*-DDE**	**0.0156**	**0.0083**	**0.0673***	-0.0690	0.0551	0.2156
PFOA	-3.9854	5.3873	0.4737	-9.6133	33.1323	0.7767
PFNA	-4.9467	22.6829	0.8310	-96.1395	130.5132	0.4755
PFHxS	**-9.9000**	**4.7173**	**0.0577***	-62.8273	41.7140	0.1579
PFOS	0.2729	0.1747	0.1443	0.1529	1.1613	0.8974
	TT					
PCB-138	0.0006	0.0004	0.1748			
PCB-153	**0.0004**	**0.0002**	**0.0365****			
PCB-180	**0.0007**	**0.0002**	**0.0006****			
HCB	0.0007	0.0013	0.5859			
β-HCH	0.0005	0.0030	0.8689			
***p,p'*-DDE**	0.0003	0.0003	0.3555			
PFOA	0.0254	0.1216	0.8383			
PFNA	0.2695	0.4818	0.5862			
PFHxS	0.2245	0.1839	0.2457			
PFOS	-0.0004	0.0043	0.9286			

PCB: polychlorinated biphenyl; OCPs: organochlorine pesticides; PFAS: per- and polyfluoroalkyl substances. SE: standard error; HCB: hexachlorobenzene; β-HCH: beta-hexachlorocyclohexane; p,p'-DDE p,p'-dichlorodiphenyldichloroethylene; PFOA: perfluorooctanoic acid; PFNA: perfluorononanoic acid; PFHxS: perfluorohexane sulfonic acid*; PFOS: perfluorooctane sulfonic acid; FSH: follicle stimulating hormone; LH: luteinizing hormone; SHBG: sex hormone binding globulin; TT: total testosterone. Significant and marginally significant associations are in bold.*

*Marginally significant association (<0.1)

*** Significant association (<0.05)*
Models were adjusted for age, BMI, smoking habit, alcohol consumption, education level, number of children, breastfeeding and use of contraceptives.
Continuous variables were entered in the models without any transformation.

Table S9. Associations of PCBs/OCPs (n = 59) and PFAS (n = 27) with sex hormones levels in postmenopausal women. Multivariable robust linear regression analyses.

	Estradiol			FSH		
	β	**SE**	**p-value**	**β**	**SE**	**p-value**
PCB-138	0.0112	0.0167	0.5074	-0.0103	0.0458	0.8231
PCB-153	-0.0047	0.0072	0.5213	-0.0036	0.0167	0.8288
PCB-180	-0.0009	0.0097	0.9245	-0.0165	0.0244	0.502
HCB	-0.0059	0.0079	0.4584	0.0087	0.0133	0.5151
β-HCH	-0.0289	0.0446	0.5209	-0.1289	0.1116	0.2541
***p,p'*-DDE**	0.016	0.0661	0.8095	-0.0263	0.111	0.814
PFOA	-1.4188	2.6005	0.5929	**8.916**	**4.8484**	**0.0846***
PFNA	-11.4944	13.8533	0.4189	34.7004	26.8668	0.2149
PFHxS	0.5195	3.6273	0.8879	3.4941	7.3699	0.6418
PFOS	-0.1112	0.2068	0.5981	0.1850	0.4194	0.6650
	LH			**SHGB**		
PCB-138	0.0074	0.0294	0.8015	0.0607	0.0386	0.1226
PCB-153	0.0067	0.0123	0.5868	0.015	0.0171	0.3856
PCB-180	0.005	0.0172	0.7715	0.0321	0.0225	0.1602
HCB	0.0113	0.0093	0.2313	0.0178	0.0128	0.1719
β-HCH	0.0084	0.0779	0.9141	0.0114	0.1105	0.918
***p,p'*-DDE**	0.0853	0.0788	0.2845	-0.0796	0.1228	0.5202
PFOA	-3.4491	3.8662	0.3855	-23.6556	42.2410	0.5832
PFNA	-5.0828	19.2406	0.7950	-10.4052	40.9552	0.8027
PFHxS	-2.2519	5.0083	0.6590	-8.9477	10.6431	0.4129
PFOS	-0.4120	0.2944	0.1807	-0.1016	0.6245	0.8728
	TT					
PCB-138	0.000	0.000	0.651			
PCB-153	0.000	0.000	0.899			
PCB-180	0.000	0.000	0.794			
HCB	0.000	0.000	0.257			
β-HCH	0.000	0.001	0.871			
***p,p'*-DDE**	0.001	0.001	0.548			
PFOA	-0.0143	0.0304	0.6455			
PFNA	0.0763	0.1494	0.6167			
PFHxS	-0.0162	0.0410	0.6982			
PFOS	0.0018	0.0019	0.3593			

PCB: polychlorinated biphenyl; OCPs: organochlorine pesticides; PFAS: per- and polyfluoroalkyl substances. SE: standard error; HCB: hexachlorobenzene; β-HCH: beta-hexachlorocyclohexane; p,p'-DDE p,p'-dichlorodiphenyldichloroethylene; PFOA: perfluorooctanoic acid; PFNA: perfluorononanoic acid; PFHxS: perfluorohexane sulfonic acid*; PFOS: perfluorooctane sulfonic acid; FSH: follicle stimulating hormone; LH: luteinizing hormone; SHBG: sex hormone binding globulin; TT: total testosterone. Significant associations are in bold.*
**Marginally significant association (<0.1)*

*** Significant association (<0.05)*
Models were adjusted for age, BMI, smoking habit, alcohol consumption, education level, number of children, breastfeeding and use of contraceptives.
Continuous variables were entered in the models without any transformation.

Table S10. Associations of the mixture of Organochlorine Pesticides (OCPs) and Polychlorinated Biphenyls (PCBs) with sex hormones levels in multipollutant analyses obtained by weighted quantile sum (WQS) models. Positive model.

	Men (n=113)		**Women (n=121)**	
	β (SE)	**p-value**	**β (SE)**	**p-value**
Estradiol	37.2171 (24.2792)	0.1290	-0.2071 (6.9430)	0.4295
FSH	**3.1809 (1.2659)**	**0.0139**	1.7931 (4.1933)	0.6700
LH	-0.4121 (1.3799)	0.7660	2.1457 (1.6694)	0.2022
SHBG	-1.2770 (0.7048)	0.7048	<u>-9.0075 (5.2493)</u>	<u>0.0899</u>
TT	3.1293 (0.3373)	0.6940	**0.4653 (0.1361)**	**<0.0001**
	Premenopausal women (n=63)		**Postmenopausal women (n=58)**	
	β (SE)	**p-value**	**β (SE)**	**p-value**
Estradiol	-5.522 (18.9330)	0.7720	1.4188 (6.5452)	0.8300
FSH	0.5367 (3.0148)	0.8596	6.7377 (6.1607)	0.2816
LH	0.6393 (2.3505)	0.7870	3.3117 (3.0523)	0.2860
SHBG	-12.5024 (9.7360)	0.2065	-0.7614 (5.571)	0.8920
TT	0.2433 (0.2721)	0.3766	-0.1690 (0.2018)	0.4000

SE: standard error; FSH: follicle stimulating hormone; LH: luteinizing hormone; SHBG: sex hormone binding globulin; TT: total testosterone. Significant associations are in bold. Marginally significant associations are underlined.

Table S11. Associations of the mixture of Organochlorine Pesticides (OCPs) and Polychlorinated Biphenyls (PCBs) with sex hormones levels in multipollutant analyses obtained by weighted quantile sum (WQS) models. Negative model.

	Men (n=113)		**Women (n=121)**	
	β (SE)	**p-value**	**β (SE)**	**p-value**
Estradiol	3.5006 (3.9820)	0.3820	5.2635 (7.6439)	0.4930
FSH	<u>2.2516 (1.3044)</u>	<u>0.0881</u>	0.4273 (3.6877)	0.9080
LH	-0.1437 (1.3294)	0.9140	2.1824 (1.8343)	0.2375
SHBG	-4.3695 (3.3676)	0.1981	<u>-9.7114 (5.5389)</u>	<u>0.0832</u>
TT	-0.1377 (0.3361)	0.6830	**0.3185 (0.1410)**	**0.0264**
	Premenopausal women (n=63)		**Postmenopausal women (n=58)**	
	β (SE)	**p-value**	**β (SE)**	**p-value**
Estradiol	-1.382 (18.1980)	0.8790	1.2389 (0.1930)	0.8480
FSH	1.1396 (2.6660)	0.67133	6.3788 (6.145)	0.3063
LH	0.6043 (2.2757)	0.7919	2.6014 (2.9978)	0.391
SHBG	-12.3197 (9.6237)	0.2079	-1.3078 (5.4498)	0.8117
TT	0.2549 (0.2747)	0.3590	-0.1459 (0.2013)	0.4733

SE: standard error; FSH: follicle stimulating hormone; LH: luteinizing hormone; SHBG: sex hormone binding globulin; TT: total testosterone. Significant associations are in bold. Marginally significant associations are underlined.

Data S1. Original R script.

#Libraries:

```
library(MASS)
library(dplyr)
library(car)
library(haven)
library(ggplot2)
library(gWQS)
library(mgcv)

data <- read_sav("Hormones.sav")
data <- subset(data, !is.na(fsh) | !is.na(lh) | !is.na(estradiol) | !is.na(shbg) | !is.na(tt))

data <- subset(data, !is.na(pcb138_lip) | !is.na(pcb153_lip) | !is.na(pcb180_lip)| !is.na(hcb_lip) | !is.na(dde_lip) | !is.na(hch_lip) | !is.na(pfoa) | !is.na(pfna)  !is.na(pfhxs) | !is.na(brpfos))

var_fac <- c("smoke_3cat", "alcohol", "study_level", "sex", "menstruation", "nchildren", "breast", "cs_rec", "anticonc")
```

```
data <- data %>%
  mutate_at(vars(all_of(var_fac)), factor)

variables <- c("pcb138_lip", "pcb153_lip","pcb180_lip","hcb_lip","dde_lip", "hch_lip", "pfoa", "pfna", "pfhxs" , "brpfos",  "fsh", "estradiol", "lh", "shbg", "tt", "age_rec", "bmi", "smoke_3cat", "alcohol", "study_level", "sex", "menstruation", "nchildren", "breast", "cs_rec", "anticonc")
pollutants <- c("pcb138_lip", "pcb153_lip","pcb180_lip","hcb_lip","dde_lip", "hch_lip", "pfoa", "pfna", "pfhxs", "brpfos")

pcbs_ocps_vars <- c("pcb138_lip", "pcb153_lip","pcb180_lip","hcb_lip","dde_lip", "hch_lip")
pfas_vars <- c("pfoa", "pfna", "pfhxs", "brpfos")

hormones <- c("fsh", "estradiol", "lh", "shbg", "tt")

data <- data %>%
  mutate_at(vars(all_of(pollutants)), as.numeric)

data <- data %>%
  mutate_at(vars(all_of(hormones)), as.numeric)

data.r <- data %>% dplyr::select(all_of(variables))
rm(data)

data.r <- subset(data.r, !is.na(data.r$sex))

#Population groups:
men <- subset(data.r, sex == 2)
women <- subset(data.r, sex == 1)
women_pre <- subset(data.r, sex == 1 & menstruation == 1)
women_post <- subset(data.r, sex == 1 & menstruation == 0)

#Pollutants groups:
pcbs_ocps <- subset(data.r, hch_lip >= 0)
pfas <- subset(data.r, brpfos >= 0)

#PCBs and OCPS and PFAS subgroup:

subgroup  <- subset(data.r, !is.na(pcb138_lip) & !is.na(pcb153_lip) & !is.na(pcb180_lip)& !is.na(hcb_lip) & !is.na(dde_lip) & !is.na(hch_lip) & !is.na(alfa_hch_det) & !is.na(pfoa) & !is.na(pfna) & !is.na(pfhxs) & !is.na(brpfos))
```

GAM models

```
gam_plots <- function(hormone, data, pollutants) {
  for (pollutant in pollutants) {
    p <- ggplot(data, aes_string(x = pollutant, y = hormone)) +
      geom_point(alpha = 0.3) +
      geom_smooth(method = "loess", color = "firebrick", se = FALSE) +
      ggtitle(pollutant) +
      xlab("") +
      ylab("") +
```

```r
    theme_minimal() +
    theme(
      axis.text = element_blank(),
      axis.ticks = element_blank()
    )

  graficos[[length(graficos) + 1]] <- p
 }

 final_plot <- wrap_plots(graficos, ncol = length(pollutants))
 print(final_plot)
}


gam_plots(hormones, men, pollutants)
gam_plots(hormones, women, pollutants)
gam_plots(hormones, women_pre, pollutants)
gam_plots(hormones, women_post, pollutants)
```

Outlier detection

```r
out_inf <- function(hormones, data, pollutants,
                    extra_formula = "") {


 outliers_lista <- list()

 for (hormone in hormones) {
  outliers_lista[[hormone]] <- list()

  for (pollutant in pollutants) {

    formula_lm <- as.formula(paste0(hormone, " ~ ", pollutant, " ", extra_formula))
    modelo <- lm(formula_lm, data = data)

    car::influencePlot(modelo,
          main = paste("Influence Plot:", hormone, "~", pollutant),
          sub = extra_formula,
          col = "red", pch = 19)

    infl_data <- influence.measures(modelo)
    infl_obs <- which(apply(infl_data$is.inf, 1, any))
    outliers_lista[[hormone]][[pollutant]] <- data[infl_obs, ]
  }
 }

 return(outliers_lista)
}
```

outliers_detectados <- out_inf(hormones, men, pollutants, extra_formula = "+ age_rec + smoke_3cat + bmi + alcohol + study_level")
outliers_detectados_PFAS <- out_inf(hormones, men, pollutants, extra_formula = "+ age_rec + smoke_3cat + alcohol + study_level")
outliers_detectados <- out_inf(hormones, women, pollutants, extra_formula = "+ age_rec + smoke_3cat + bmi + breast + nchildren + alcohol + study_level + anticonc")
outliers_detectados_PFAS <- out_inf(hormones, women, pollutants, extra_formula = "+ age_rec + smoke_3cat + breast + nchildren + alcohol + study_level + anticonc")
outliers_detectados <- out_inf(hormones, women_pre, pollutants, extra_formula = "+ age_rec + smoke_3cat + bmi + breast + nchildren + alcohol + study_level + anticonc")
outliers_detectados_PFAS <- out_inf(hormones, women_pre, pollutants, extra_formula = "+ age_rec + smoke_3cat + breast + nchildren + alcohol + study_level + anticonc")
outliers_detectados <- out_inf(hormones, women_post, pollutants, extra_formula = "+ age_rec + smoke_3cat + bmi + breast + nchildren + alcohol + study_level + anticonc")
outliers_detectados_PFAS <- out_inf(hormones, women_post, pollutants, extra_formula = "+ age_rec + smoke_3cat + breast + nchildren + alcohol + study_level + anticonc")

 # Robust linear regression models.

Men. Adjusted for age, smoke habit, bmi, alcohol consumption and study level.

```
mod_rob_h <- function(data, hormone, pollutant) {
  data <- droplevels(data)
  formula <- as.formula(paste(hormone, "~", pollutant, "+ age_rec + smoke_3cat + bmi + alcohol
+ study_level"))
  modelo_robusto <- rlm(formula, method = c("MM"), maxit = 50,
  model = TRUE, x.ret = TRUE, y.ret = FALSE, contrasts = NULL, data = data)
  coeficientes_robusto <- as.data.frame(summary(modelo_robusto)$coefficients)
  coeficientes_robusto$p_value <- 2 * (1 - pt(q = abs(coeficientes_robusto$`t value`), df =
nrow(data) - length(coef(modelo_robusto))))

    return(list(
      modelo_robusto = modelo_robusto,
      coeficientes_robusto = coeficientes_robusto
    ))
  }

comparar_modelos <- function(data, hormones, pollutants) {
  resultados <- list()

  for (hormone in hormones) {
    for (pollutant in pollutants) {
      if (hormone %in% colnames(data) && pollutant %in% colnames(data)) {
        resultado_original <- mod_rob_h(data, hormone, pollutant)

        resultados[[paste(hormone, pollutant)]] <- list(
          original = resultado_original
        )

        if (!is.null(resultado_original$modelo_robusto)) {
          cat("\nModelo Robusto Original para:", hormone, "con", pollutant, "\n")
```

```
          print(summary(resultado_original$modelo_robusto))
          cat("\nCoeficientes del Modelo Robusto:\n")
          print(resultado_original$coeficientes_robusto)
        }
      }
    }
  }

  return(resultados)
}

evaluar_significancia <- function(p_vals) {
  significancia <- character(length(p_vals))

  for (i in seq_along(p_vals)) {
    p <- p_vals[i]
    if (p < 0.05) {
      significancia[i] <- "Sig"
    } else if (p < 0.1) {
      significancia[i] <- "Border"
    } else {
      significancia[i] <- "No sig"
    }
  }

  return(significancia)
}

extraer_coeficientes <- function(resultados_modelos, pollutants) {
  coeficientes <- data.frame()

  for (nombre in names(resultados_modelos)) {
    partes <- unlist(strsplit(nombre, " "))
    hormone <- partes[1]
    pollutant <- partes[2]

    if (pollutant %in% pollutants) {
      robusto <- resultados_modelos[[nombre]]$original$coeficientes_robusto

      if (!is.null(robusto) && pollutant %in% rownames(robusto)) {
        liliefors_robusto <- resultados_modelos[[nombre]]$original$liliefors_test_robusto
        coeficientes <- rbind(coeficientes, data.frame(
          Modelo = "Robust",
          Hormone = hormone,
          Pollutant = pollutant,
          Estimate = robusto[pollutant, "Value"],
          `P-valor` = robusto[pollutant, "p_value"],
          Sig = evaluar_significancia(robusto[pollutant, "p_value"])
        ))
      }
    }
```

```
  }

  return(coeficientes)
}

resultados_modelos <- comparar_modelos(men, hormones, pollutants)
resultados_modelos

table_coeficientes <- extraer_coeficientes(resultados_modelos, pollutants)
```

PFAs in men. Adjusted for age, smoke habit, alcohol consumption and study level.

```
mod_rob_h <- function(data, hormone, pollutant) {
  data <- droplevels(data)
  formula <- as.formula(paste(hormone, "~", pollutant, "+ age_rec + smoke_3cat + alcohol + study_level"))
  modelo_robusto <- rlm(formula, method = c("MM"), maxit = 50,
  model = TRUE, x.ret = TRUE, y.ret = FALSE, contrasts = NULL, data = data)
  coeficientes_robusto <- as.data.frame(summary(modelo_robusto)$coefficients)
  coeficientes_robusto$p_value <- 2 * (1 - pt(q = abs(coeficientes_robusto$`t value`), df = nrow(data) - length(coef(modelo_robusto))))

    return(list(
      modelo_robusto = modelo_robusto,
      coeficientes_robusto = coeficientes_robusto
    ))
  }


comparar_modelosPFAS <- function(data, hormones, pollutants) {
  resultados <- list()

  for (hormone in hormones) {
    for (pollutant in pollutants) {
      if (hormone %in% colnames(data) && pollutant %in% colnames(data)) {
        resultado_original <- mod_rob_h(data, hormone, pollutant)

        resultados[[paste(hormone, pollutant)]] <- list(
          original = resultado_original
        )


        if (!is.null(resultado_original$modelo_robusto)) {
          cat("\nModelo Robusto Original para:", hormone, "con", pollutant, "\n")
          print(summary(resultado_original$modelo_robusto))
          cat("\nCoeficientes del Modelo Robusto:\n")
          print(resultado_original$coeficientes_robusto)
        }
      }
    }
  }
```

```
  return(resultados)
}



men_pfas <- subset(men, !is.na(men$brpfos))
resultados_modelos <- comparar_modelosPFAS(men_pfas, hormones, pfas_vars)
resultados_modelos
table_coeficientes <- extraer_coeficientes(resultados_modelos, pollutants)
```

Women. Adjusted for age, smoke habit, bmi, alcohol consumption, study level, breastfeeding, number of children and contraceptive use.

```
## Total women
mod_rob_m <- function(data, hormone, pollutant) {
  data <- droplevels(data)
  formula <- as.formula(paste(hormone, "~", pollutant, "+ age_rec + smoke_3cat + bmi + breast+ nchildren_cat + alcohol + study_level + anticonc"))
  modelo_robusto <- rlm(formula, method = c("MM"), maxit = 50,
  model = TRUE, x.ret = TRUE, y.ret = FALSE, contrasts = NULL, data = data)
  coeficientes_robusto <- as.data.frame(summary(modelo_robusto)$coefficients)
  coeficientes_robusto$p_value <- 2 * (1 - pt(q = abs(coeficientes_robusto$`t value`), df = nrow(data) - length(coef(modelo_robusto))))

    return(list(
      modelo_robusto = modelo_robusto,
      coeficientes_robusto = coeficientes_robusto
    ))
  }

comparar_modelos <- function(data, hormones, pollutants) {
  resultados <- list()

  for (hormone in hormones) {
    for (pollutant in pollutants) {
      if (hormone %in% colnames(data) && pollutant %in% colnames(data)) {
        resultado_original <- mod_rob_m(data, hormone, pollutant)

        resultados[[paste(hormone, pollutant)]] <- list(
          original = resultado_original
        )

        if (!is.null(resultado_original$modelo_robusto)) {
          cat("\nModelo Robusto Original para:", hormone, "con", pollutant, "\n")
          print(summary(resultado_original$modelo_robusto))
          cat("\nCoeficientes del Modelo Robusto:\n")
          print(resultado_original$coeficientes_robusto)
        }
      }
```

```
    }
  }

  return(resultados)
}


resultados_modelos <- comparar_modelos(women, hormones, pollutants)
resultados_modelos
table_coeficientes <- extraer_coeficientes(resultados_modelos, pollutants)
```

PFAs in women. Adjusted for age, smoke habit, alcohol consumption, study level, breastfeeding, number of children and contraceptive use.

```
mod_rob_m <- function(data, hormone, pollutant) {
  data <- droplevels(data)
  formula <- as.formula(paste(hormone, "~", pollutant, "+ age_rec + smoke_3cat + breast + nchildren + alcohol + study_level + anticonc"))
  modelo_robusto <- rlm(formula, method = c("MM"), maxit = 50,
  model = TRUE, x.ret = TRUE, y.ret = FALSE, contrasts = NULL, data = data)
  coeficientes_robusto <- as.data.frame(summary(modelo_robusto)$coefficients)
  coeficientes_robusto$p_value <- 2 * (1 - pt(q = abs(coeficientes_robusto$`t value`), df = nrow(data) - length(coef(modelo_robusto))))

    return(list(
      modelo_robusto = modelo_robusto,
      coeficientes_robusto = coeficientes_robusto
    ))
  }
comparar_modelosPFAS <- function(data, hormones, pollutants) {
  resultados <- list()

  for (hormone in hormones) {
    for (pollutant in pollutants) {
      if (hormone %in% colnames(data) && pollutant %in% colnames(data)) {
        resultado_original <- mod_rob_m(data, hormone, pollutant)

        resultados[[paste(hormone, pollutant)]] <- list(
          original = resultado_original
        )


        if (!is.null(resultado_original$modelo_robusto)) {
          cat("\nModelo Robusto Original para:", hormone, "con", pollutant, "\n")
          print(summary(resultado_original$modelo_robusto))
          cat("\nCoeficientes del Modelo Robusto:\n")
          print(resultado_original$coeficientes_robusto)
        }
      }
    }
  }
```

```
  return(resultados)
}

women_pfas <- subset(women, !is.na(women$brpfos))
resultados_modelos <- comparar_modelosPFAS(women_pfas, hormones, pfas_vars)
resultados_modelos
```

Premenopausal women. Adjusted for age, smoke habit, bmi, alcohol consumption, study level, breastfeeding, number of children and contraceptive use.

```
resultados_modelos <- comparar_modelos(women_pre, hormones, pollutants)
resultados_modelos

table_coeficientes <- extraer_coeficientes(resultados_modelos, pollutants)
```

PFAs in premenopausal women. Adjusted for age, smoke habit, alcohol consumption, study level, breastfeeding, number of children and contraceptive use.

```
women_pre_pfas <- subset(women_pre, !is.na(women_post$brpfos))
resultados_modelos <- comparar_modelos(women_pre_pfas, hormones, pfas_vars)
resultados_modelos
```

Postmenopausal women. Adjusted for age, smoke habit, bmi, alcohol consumption, study level, breastfeeding, number of children and contraceptive use.

```
resultados_modelos <- comparar_modelos(women_post, hormones, pcbs_ocps_vars)
resultados_modelos

table_coeficientes <- extraer_coeficientes(resultados_modelos, pollutants)
```

PFAs in postmenopausal women. Adjusted for age, smoke habit, alcohol consumption, study level, breastfeeding, number of children and contraceptive use.

```
women_post_pfas <- subset(women_post, !is.na(women_post$brpfos))
resultados_modelos <- comparar_modelos(women_post_pfas, hormones, pfas_vars)
resultados_modelos


table_coeficientes <- extraer_coeficientes(resultados_modelos, pollutants)
```

WQS men:

Positive:

```
wqs_fun_pos <- function(data, hormones, mix_name) {
  resultados <- list()

  for (hormone in hormones) {
```

```
    formula <- as.formula(paste(hormone, "~ wqs + age_rec + smoke_3cat + bmi + alcohol +
study_level"))


    model <- gwqs(formula,
                  mix_name = mix_name,
                  data = data,
                  q = 4,
                  b = 100,
                  b1_pos = TRUE,
                  validation = 0.75,
                  family = gaussian,
                  b_constr = FALSE,
                  zero_infl = FALSE,
                  seed = 2016,
                  plots = TRUE,
                  tables = TRUE)

    resumen <- summary(model)
    pesos_finales <- model$final_weights
    intervalos <- confint(model)
    resultado <- list(
      hormone = hormone,
      resumen = resumen,
      pesos_finales = pesos_finales,
      intervalos = intervalos
    )
    resultados[[hormone]] <- resultado
    gwqs_barplot(model)
  }
  return(resultados)
}

resultados_men <- wqs_fun_pos(men, hormones, pcbs_ocps_vars)
resultados_men
```

Negative:

```
wqs_fun_neg <- function(data, hormones, mix_name) {
  resultados <- list()
  for (hormone in hormones) {
    formula <- as.formula(paste(hormone, "~ wqs + age_rec + smoke_3cat + bmi + alcohol +
study_level"))

    model <- gwqs(formula,
                  mix_name = mix_name,
                  data = data,
                  q = 4,
                  b = 100,
                  b1_pos = FALSE,
```

```
              validation = 0.75,
              family = gaussian,
              b_constr = FALSE,
              zero_infl = FALSE,
              seed = 2016,
              plots = TRUE,
              tables = TRUE)
    resumen <- summary(model)
    pesos_finales <- model$final_weights
    intervalos <- confint(model)

    resultado <- list(
      hormone = hormone,
      resumen = resumen,
      pesos_finales = pesos_finales,
      intervalos = intervalos
    )
    resultados[[hormone]] <- resultado
    gwqs_barplot(model)
  }
  return(resultados)
}

resultados_men <- wqs_fun_neg(men, hormones, pcbs_ocps_vars)
```

WQS women:

Positive:

```
wqs_fun_pos <- function(data, hormones, mix_name) {
  resultados <- list()
  for (hormone in hormones) {
    formula <- as.formula(paste(hormone, "~ wqs + age_rec + smoke_3cat + bmi + breast+
nchildren_cat + alcohol + study_level + anticonc"))

    model <- gwqs(formula,
              mix_name = mix_name,
              data = data,
              q = 4,
              b = 100,
              b1_pos = TRUE,
              validation = 0.75,
              family = gaussian,
              b_constr = FALSE,
              zero_infl = FALSE,
              seed = 2016,
              plots = TRUE,
              tables = TRUE)

    resumen <- summary(model)
    pesos_finales <- model$final_weights
```

```
    intervalos <- confint(model)
    resultado <- list(
      hormone = hormone,
      resumen = resumen,
      pesos_finales = pesos_finales,
      intervalos = intervalos
    )
    resultados[[hormone]] <- resultado
    gwqs_barplot(model)
  }
  return(resultados)
}

resultados_women_pos <- wqs_fun_pos(women, hormones, pcbs_ocps_vars)
```

Negative:

```
wqs_fun_neg <- function(data, hormones, mix_name) {
  resultados <- list()
  for (hormone in hormones) {
    formula <- as.formula(paste(hormone, "~ wqs + age_rec + smoke_3cat + bmi + breast+
nchildren_cat + alcohol + study_level + anticonc"))

    model <- gwqs(formula,
              mix_name = mix_name,
              data = data,
              q = 4,
              b = 100,
              b1_pos = FALSE,
              validation = 0.75,
              family = gaussian,
              b_constr = FALSE,
              zero_infl = FALSE,
              seed = 2016,
              plots = TRUE,
              tables = TRUE)

    resumen <- summary(model)
    pesos_finales <- model$final_weights
    intervalos <- confint(model)
    resultado <- list(
      hormone = hormone,
      resumen = resumen,
      pesos_finales = pesos_finales,
      intervalos = intervalos
    )
    resultados[[hormone]] <- resultado
    gwqs_barplot(model)
  }

  return(resultados)
```

}

resultados_women_neg <- wqs_fun_neg(women, hormones, pcbs_ocps_vars)

WQS premenopausal women:

resultados_women_pre_pos <- wqs_fun_pos(women_pre, hormones, pcbs_ocps_vars)

resultados_women_pre_neg <- wqs_fun_neg(women_pre, hormones, pcbs_ocps_vars)

WQS postmenopausal women:

resultados_women_pos_pos <- wqs_fun_pos(women_post, hormones, pcbs_ocps_vars)

resultados_women_pos_neg <- wqs_fun_neg(women_post, hormones, pcbs_ocps_vars)