

## Supplementary Material

### **Kisspeptin as potential biomarker of environmental chemical mixture effect on reproductive hormone profile: a pilot study in adolescent males**

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**Table S1.** Distribution of LOD-LOQ values of urinary environmental contaminants measured in adolescent males from the INMA-Granada cohort (n=133)

**Table S2.** Primer and probe information for real-time PCR and pyrosequencing

**Table S3.** Spearman’s correlation analyses of environmental chemical contaminants

**Table S4.** Associations between the joint effect of environmental chemical mixtures with kisspeptin and hormones biomarkers (n=133)

**Table S5.** Association between kisspeptin effect biomarkers and hormone concentrations (n=133)

**Figure S1.** Flow-chart showing the time-line of follow-ups conducted in the INMA-Granada cohort and the final sample of 15-17-year-old adolescents in the present study.

**Figure S2.** Correlation analyses among kisspeptin effect biomarkers (n=133).

### **Sensitivity Analyses**

**Table S6.** Associations between individual environmental chemicals and kisspeptin biomarkers without adjusting for Tanner stage (n=133)

**Table S7.** Associations between the joint effect of environmental chemical mixtures with kisspeptin and hormones biomarkers without adjusting for Tanner stage (n=133)

**Table S8.** Associations between kisspeptin biomarkers and serum hormone levels without adjusting for Tanner stage (n=133)

**Table S9.** Associations between individual environmental chemicals and kisspeptin biomarkers without multiple imputation (n=104 for serum kiss43; n= 117 for KISS1 DNA methylation)

**Table S10.** Associations between the joint effect of environmental chemical mixtures with kisspeptin and hormones biomarkers without

**Table S11.** Associations between kisspeptin biomarkers and serum hormone levels without multiple imputation (n=104 for serum kiss43; n= 117 for KISS1 DNA methylation) multiple imputation (n=104 for serum kiss43; n= 117 for KISS1 DNA methylation)

### **Sociodemographic characteristics across INMA-Granada follow-ups**

**Table S12.** General characteristics of study participants at follow-ups

**Table S1. Distribution of LOD-LOQ values of urinary environmental contaminants and normal values for hormonal levels measured in adolescent males from the INMA-Granada cohort (n=130)**

<b>Environmental chemicals</b>	<b>BPA</b>	<b>MPB</b>	<b>BP3</b>	<b>As</b>	<b>Cd</b>	<b>IMPy</b>	<b>MDA</b>	<b>DCCA</b>	<b>ETU</b>
LOD (ng/mL)	0.1	0.1	0.1	0.60	0.01	0.117	0.052	0.055	0.072
LOQ (ng/mL)	0.3	0.3	0.3	1.81	0.04	0.391	0.172	0.184	0.241
<b>Reproductive hormone levels reference values</b>									
<b>Hormones</b>	<b>T (nmol/L)</b>	<b>DHEAS (µmol/L)</b>	<b>E2 (pmol/L)</b>	<b>SHBG (nmol/L)</b>	<b>LH (mU/L)</b>	<b>FSH (mU/L)</b>			
Reference value (lower-upper limits)	11.1-26.9	2.3-10	<10-82	10.46-75.25	1.7-8.6	1.54-12.4			

BPA= bisphenol A; MPB= methylparaben; BP3= benzophenone 3; As= arsenic; Cd= cadmium; IMPy= 2-isopropyl-4-methyl-6-hydroxypyrimidine; MDA= malathion dicarboxylic acid; DCCA= 2,2-dichlorovinyl-2,2-dimethylcyclopropane-1-carboxylic acid; ETU= ethylene thiourea. T = testosterone; DHEAS= dehydroepiandrosterone sulphate; E2= estradiol; SHBG= sex hormone binding-globulin; LH= luteinizing hormone; FSH= follicle stimulating hormone. LOD: Limit of detection; LOQ: Limit of quantification; Q-SRM: Selected reaction monitoring; tR: Retention time; RSD= Relative standard deviation; SD= standard deviation.

Reference levels of male reproductive hormones were taken from Howard, 2021; Karbasy et al., 2016; and Yamamoto et al., 1995.

**Table S2. Primer and probe information for real-time PCR and pyrosequencing**

Gene Symbol	Chromosome location	Accession#	Amplicon length	ABI assay ID / part#
<i>CDH1</i>	16q22.1	NM_004360.2	117	Hs00170423_m1
<i>CDH11</i>	16q22.1	NM_001797.2	96	Hs00156438_m1
<i>KISS1</i>	1q32	NM_002256.2	71	Hs00158486_m1
<i>ID2</i>	2p25	NM_002166.4	112	Hs00747379_m1
<i>PLAC1</i>	Xq26	NM_021796.3	81	Hs00222307_m1
<i>ACTB</i>	7p15- p12	NM_001101.2	171	4352935E
<i>TBP</i>	6q27	M55654.1	127	4333769F
<i>HPRT1</i>	Xq26.1	NM_000194.1	100	4333768F

**Primer information for pyrosequencing**

Gene symbol	Chromosome location	Region	Primer type <sup>a</sup>	Primer sequence (5'→3')
<i>APC</i>	5q21-q22	promoter	F	TTTTTTGTTTGTGGGGATTG
			R	5' Biotin- ATCC(A/G)ACAACACCTCCATTCTAT
			S	TTTGTTGGGGATTGG
<i>KISS1</i>	1q32	promoter	F	GGTGTGGAGGATGGAAAGAGT
			R	5' Biotin- TCCCTAATCACATCCCTTTTATAAAA
			S	GGAGAGGTTGTTTTAGTTA
<i>CASP8</i>	2q33-q34	exon 1	F	GGTTATGGAGGGAGGTAGAAGAGTT
			R	5' Biotin- AACAAACACCCAAAAATATCCTCAA
			S	ATTTTTTTTTTTGTTGAGTA
<i>PTPN6</i>	12p13	promoter	F	TTTTTTATTTGGTTTGGGTTATTGTGTA
			R	5' Biotin- TTCACACAC(A/G)TC(A/G)AAATAAACATCA
			S	TTTTTTTTTTTTATAGAGAGATG

<sup>a</sup> F, forward primer; R, reverse primer; S, sequencing primer

Table S3. Spearman's correlation analyses of environmental chemical contaminants

		BPA	MPB	BP3	As	Cd	IMPy	MDA	DCCA	ETU
BPA	Rho	1.00	<b>0.30</b>	<b>0.44</b>	0.04	0.19	-0.10	0.07	0.11	-0.04
	p		<b>0.00</b>	<b>0.00</b>	0.63	0.03	0.23	0.45	0.20	0.67
MPB	Rho	<b>0.30</b>	1.00	<b>0.50</b>	<b>0.15</b>	<b>0.22</b>	-0.03	0.05	0.10	0.06
	p	<b>0.00</b>		<b>0.00</b>	<b>0.09</b>	<b>0.01</b>	0.73	0.55	0.27	0.46
BP3	Rho	<b>0.44</b>	<b>0.50</b>	1.00	<b>0.25</b>	0.08	0.03	0.08	<b>0.17</b>	0.11
	p	<b>0.00</b>	<b>0.00</b>		<b>0.00</b>	0.33	0.71	0.37	<b>0.05</b>	0.21
As	Rho	0.04	<b>0.15</b>	<b>0.25</b>	1.00	<b>0.21</b>	0.08	0.09	<b>0.18</b>	0.06
	p	0.63	<b>0.09</b>	<b>0.00</b>		<b>0.02</b>	0.38	0.31	<b>0.04</b>	0.51
Cd	Rho	<b>0.19</b>	<b>0.22</b>	0.08	<b>0.21</b>	1.00	0.08	0.04	<b>0.20</b>	0.07
	p	<b>0.03</b>	<b>0.01</b>	0.33	<b>0.02</b>		0.36	0.66	<b>0.02</b>	0.43
IMPy	Rho	-0.10	-0.03	0.03	0.08	0.08	1.00	0.09	0.06	0.09
	p	0.23	0.73	0.71	0.38	0.36		0.30	0.46	0.31
MDA	Rho	0.07	0.05	0.08	0.09	0.04	0.09	1.00	0.02	0.02
	p	0.45	0.55	0.37	0.31	0.66	0.30		0.78	0.85
DCCA	Rho	0.11	0.10	<b>0.17</b>	<b>0.18</b>	<b>0.20</b>	0.06	0.02	1.00	0.08
	p	0.20	0.27	<b>0.05</b>	<b>0.04</b>	<b>0.02</b>	0.46	0.78		0.38
ETU	Rho	-0.04	0.06	0.11	0.06	0.07	0.09	0.02	0.08	1.00
	p	0.67	0.46	0.21	0.51	0.43	0.31	0.85	0.38	

BPA= bisphenol A; MPB= methylparaben; BP3= benzophenone 3; As= arsenic; Cd= cadmium; IMPy= 2-isopropyl-4-methyl-6-hydroxypyrimidine; MDA= malathion dicarboxylic acid; DCCA= 2,2-dichlorovinyl-2,2-dimethylcyclopropane-1-carboxylic acid; ETU= ethylene thiourea.

**Table S4. Associations between the joint effect of environmental chemical mixtures with kisspeptin and hormones biomarkers (n=133)**

	Kiss54			CpG1			CpG2			CpG3			CpG4			CpGt		
	%	LL	UL	%	LL	UL	%	LL	UL	%	LL	UL	%	LL	UL	%	LL	UL
<b>Phenols</b>	2.36	-1.38	6.25	-0.88	-2.59	0.85	-2.24	-5.59	1.22	-0.57	-2.28	1.17	-2.26	-5.52	1.12	-1.32	-3.03	0.43
<b>Metals</b>	1.99	-2.06	6.21	-0.03	-1.96	1.94	-0.31	-4.13	3.66	0.29	-1.62	2.24	0.24	-3.52	4.14	0.11	-1.85	2.11
<b>Pesticides</b>	<b>9.09**</b>	3.29	15.21	-2.07	-4.54	0.47	<b>-5.26*</b>	-9.96	-0.32	-1.16	-3.67	1.42	<b>-6.03*</b>	-10.63	-1.19	<b>-3.34*</b>	-5.81	-0.81
<b>Whole Mixture</b>	<b>11.61**</b>	3.96	19.82	<b>-3.10†</b>	-6.35	0.27	<b>-7.19*</b>	-13.26	-0.71	-1.53	-4.83	1.90	<b>-7.87*</b>	-13.81	-1.52	<b>-4.48*</b>	-7.68	-1.16
	<b>Hormones</b>																	
	T			DHEAS			E2			SHBG			LH			FSH		
	%	LL	UL	%	LL	UL	%	LL	UL	%	LL	UL	%	LL	UL	%	LL	UL
<b>Phenols</b>	11.08	-3.19	27.46	9.72	-2.43	23.38	3.93	-17.81	31.43	<b>15.67*</b>	4.89	27.56	6.62	-8.27	23.93	7.42	-21.03	46.13
<b>Metals</b>	11.97	-4.04	30.66	3.60	-9.20	18.20	-0.66	-23.64	29.24	2.16	-8.75	14.38	7.60	-8.94	27.15	-19.34	-42.78	13.70
<b>Pesticides</b>	6.99	-13.28	32.01	-1.93	-18.00	17.28	14.88	-19.22	63.37	-10.10	-22.74	4.61	<b>-23.34*</b>	-38.63	-4.24	-20.38	-49.69	26.02
<b>Whole Mixture</b>	24.10	-5.98	63.80	14.31	-9.64	44.60	12.85	-29.55	80.78	3.86	-14.67	26.41	-12.75	-35.19	17.45	-23.97	-58.65	39.78

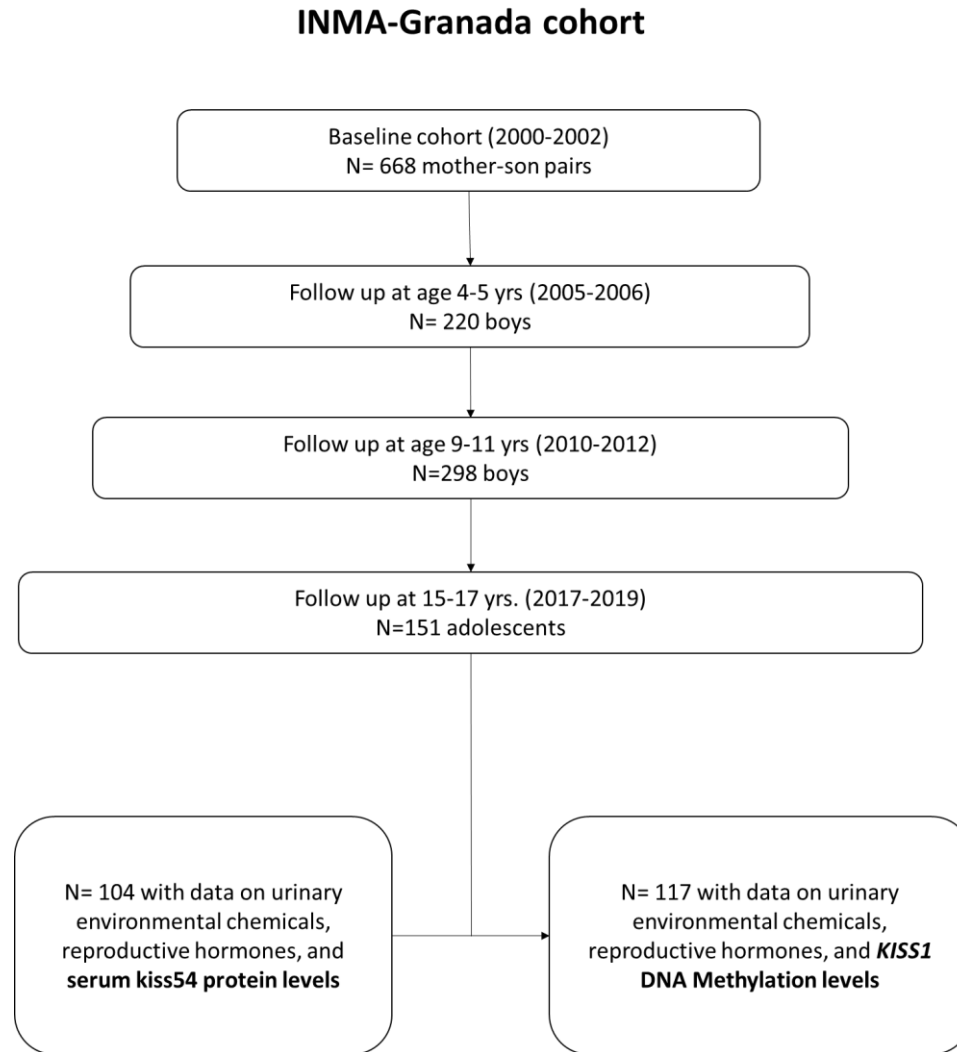
DHEAS= dehydroepiandrosterone sulfate; E2= estradiol; SHBG= sex hormone binding-globulin; FSH= follicle stimulation hormone. Model was adjusted for adolescents' age (continuous), waist to height ratio (continuous), Tanner Stage ( $\leq G4 / > G4$ ), urine creatinine concentrations (continuous), annual family income (<25000/2500-35000/>35000), season of sample collection (spring/summer/autumn/winter). LL: lower limit, and UL: upper limit of the confidence interval (CI).

\*\*p<0.01; \*p<0.05; †p<0.10.

**Table S5. Association between kisspeptin effect biomarkers and hormone concentrations (n=133)**

	TT		DHEAS			E2			SHBG			LH			FSH			
	%Change	95%CI		% Change	95%CI		% Change	95%CI		% Change	95%CI		% Change	95%CI				
		LL	UL		LL	UL		LL	UL		LL	UL		LL	UL			
<b>kiss54</b>	38.08	-28.87	168.04	8.62	-37.51	88.81	-5.13	-68.03	181.58	43.17	-10.00	127.75	<b>131.01*</b>	14.79	364.92	<b>-83.51*</b>	-95.94	-33.02
<b>CpG1</b>	<b>429.72*</b>	27.31	2104.1	-44.93	-83.49	83.72	-40.82	-94.51	537.45	-51.48	-82.44	34.09	185.37	-39.55	1247.08	-68.41	-98.63	627.06
<b>CpG2</b>	-13.86	-58.26	77.76	-28.26	-60.60	30.65	-58.94	-87.31	32.85	<b>-44.01*</b>	-66.04	-7.70	35.66	-37.60	194.91	-37.29	-86.86	199.33
<b>CpG3</b>	-42.86	-86.59	143.36	-23.43	-77.07	155.67	-68.82	-97.07	231.74	44.09	-48.05	299.65	-12.83	-81.67	314.52	49.18	-93.51	3329.02
<b>CpG4</b>	-10.95	-57.74	87.66	-19.08	-56.40	50.18	-58.84	-87.71	37.82	<b>-38.40†</b>	-63.30	3.38	25.23	-43.71	178.60	-3.10	-80.63	384.66
<b>CpGt</b>	1.18	-75.90	324.84	-49.37	-84.53	65.76	<b>-87.14†</b>	-98.73	30.44	<b>-62.77†</b>	-86.23	0.65	96.05	-57.80	810.72	-63.17	-98.33	711.52

DHEAS= dehydroepiandrosterone sulfate; E2= estradiol; SHBG= sex hormone binding-globulin; FSH= follicle stimulation hormone. Model was adjusted for adolescents' age (continuous), waist to height ratio (continuous), Tanner Stage ( $\leq$ G4/ $>$ G4), urine creatinine concentrations (continuous), annual family income (<25000/2500-35000/ $>$ 35000), season of sample collection (spring/summer/autumn/winter). LL: lower limit, and UL: upper limit of the confidence interval (CI). \*p<0.05; †p<0.10



**Figure S1.** Flow-chart showing the time-line of follow-ups conducted in the INMA-Granada cohort and the final sample of 15-17-year-old adolescents in the present study.

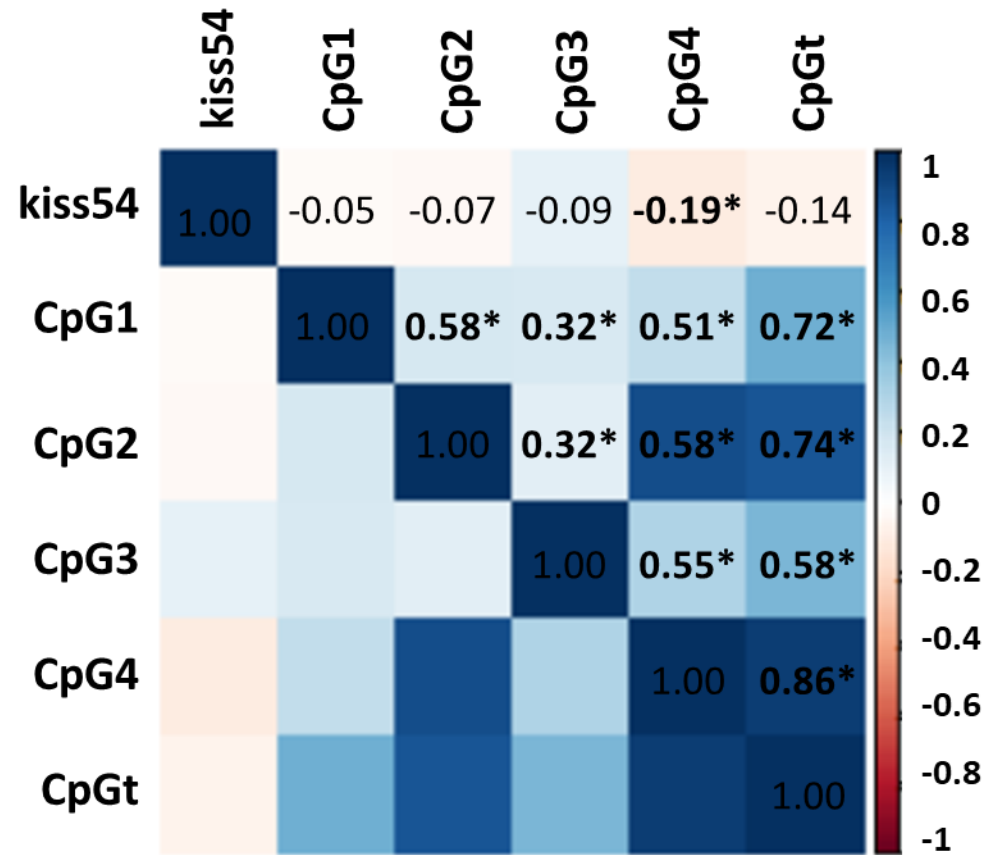


Figure S2. Correlation analyses among kisspeptin effect biomarkers (n=133). Spearman's Rho is showed. \* $p < 0.05$



**Table S6. Associations between individual environmental chemicals and kisspeptin biomarkers without adjusting for Tanner stage (n=133)**

	Kiss54			CpG1			CpG2			CpG3			CpG4			CpGt		
	%Change	95%CI		%Change	95%CI		%Change	95%CI		%Change	95%CI		%Change	95%CI		%Change	95%CI	
		LL	UL		LL	UL		LL	UL		LL	UL		LL	UL		LL	UL
<b>BPA</b>	0.24	-1.17	1.67	<b>-0.54</b> †	-1.18	0.10	-0.53	-1.82	0.79	-0.27	-0.91	0.38	-1.01	-2.25	0.24	<b>-0.58</b> †	-1.23	0.07
<b>MPB</b>	0.78	-0.22	1.79	0.02	-0.44	0.48	-0.50	-1.42	0.43	0.09	-0.37	0.55	-0.17	-1.07	0.73	-0.10	-0.57	0.37
<b>BP3</b>	0.56	-0.56	1.69	-0.27	-0.78	0.25	<b>-1.04</b> *	-2.06	-0.02	-0.27	-0.79	0.24	-0.80	-1.79	0.20	<b>-0.52</b> †	-1.03	0.00
<b>As</b>	<b>1.74</b> †	-0.08	3.60	-0.30	-1.14	0.55	-0.46	-2.15	1.26	-0.47	-1.31	0.37	0.06	-1.57	1.73	-0.24	-1.09	0.62
<b>Cd</b>	<b>-3.93</b> *	-7.37	-0.36	-0.29	-1.98	1.43	-0.23	-3.63	3.28	0.34	-1.36	2.06	-0.65	-3.90	2.72	-0.11	-1.83	1.64
<b>IMPy</b>	0.67	-1.16	2.54	<b>-1.17</b> *	-1.98	-0.35	-1.08	-2.76	0.62	-0.32	-1.16	0.53	-1.33	-2.94	0.29	<b>-0.92</b> *	-1.75	-0.08
<b>MDA</b>	<b>2.98</b> *	0.41	5.61	0.73	-0.45	1.93	<b>2.66</b> *	0.27	5.11	<b>-1.10</b> †	-2.25	0.07	0.69	-1.61	3.04	0.50	-0.70	1.72
<b>DCCA</b>	<b>1.93</b> *	0.45	3.43	-0.48	-1.16	0.21	<b>-1.83</b> *	-3.16	-0.49	0.45	-0.24	1.14	<b>-1.35</b> *	-2.65	-0.04	<b>-0.69</b> *	-1.38	-0.01
<b>ETU</b>	0.86	-0.71	2.45	0.38	-0.34	1.11	-0.59	-2.03	0.88	0.49	-0.23	1.21	-0.07	-1.47	1.35	0.12	-0.62	0.85

kiss54: kisspeptin 54; CpGt: Total DNA methylation [(CpG1 + CpG2 + CpG3 + CpG4)/4]; BPA: Bisphenol A; MPB: methylparaben; BP3: benzophenone 3; As: arsenic (total), Cd: cadmium; IMPy: 2-isopropyl-6-methyl-4-pyrimidinol; MDA: malathion diacid; DCCA: dimethylcyclopropane carboxylic acid; ETU: ethylene thiourea. LL: lower limit, and UL: upper limit of the confidence interval (CI). All models were adjusted for age (continuous), waist-to-height ratio (continuous), urine creatinine concentrations (continuous), annual family income (<25,000/25,000-35,000/>35,000), season of biological sample collection (spring/summer/autumn/winter). \*p<0.05; †p<0.10

**Table S7. Associations between the joint effect of environmental chemical mixtures with kisspeptin and hormones biomarkers without adjusting for Tanner stage (n=133)**

	Kisspeptin effect biomarkers																	
	Kiss54			CpG1			CpG2			CpG3			CpG4			CpGt		
	%	LL	UL	$\beta$	LL	UL	$\beta$	LL	UL	$\beta$	LL	UL	$\beta$	LL	UL	$\beta$	LL	UL
<b>Phenols</b>	1.31	-1.72	4.43	-1.10	-2.74	0.57	-1.87	-5.14	1.51	-0.36	-1.97	1.27	-1.98	-5.09	1.24	-1.18	-2.84	0.52
<b>Metals</b>	1.74	-1.52	5.09	-0.38	-2.18	1.46	0.13	-3.49	3.88	0.49	-1.26	2.27	0.50	-2.97	4.09	0.24	-1.60	2.12
<b>Pesticides</b>	<b>10.41*</b>	5.83	15.20	-1.44	-3.84	1.02	-3.24	-7.90	1.66	-0.73	-3.09	1.69	<b>-4.30†</b>	-8.75	0.37	<b>-2.24†</b>	-4.67	0.26
<b>Whole Mixture</b>	<b>11.60*</b>	5.62	17.93	<b>-2.82†</b>	-5.89	0.36	-4.53	-10.51	1.84	-0.81	-3.88	2.35	<b>-5.91†</b>	-11.56	0.10	<b>-3.20†</b>	-6.30	0.01
	Hormones																	
	T			DHEAS			E2			SHBG			LH			FSH		
	$\beta$	LL	UL	$\beta$	LL	UL	$\beta$	LL	UL	$\beta$	LL	UL	$\beta$	LL	UL	$\beta$	LL	UL
<b>Phenols</b>	6.33	-7.48	22.21	7.64	-3.98	20.66	1.16	-19.50	27.12	<b>15.38*</b>	4.84	26.97	7.74	-7.36	25.30	4.53	-22.74	41.41
<b>Metals</b>	2.47	-11.94	19.22	0.02	-11.69	13.28	-2.04	-23.56	25.55	1.57	-8.74	13.05	6.58	-9.45	25.44	-14.68	-38.57	18.51
<b>Pesticides</b>	2.07	-17.18	25.79	-4.07	-19.18	13.87	18.71	-15.28	66.35	-8.44	-20.91	5.99	<b>-22.94*</b>	-38.19	-3.92	-12.11	-43.79	37.43
<b>Whole Mixture</b>	7.44	-18.30	41.28	6.88	-14.44	33.52	11.69	-28.32	74.05	4.52	-13.30	26.02	-12.69	-34.67	16.68	-14.78	-52.39	52.55

DHEAS= dehydroepiandrosterone sulfate; E2= estradiol; SHBG= sex hormone binding-globulin; FSH= follicle stimulation hormone. LL: lower limit, and UL: upper limit of the confidence interval (CI). Model was adjusted for adolescents' age (continuous), waist to height ratio (continuous), urine creatinine concentrations (continuous), annual family income (<25000/2500-35000/>35000), season of sample collection (spring/summer/autumn/winter). \*\*p<0.01; \*p<0.05; †p<0.10

**Table S8. Associations between kisspeptin biomarkers and serum hormone levels without adjusting for Tanner stage (n=133)**

		T		DHEAS			E2		SHBG		LH		FSH						
		%Change	95%CI		%Change	95%CI		%Change	95%CI		%Change	95%CI		%Change	95%CI				
			LL	UL		LL	UL		LL	UL		LL	UL		LL	UL			
<b>Kiss54</b>	<b>T2</b>	-3.66	-21.85	18.75	-5.78	-20.98	12.35	-1.02	-30.07	40.10	8.14	-6.94	25.68	<b>23.66†</b>	-1.31	54.95	-27.08	-53.91	15.36
	<b>T3</b>	<b>22.38†</b>	-0.95	51.21	-4.41	-19.99	14.21	22.81	-13.56	74.50	9.48	-5.95	27.45	<b>30.17*</b>	3.63	63.51	<b>-38.25*</b>	-61.17	-1.82
<b>CpG1</b>	<b>T2</b>	7.87	-12.42	32.87	-4.53	-19.57	13.31	-4.48	-32.02	34.22	-10.38	-22.53	3.67	<b>40.72*</b>	13.31	74.76	<b>-44.61*</b>	-64.20	-14.32
	<b>T3</b>	7.88	-12.45	32.94	-7.54	-22.13	9.79	-15.19	-39.69	19.25	0.59	-13.07	16.39	16.62	-6.14	44.89	<b>-49.04*</b>	-67.08	-21.09
<b>CpG2</b>	<b>T2</b>	5.27	-14.33	29.36	<b>-17.23*</b>	-29.91	-2.26	-18.21	-41.10	13.56	-7.97	-20.36	6.35	10.50	-11.45	37.90	<b>-37.55*</b>	-59.42	-3.89
	<b>T3</b>	-1.93	-20.67	21.23	<b>-13.66†</b>	-27.24	2.45	<b>-36.40*</b>	-54.63	-10.85	-8.76	-21.38	5.88	8.80	-13.38	36.64	<b>-51.73*</b>	-69.03	-24.79
<b>CpG3</b>	<b>T2</b>	1.11	-17.50	23.91	-11.00	-24.97	5.58	2.55	-27.13	44.31	-0.10	-13.80	15.78	3.75	-17.08	29.82	2.62	-34.41	60.55
	<b>T3</b>	<b>-20.30*</b>	-34.76	-2.63	-7.43	-21.75	9.52	-2.48	-30.33	36.51	-2.91	-16.03	12.26	-7.82	-26.07	14.93	<b>51.29†</b>	-2.61	135.04
<b>CpG4</b>	<b>T2</b>	0.38	-18.29	23.31	-0.75	-16.25	17.61	-21.44	-43.33	8.91	-6.59	-19.27	8.07	<b>9.88†</b>	-12.00	37.20	2.37	-34.68	60.41
	<b>T3</b>	-12.03	-28.57	8.34	-8.64	-23.07	8.49	<b>-40.44*</b>	-57.21	-17.11	-1.30	-14.85	14.40	-3.75	-23.12	20.51	-19.85	-49.13	26.29
<b>CpGt</b>	<b>T2</b>	8.76	-11.78	34.10	-0.31	-16.14	18.50	-4.62	-31.74	33.29	-10.15	-22.46	4.10	0.03	-20.26	25.48	-12.99	-44.81	37.19
	<b>T3</b>	-4.42	-22.35	17.66	-6.28	-21.05	11.26	<b>-33.70*</b>	-52.44	-7.59	-1.79	-15.15	13.66	-3.85	-23.22	20.41	-26.91	-53.48	14.84

T: Total testosterone; DHEAS= dehydroepiandrosterone sulfate; E<sub>2</sub>= estradiol; SHBG= sex hormone binding-globulin; LH=luteinizing hormone; FSH= follicle stimulation hormone; kiss54: kisspeptin 54; CpGt: Total DNA methylation [(CpG1 + CpG2 + CpG3 + CpG4)/4]. LL: lower limit, and UL: upper limit of the confidence interval (CI). Model was adjusted for adolescents' age (continuous), waist to height ratio (continuous), urine creatinine concentrations (continuous), annual family income (<25,000/25,000-35,000/>35,000), season of biological sample collection (spring/summer/autumn/winter). \*p<0.05; †p<0.10

**Table S9. Associations between individual environmental chemicals and kisspeptin biomarkers without multiple imputation (n=104 for serum kiss43; n= 117 for *KISS1* DNA methylation)**

	Kiss54		KISS1 gene DNA methylation															
			CpG1		CpG2		CpG3		CpG4		CpGt							
	%Change	95% CI		%Change	95% CI		%Change	95% CI		%Change	95% CI		%Change	95% CI		%Change	95% CI	
	LL	UP		LL	UP		LL	UP		LL	UP		LL	UP		LL	UP	
<b>BPA</b>	0.07	-1.35	1.51	<b>-0.74</b> †	-1.50	0.02	-0.80	-2.33	0.74	-0.34	-1.10	0.44	<b>-1.41</b> †	-2.84	0.04	<b>-0.79</b> *	-1.54	-0.03
<b>MPB</b>	0.52	-0.51	1.57	0.01	-0.53	0.55	-0.61	-1.67	0.46	0.16	-0.37	0.70	-0.33	-1.35	0.71	-0.13	-0.66	0.41
<b>BP3</b>	0.73	-0.46	1.95	-0.40	-0.98	0.18	<b>-1.32</b> *	-2.45	-0.17	-0.26	-0.84	0.33	<b>-1.11</b> *	-2.20	-0.00	<b>-0.65</b> *	-1.23	-0.08
<b>As</b>	1.29	-0.66	3.27	-0.38	-1.32	0.56	-0.79	-2.64	1.10	-0.52	-1.45	0.41	-0.34	-2.13	1.48	-0.39	-1.32	0.55
<b>Cd</b>	-3.01	-6.67	0.80	-0.10	-1.99	1.82	0.17	-3.58	4.06	0.28	-1.61	2.20	-0.32	-3.90	3.39	0.07	-1.82	1.99
<b>IMPy</b>	<b>1.76</b> †	-0.19	3.75	<b>-1.19</b> *	-2.10	-0.26	-0.80	-2.68	1.11	-0.31	-1.25	0.65	-1.25	-3.03	0.57	<b>-0.85</b> †	-1.78	0.08
<b>MDA</b>	<b>2.66</b> †	-0.04	5.44	0.56	-0.77	1.92	<b>2.47</b> †	-0.20	5.22	<b>-1.20</b> †	-2.49	0.11	0.50	-2.05	3.12	0.32	-1.02	1.67
<b>DCCA</b>	<b>0.02</b> *	0.00	0.03	0.00	-0.01	0.00	<b>-0.02</b> *	-0.03	-0.00	<b>0.01</b> *	0.00	0.01	-0.01	-0.03	0.00	-0.01	-0.01	0.00
<b>ETU</b>	1.18	-0.47	2.85	0.52	-0.31	1.35	-0.70	-2.33	0.95	0.51	-0.32	1.34	-0.16	-1.74	1.44	0.12	-0.71	0.95

kiss54: kisspeptin 54; CpGt: Total DNA methylation [(CpG1 + CpG2 + CpG3 + CpG4)/4]; BPA: Bisphenol A; MPB: methylparaben; BP3: benzophenone 3; As: arsenic (total), Cd: cadmium; IMPy: 2-isopropyl-6-methyl-4-pyrimidinol; MDA: malathion diacid; DCCA: dimethylcyclopropane carboxylic acid; ETU: ethylene thiourea. LL: lower limit, and UL: upper limit of the confidence interval (CI). All models were adjusted for age (continuous), waist-to-height ratio (continuous), Tanner Stage (<G5/G5), urine creatinine concentrations (continuous), annual family income (<25,000/25,000-35,000/>35,000), season of biological sample collection (spring/summer/autumn/winter). \*p<0.05; †p<0.10

**Table S10. Associations between the joint effect of environmental chemical mixtures with kisspeptin and hormones biomarkers without multiple imputation (n=104 for serum kiss43; n= 117 for *KISS1* DNA methylation)**

	Kisspeptin effect biomarkers																	
	Kiss54			CpG1			CpG2			CpG3			CpG4			CpGt		
	%Change	LL	UL	%Change	LL	UL	%Change	LL	UL	%Change	LL	UL	%Change	LL	UL	%Change	LL	UL
<b>Phenols</b>	1.46	-2.39	5.46	-1.50	-3.45	0.48	-2.33	-6.18	1.68	-0.47	-2.45	1.54	-2.68	-6.34	1.12	-1.56	-3.49	0.42
<b>Metals</b>	2.00	-2.13	6.31	-0.34	-2.35	1.72	0.37	-3.63	4.54	0.51	-1.49	2.56	0.59	-3.26	4.60	0.35	-1.66	2.41
<b>Pesticides</b>	<b>11.86*</b>	6.30	17.70	-1.68	-4.40	1.11	-3.97	-9.17	1.53	-0.56	-3.30	2.26	<b>-5.04†</b>	-10.02	0.22	<b>-2.62†</b>	-5.31	0.15
<b>Whole Mixture</b>	<b>13.76*</b>	6.17	21.89	<b>-3.19†</b>	-6.62	0.37	-5.15	-11.74	1.93	-0.78	-4.30	2.87	<b>-6.80*</b>	-13.04	-0.11	<b>-3.64*</b>	-7.03	-0.12
	Hormones																	
	T			DHEAS			E2			SHBG			LH			FSH		
	%Change	LL	UL	%Change	LL	UL	%Change	LL	UL	%Change	LL	UL	%Change	LL	UL	%Change	LL	UL
<b>Phenols</b>	6.31	-7.58	22.30	7.74	-3.96	20.87	1.20	-19.59	27.36	<b>14.73**</b>	4.30	26.21	7.27	-7.82	24.83	6.23	-21.43	43.61
<b>Metals</b>	2.48	-11.98	19.31	0.02	-11.74	13.33	-2.08	-23.67	25.62	1.73	-8.52	13.13	6.71	-9.36	25.62	-15.09	-38.71	17.65
<b>Pesticides</b>	2.10	-17.25	25.97	-4.11	-19.29	13.92	18.82	-15.36	66.80	-9.06	-21.36	5.17	<b>-23.36*</b>	-38.55	-4.41	-10.53	-42.68	39.67
<b>Whole Mixture</b>	7.47	-18.42	41.58	7.02	-14.45	33.88	11.65	-28.56	74.49	3.39	-14.16	24.53	-13.50	-35.33	15.71	-12.26	-50.92	56.86

DHEAS= dehydroepiandrosterone sulfate; E2= estradiol; SHBG= sex hormone binding-globulin; FSH= follicle stimulation hormone. LL: lower limit, and UL: upper limit of the confidence interval (CI). Model was adjusted for adolescents' age (continuous), waist to height ratio (continuous), Tanner Stage ( $\leq G4$ / $>G4$ ), urine creatinine concentrations (continuous), annual family income ( $<25000/2500-35000/>35000$ ), season of sample collection (spring/summer/autumn/winter).

\*\*p<0.01; \*p<0.05; †p<0.10

**Table S11. Associations between kisspeptin biomarkers and serum hormone levels without multiple imputation (n=104 for serum kiss43; n= 117 for *KISS1* DNA methylation)**

		T		DHEAS			E2		SHBG			LH		FSH					
		%Change	95%CI		%Change	95%CI		%Change	95%CI		%Change	95%CI		%Change	95%CI				
			LL	UL		LL	UL		LL	UL		LL	UL		LL	UL			
<b>Kiss54</b>	<b>T2</b>	4.52	-18.79	34.51	-7.78	-25.46	14.08	12.86	-22.68	64.73	8.25	-8.91	28.66	19.26	-6.93	52.82	-8.09	-32.05	24.32
	<b>T3</b>	<b>33.16*</b>	2.51	72.95	-8.48	-26.60	14.10	<b>59.00*</b>	7.43	135.33	3.99	-13.05	24.37	18.49	-8.36	53.22	-14.59	-37.55	16.81
<b>CpG1</b>	<b>T2</b>	-3.91	-23.63	20.92	-6.52	-22.37	12.56	0.25	-30.01	43.58	<b>-16.26*</b>	-27.97	-2.66	22.58	-3.78	56.16	-25.00	-54.12	22.58
	<b>T3</b>	1.77	-19.50	28.65	-10.62	-26.05	8.03	-23.83	-47.20	9.89	-1.64	-15.65	14.68	12.83	-11.86	44.45	-39.15	-63.14	0.46
<b>CpG2</b>	<b>T2</b>	4.38	-16.88	31.08	<b>-19.27*</b>	-32.54	-3.39	-26.21	-48.01	4.74	-7.16	-20.42	8.31	10.97	-12.95	41.46	-48.56	-68.02	-17.24
	<b>T3</b>	-9.90	-28.52	13.57	<b>-22.00*</b>	-35.01	-6.39	-40.08	-58.02	-14.46	-5.43	-19.14	10.61	17.80	-7.96	50.76	<b>-51.62*</b>	-70.16	-21.57
<b>CpG3</b>	<b>T2</b>	9.40	-12.48	36.75	-5.37	-21.60	14.23	19.52	-17.08	72.27	4.98	-10.19	22.72	4.02	-18.73	33.13	-21.43	-51.90	28.32
	<b>T3</b>	<b>-20.13*</b>	-36.00	-0.33	-0.57	-17.52	19.85	6.15	-26.15	52.59	-0.15	-14.48	16.59	-4.77	-25.46	21.66	42.26	-12.59	131.52
<b>CpG4</b>	<b>T2</b>	-3.45	-23.09	21.20	4.87	-12.80	26.12	-24.57	-47.00	7.34	-8.80	-21.75	6.30	16.28	-8.69	48.09	-4.42	-41.71	56.73
	<b>T3</b>	-11.74	-29.79	10.96	-6.65	-22.47	12.41	<b>-34.21*</b>	-53.88	-6.17	-3.05	-16.90	13.11	1.70	-20.27	29.72	-22.51	-52.89	27.47
<b>CpGt</b>	<b>T2</b>	8.11	-13.94	35.81	0.99	-16.03	21.45	8.97	-23.14	54.50	<b>-15.70*</b>	-27.46	-2.03	21.25	-4.76	54.37	-0.01	-39.15	64.29
	<b>T3</b>	-6.34	-25.55	17.82	-11.74	-26.70	6.27	<b>-33.85</b>	-53.45	-6.01	0.02	-14.02	16.34	1.61	-20.31	29.56	-21.49	-52.37	29.40

T: Total testosterone; DHEAS= dehydroepiandrosterone sulfate; E<sub>2</sub>= estradiol; SHBG= sex hormone binding-globulin; LH=luteinizing hormone; FSH= follicle stimulation hormone; kiss54: kisspeptin 54; CpGt: Total DNA methylation [(CpG1 + CpG2 + CpG3 + CpG4)/4]. LL: lower limit, and UL: upper limit of the confidence interval (CI). Model was adjusted for adolescents' age (continuous), waist to height ratio (continuous), Tanner Stage (<G5/>G5), urine creatinine concentrations (continuous), annual family income (<25,000/25,000-35,000/>35,000), season of biological sample collection (spring/summer/autumn/winter). \*p<0.05; †p<0.10

**Table S12.** General characteristics of study participants at follow-ups.

<b>Characteristics</b>		<b>15-17 yrs (n=133)</b>	<b>9-11 yrs (n=300)</b>	<b>4-5 yrs (n=220)</b>
BMI (kg/m <sup>2</sup> ), mean		23.1	19.1	17.0
Area of residence, %	Urban	71.5	24.7*	64.3
	Suburban/rural	28.5	75.3	35.7
Passive smoking, %	Yes	41.2*	72.1	53.0*
	No	58.8	27.9	47.0
Annual family income (euros), %	<25,000	36.1*	66.2	N.A.
	25,000-35,000	39.1	24.1	N.A.
	>35,000	24.8	9.7	N.A.

BMI: Body mass index. N.A.: Not available. \*p<0.05