A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP15117.

Prenatal Exposure to Synthetic Phenols Assessed in Multiple Urine Samples and Dysregulation of Steroid Hormone Homeostasis in Two European Cohorts

Paulina Jedynak,[1,2,3,4] Mariona Bustamante,[2,3,4] Matthieu Rolland,[1] Vicente Mustieles,[4,5,6] Cathrine Thomsen,[7] Amrit K. Sakhi,[7] Azemira Sabaredzovic,[7] Maria Foraster,[2,3,4] Mireia Gascon,[8,2,3,4] Maria Dolores Gómez-Roig,[9,10] Elisa Llurba,[2,3,4] Ioar Rivas,[2,3,4] Isabelle Ouellet-Morin,[11] Sam Bayat,[12] Sarah Lyon-Caen,[1] Oscar J. Pozo,[13] Martine Vrijheid,[2,3,4] Jordi Sunyer,[2,3,4,14] Rémy Slama,[1] Payam Dadvand,[2,3,4] and Claire Philippat[1]

[1]University Grenoble Alpes, Inserm U 1209, CNRS UMR 5309, Institute for Advanced Biosciences, 38000 Grenoble, France
[2]ISGlobal, Barcelona, Spain
[3]Universitat Pompeu Fabra (UPF), Barcelona, Spain
[4]CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain
[5]Instituto de Investigación Biosanitaria (ibs.GRANADA), Granada, Spain
[6]Biomedical Research Center (CIBM), University of Granada, Granada, Spain
[7]Department of Food Safety, Norwegian Institute of Public Health, Oslo, Norway
[8]Unitat de Suport a la Recerca de la Catalunya Central, Fundació Institut Universitari per a la Recerca a l'Atenció Primária de Salut Jordi Gol i Gurina (IDIAPJGol), Manresa, Spain
[9]Barcelona Centre for Maternal-Fetal and Neonatal Medicine (Hospital Sant Joan de Déu and Hospital Clínic), Institut de Recerca Sant Joan de Déu (IR-SJD), Barcelona, Spain
[10]Primary Care Interventions to Prevent Maternal and Child Chronic Diseases of Perinatal and Developmental Origin Network (RICORS-SAMID) (RD21/0003), Instituto de Salud Carlos III, Madrid, Spain
[11]School of Criminology, Université de Montréal and Research Center of the Institut Universitaire en Santé Mentale de Montréal, Montreal, Québec, Canada
[12]Department of Pulmonology and Physiology, CHU Grenoble Alpes, Grenoble, France
[13]Applied Metabolomics Research Group, Hospital del Mar Research Institute, Barcelona, Spain
[14]Hospital del Mar Medical Research Institute, Barcelona, Spain

Background: Some synthetic phenols alter hormonal pathways involved in successful pregnancy and fetal development. Despite high within-subject temporal variability of phenols, previous studies mostly utilized spot urine samples to assess pregnancy exposure. Herein, we investigated associations between pregnancy exposure to eight phenols assessed in multiple pooled urine samples and steroid hormones assessed in maternal hair reflecting cumulative hormone levels over the previous weeks to months.

Methods: We assessed phenol–hormone associations in 928 pregnant women from two pooled cohorts recruited in Spain [Barcelona Life Study Cohort (BiSC), 2018–2021] and France [Assessment of Air Pollution exposure during Pregnancy and Effect on Health (SEPAGES), 2014–2017] using pools of up to 21 samples each, collected in early pregnancy (median gestational age: 18.0 wk), as well as hair collected in late pregnancy (BiSC) or at birth (SEPAGES). We measured two bisphenols, four parabens, benzophenone-3, and triclosan along with metabolites of three adrenal ($\sum$cortisol, $\sum$cortisone, and 11-dehydrocorticosterone) and two reproductive (progesterone and testosterone) hormones. We ran adjusted linear regressions for each exposure biomarker–outcome pair and Bayesian kernel machine regression for phenols mixture.

Results: Bisphenol S was associated with higher cortisol and 11-dehydrocorticosterone concentrations. Propylparaben was associated with lower levels of cortisol, cortisone, and 11-dehydrocorticosterone, while methylparaben was linked to a reduction in cortisol levels. Interestingly, associations identified for parabens were stronger for women carrying female fetuses. No associations for phenol mixture were detected.

Conclusions: Our study suggests that pregnancy exposure to bisphenol S and some parabens (propyl- and methylparaben) may affect production of maternal corticosteroid hormones that are important for a successful pregnancy and fetal development. https://doi.org/10.1289/EHP15117

Introduction

The hypothalamus–pituitary–gonadal (HPG) axis regulates the synthesis of sex hormones, such as testosterone, estrogen, and progesterone, while the hypothalamus–pituitary–adrenal (HPA) axis synthesizes glucocorticoids, like cortisol, which mediates the body's adaptive response to stress.[1] Both axes and their interaction are critical for maintaining pregnancy and supporting the normal development of the fetus.[2] For instance, progesterone and progesterone/cortisol balance are vital for sustaining pregnancy and modulating the maternal immune response to prevent rejection of the developing fetus and placenta.[3] Estrogens promote fetal organs development while testosterone is involved in sexual differentiation.[2] Dysregulation of steroid hormone homeostasis during pregnancy could impact fetal development and the child's health later in life.[3,4] For example, increased maternal salivary cortisol has been linked with lower weight at birth[5] while prenatal exposure to synthetic glucocorticoids has been associated with behavioral problems in children.[6,7]

Factors that may alter the HPA/HPG axes include endocrine-disrupting chemicals (EDCs),[8] such as synthetic phenols used in the manufacture of household items and personal care products.[9] EDCs can affect steroid hormone homeostasis through various mechanisms.[10] Bisphenol A, for example, can bind to the glucocorticoid receptor as an agonist[11] and can increase circulating levels of the sex hormone binding globulin that binds to circulating sex hormones. Alteration of estrogen[12,13] and glucocorticoid receptors expression[14,15] has also been reported as linked to bisphenol A exposure.

Address correspondence to Claire Philippat, University Grenoble Alpes, Inserm U 1209, CNRS UMR 5309, Team of Environmental Epidemiology applied to Development and Respiratory Health, Institute for Advanced Biosciences, Grenoble, France. Email: claire.philippat@inserm.fr

Supplemental Material is available online (https://doi.org/10.1289/EHP15117).

The authors declare they have no conflicts of interest related to this work to disclose.

Received 6 April 2024; Revised 6 February 2025; Accepted 19 March 2025; Published 22 May 2025.

Note to readers with disabilities: *EHP* strives to ensure that all journal content is accessible to all readers. However, some figures and Supplemental Material published in *EHP* articles may not conform to 508 standards due to the complexity of the information being presented. If you need assistance accessing journal content, please contact ehpsubmissions@niehs.nih.gov. Our staff will work with you to assess and meet your accessibility needs within 3 working days.

Despite growing toxicological evidence, only a few epidemiological studies have explored associations between prenatal phenol exposure and maternal steroid hormone concentrations. Concerning HPG hormones, one large study ($n = 602$) found higher bisphenol A urinary concentrations associated with lower maternal serum testosterone concentrations, while most studies measuring progesterone did not find any association with this phenol.[2] Regarding HPA hormones, Giesbrecht et al. reported an association between higher maternal bisphenol A urinary concentrations and lower saliva cortisol concentrations in pregnant women ($n = 174$)[16] and in male infants ($n = 66$),[16] while for female infants ($n = 66$) cortisol levels were higher.[17] The limitations of those studies included low sample size ($n \leq 174$) as well as assessment of phenols in spot urine, which does not account for their short half-life and causes exposure misclassification and effect estimates biased toward the null.[18]

We hypothesized that exposure to synthetic phenols during pregnancy alters maternal steroid hormones homeostasis. To address this, we pooled data from two European mother–child cohorts in which special care was taken to minimize exposure and outcome misclassification. We therefore assessed phenols in a pool of repeated urine samples collected during pregnancy (corresponding to up to 21 samples per woman) and steroid hormones in maternal hair, a matrix allowing for measurement of hormone production over several weeks to months.

Methods

Study Participants

We included pregnant women from two European mother–child cohorts, namely the Barcelona Life Study Cohort (BiSC) recruited between 2018 and 2021 in Barcelona, Spain[19] and SEPAGES (Assessment of Air Pollution exposure during Pregnancy and Effect on Health) recruited between 2014 and 2017 in Grenoble area, France.[20] Pregnant women participating in the BiSC study were recruited during their first routine hospital visit (between 11 and 14 wk of gestation) at three different university hospitals in Barcelona, Spain: Hospital Sant Joan de Déu, Hospital Clínic de Barcelona, and Hospital de la Santa Creu i Sant Pau, along with their corresponding primary health care centers. The SEPAGES women were recruited before their 19 gestational weeks at eight obstetrical ultrasonography practices of the Grenoble metropolitan area. Inclusion criteria were as follows: being pregnant (singleton pregnancy) by <19 gestational weeks at the time of inclusion, being at least 18 years old, being maximally 45 years old (BiSC), being able to communicate in Spanish/Catalan (BiSC) or read and speak fluently in French (SEPAGES), living in the catchment area of the maternity clinics (BiSC) or intending to give birth in one of the maternity clinics in the recruitment area (SEPAGES), and being a member of the national social security system (SEPAGES). For both cohorts, ethical approvals were obtained from the relevant ethical committees and both parents/legal representatives of the anticipated child signed an informed consent form. For both cohorts, both parents/legal representatives of the anticipated child signed an informed consent, and ethical approvals were obtained from the relevant ethical committees. For BiSC, ethical approvals were obtained from Clinical Research Ethics Committee of the Parc de Salut Mar (2018/8050/I), Medical Research Committee of the Fundació de Gestió Sanitària del Hospital de la Santa Creu i Sant Pau de Barcelona (EC/18/206/5272), and Ethics Committee of the Fundació Sant Joan de Déu (PIC-27-18). For SEPAGES, ethical approvals were obtained from the Comité de Protection des Personnes Sud-Est V (13-CHUG-44, ID RCB: 2013-A01491-44) and the Commission Nationale de l'Informatique et des Libertés (914138).

We included women with urine collected at the second trimester and hair samples available for phenol biomarker and hormone assessments. Out of the total 1,564 participants included in both cohorts, our final sample size was 928 for $\sum$cortisone, 11-dehydrocorticosterone, and progesterone and 927 for $\sum$cortisol, testosterone, and $\sum$cortisol/$\sum$cortisone (Figure S1).

Hair Collection and Assessment of Steroid Hormones

Maternal hair samples were collected by a nurse during a follow-up visit (BiSC, median 31.7 gestational weeks) or by the mothers themselves in the days following the delivery (SEPAGES, 1–3 days postpartum) using a similar protocol. Hair strands were cut within 1 mm of the scalp from the posterior vertex area of the head, which has been shown to have the lowest coefficient of variation,[21] following the guidelines of the Society of Hair Testing.[22] In the event that the mother had short hair, strands were cut in several places. After collection, hair samples were stored at room temperature at the cohort biobanks before they were sent to the Applied Metabolomics Research Group of the IMIM-Hospital del Mar Medical Research Institute (Barcelona, Spain) in charge of hormone concentrations assessment for both cohorts. We measured nine hormones related to the HPA axis using liquid chromatography–tandem mass spectrometry,[23] cortisol and its metabolites (20α- and 20β-dihydrocortisol), cortisone and its metabolites (20α- and 20β-dihydrocortisone, β-cortolone), 11-dehydrocorticosterone, and androstenedione, along with two reproductive hormones, testosterone and progesterone.

Briefly, 3-cm-long scalp side strands corresponding to the hair growth during the last 3 months[24,25] were cut and washed two times for 10 min with dichloromethane. The hair was dried overnight and then pulverized for 2 min using a ball mill. After addition of isotopically labeled internal standard, 50 mg of milled washed hair was extracted with 1 mL of methanol. After centrifugation, the methanolic extract was separated and evaporated under nitrogen stream at 40°C. Then, a liquid–liquid extraction was performed by addition of 1 mL of saturated NaCl solution, 250 µL of K_2CO_3 (25%, wt/wt), and 5 mL of ethyl acetate to the dry extract. The organic layer was extracted and evaporated to dryness under nitrogen stream. The extract was reconstituted with 100 µL of water:methanol (1:1), and 10 µL were injected into the ultra-high performance liquid chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS) system. The detection of the steroid profile was performed by a selected reaction monitoring method including two ion transitions per analyte. The hair hormone concentrations (pg/mg of hair) were obtained by dividing of the hormone quantity (pg) by the corresponding hair weight of each sample. The method accuracy ranged from 80% to 120%, and the precision given as relative standard deviation was below 20% for all steroids. Two quality controls (QCs) consisting of known real samples were injected every 20 samples with a minimum of two QCs per batch. In total, 95 QCs were analyzed together with the samples of the project. The limits of detection (LOD) and quantification (LOQ) of the method ranged from 0.01 to 0.5 and 0.02 to 0.8 pg/mg of hair, respectively (Table S2).

Urine Collection and Assessment of Phenol Concentrations

In both cohorts, women were asked to collect repeated urine samples during pregnancy (median gestational age: 18.4 and 17.6 gestational weeks in BiSC and SEPAGES, respectively). BiSC participants were asked to collect two samples per day (morning and evening) for six consecutive days, resulting in 12 urine samples per women (nine participants who collected lower numbers of samples were excluded from the study). SEPAGES women were asked to collect three samples (morning, midday, and evening) for seven consecutive days, resulting in a median of 21 samples (25th centile: 20; 75th centile: 21) collected per

trimester. Women stored urine samples in their home freezer at −20°C until the field workers transported them to a biobank where the samples were thawed. As detailed in Figure S2, for each participant, equal volumes of all samples collected during the collection week were combined in a single pool.[26,27] Aliquots of the pools were stored at −80°C. For both cohorts, an aliquot of the pooled sample from each participant was shipped on dry ice to the Norwegian Institute of Public Health for phenol assessment. Total (free plus conjugated) concentrations of 12 phenols (benzophenone-3; bisphenols A, AF, B, F, and S; butyl-, ethyl-, methyl-, and propylparaben; triclosan; and triclocarban) were measured using ultraperformance liquid chromatography coupled to mass spectrometry (UPLC-MS/MS).[28] Briefly, 200 µL of urine sample were mixed with internal standards and enzyme solution (beta-glucuronidase/sulfatase in ammonium acetate buffer, pH 5.0) and incubated for 4 h at 37°C. Then, the enzymatic reaction was stopped by addition of 40% formic acid. The samples were then centrifuged and 80 µL of the supernatant was transferred into the UPLC-MS/MS system. For BiSC samples, this method was slightly modified: *a*) the amount of enzyme used was 20 mg/mL, *b*) incubation time was 24 h, and *c*) 50 µL of the supernatant was transferred into the UPLC-MS/MS system. These modifications increased LOD and LOQ of bisphenol F only (LOD: 0.3 ng/mL and LOQ: 1.0 ng/mL). For quality assurance, blanks samples, in-house control samples, standard reference material provided by the National Institute of Standards and Technology (USA), and external controls (in some batches) were analyzed along with the samples. The accuracy ranged from 70% to 130% and the precision given as relative standard deviation was below 30% for most of the phenols. The LOD and LOQ ranged from 0.04 to 0.3 and 0.1 to 1.0 ng/mL, respectively (Table S2).

Statistical Analyses: Hormones and Phenols

Compounds detected in <2% of the samples in at least one cohort (androstenedione; β-cortolone; bisphenols AF, B, and F; and triclocarban) were excluded. Compounds detected in <70% of the samples (bisphenol S and butylparaben) were dichotomized (<LOD, ≥LOD). For compounds with detection rates higher than 70%, the imputation was done as follows: for hormones, the nondetected concentration values were singly imputed with *NADA*[29] and *msm*[30,31] R packages by randomly drawing values below the LOD from the estimated distribution of the compound.[32,33] Phenol concentration values below LOD and those between the LOD and LOQ were singly imputed using the same approach.

Accounting for Analytical Batch and Assay Conditions

Both recruitment center and hair analytical batch were potential predictors of hormone concentrations but could not be included in the same regression equation due to their multicollinearity (nested variables). Thus, we standardized hormone concentrations for analytical batch [all hormones except for corticosterone in SEPAGES that was not affected by batch (Table S1)] using a two-step approach.[34,35] First, we estimated the associations between each ln-transformed hormone concentration and analytical batch using linear regression adjusted for maternal age, education, ancestry, body mass index, active and passive smoking, and alcohol consumption, as well as for parity, child's phenotypic sex, season of hair samples collection, and time elapsed from hair collection to hormone assessment. We then used the measured hormone concentrations and the estimated effects of analytical batch associated with the biomarker hair concentrations ($p < 0.2$) to predict standardized concentrations, i.e., the concentrations that would have been observed if all samples had been processed under the same conditions and assayed in the same batch.

Similarly to hormones, all continuous exposures but propylparaben in BiSC and triclosan in SEPAGES that were not affected by urine processing and assay condition, were standardized using the two-step approach described above (Table S1). For phenols, conditions considered for standardization were analytical batches, individual samples thawing time at 4°C during the pooling procedure, and sample transport time from participant's home to the biobank (for SEPAGES cohort only).

We computed molar sums of cortisol and cortisone and their metabolites[36] and used those in our analyses as an indication of cortisol and cortisone production. Additionally, we computed $\sum$cortisol to $\sum$cortisone ratio serving as an indicator of 11β-hydroxysteroid dehydrogenase (11β-HSD) activity.

Hair hormone (including molar sums and ratios) and urinary phenol concentrations were ln-transformed prior to statistical analyses.

Associations between Phenols and Hormones

Covariables. Adjustment factors were selected *a priori*. These were variables likely to be common causes of both phenol and hormone levels, without being likely consequences thereof, and factors that were possible predictors of the hormone concentrations only (see "Directed acyclic graph" in Figure S3).[37] Based on this approach, we included the following: maternal age (continuous), maternal active smoking anytime during pregnancy (no/yes), maternal passive tobacco exposure 3 months before and anytime during pregnancy (BiSC) or anytime during pregnancy (SEPAGES, ≥1 hour/week; indoors, no/yes), maternal alcohol consumption during pregnancy (no/yes), maternal body mass index (BMI) at inclusion (BiSC) or before pregnancy (SEPAGES, continuous), maternal education level (below university/university degree below master/university master degree or higher), self-reported maternal ancestry (Caucasian/Latin American/other), parity (nulliparous/one or more children), fetus' phenotypic sex (female/male), season of hair collection (winter/other seasons, as hair samples collected in winter showed significantly lower corticosteroid levels compared to other seasons), and center. With the exception of BMI computed from weight and height measured during a study visit, data on covariates was collected through questionnaires and face to face interviews with fieldworkers. We used the following three categories for center: one representing the SEPAGES cohort and two representing BiSC. Two of the three BiSC hospitals (Hospital Sant Joan de Deu and Hospital Clinic) were combined in one category as they recruited patients from a similar geographic area. We adjusted for self-reported maternal ancestry, as it can affect HPA hormone concentrations. The original reporting categories differed between BiSC [Caucasian (European), Caucasian (Iranian), Latin American, Arabian, sub-Saharan African, South Asian, Far East Asian, other, and an option for "I don't know/I would rather not answer"] and SEPAGES (Africa, America, South East Asia, Europe, Oriental Mediterranean, West Pacific, and other). Due to small sample sizes in certain categories, participants were grouped into three final categories for analysis: Caucasian, Latin American, and other.

Missing values for maternal active ($n = 44$) and passive ($n = 50$) smoking, alcohol intake ($n = 51$), education level ($n = 2$), and ancestry ($n = 5$) were replaced by the mode, while missing values for maternal BMI ($n = 43$) were replaced by the median. Single imputation was chosen because the rate of missing values was low (maximum of 5.5%).

Uni-pollutant models. We fitted adjusted linear regression models for each pair of hormone–phenol concentrations, separately for each urine collection time point. For continuous phenol biomarker concentrations (all except for bisphenol S and butylparaben), β regression estimates were expressed as percent change (PC) for each two-fold increase in standardized urinary

phenol biomarkers using the following formula: $(2^{\beta}-1)\times 100$. For dichotomized phenol biomarker concentrations (bisphenol S and butylparaben), β regression estimates were expressed as PC for urinary phenol biomarker concentrations ≥LOD vs. <LOD (reference category) using the following formula: $(e^{\beta}-1)\times 100$.

Fetal sex can influence maternal steroid hormone levels during pregnancy through its hormone production.[38,39] Additionally, some previous studies assessing steroid hormones in maternal or cord blood have reported associations with phenol exposure that vary by fetal sex.[2] Therefore, we further explored effect modification by child's phenotypic sex by adding an interaction term between each phenol and child's sex in multiple linear regression models. For interaction *t*-tests with *p*-value below 0.1, sex-stratified analyses were performed.

Due to the exploratory nature of our study, which is one of the first on this topic, and biological plausibility of phenols affecting hormone levels,[11,14,15,40,41] we did not formally correct for multiple testing.[42,43]

Mixture models. The overall effect of phenols mixture was estimated using adjusted Bayesian kernel machine regression (BMKR) from *bkmr* and *bkmrhat* R packages.[44,45]

As recommended by Bobb et al.,[44,45] each mixture model was run in 50,000 iterations, and the first 50% of samples were discarded and every 10th iteration was retained for the posterior analyses. The overall effect of the exposures was evaluated graphically. Individual effects of each exposure on a given hormone and posterior inclusion probabilities were analyzed only if a mixture effect was detected. All continuous phenol concentrations (i.e., detected in >70% of the samples) were included in BKMR models. Categorized exposures were included in the mixture only if identified as associated with the outcome in the uni-pollutant model. In such case, they were included as continuous variables after concentrations below LOD were imputed using the "fill-in" method.

Sensitivity Analyses

To test the robustness of our observed exposure–outcome associations, we conducted several sensitivity analyses for the uni-pollutant models. First, we stratified by center, grouping participants into three categories [BiSC (hospitals 1 and 2), BiSC (hospital 3), and SEPAGES]. We also tested for interaction between center and exposure to evaluate potential effect modification ($p<0.1$). Next, we adjusted the models for maternal hair dyeing (BiSC only), as it could affect hair cortisol concentrations.

In another round of sensitivity analyses, we adjusted the uni-pollutant regression models for urine specific gravity measured using a handheld refractometer. We did not adjust the main models for specific gravity, as it has been suggested that correcting for urine dilution when using pooled samples is unnecessary.[26] To assess the potential impact of outliers, we applied winsorization. This approach consisted of replacing values below the first percentile with the value at the first percentile and values above the 99th percentile with the value at the 99th percentile prior to fitting the linear models for both the outcome and exposure.

Finally, we conducted a sensitivity analysis using a mixed effect model, treating center as a random effect to account for both within- and between-center variation.

Research Data and Code

The analyses were conducted using R version 4.3.2 (R Core Team) and RStudio 2023.12.0-369 (Posit Team). The code and statistical plan are available at https://gricad-gitlab.univ-grenoble-alpes.fr/iab-env-epi/jedynak-prenatal-2024. The code is also provided in the supplemental material.

Results

Study Participants

Out of the 928 women included in our study, 546 were enrolled in the BiSC and 382 in the SEPAGES cohort (Table 1). Participants had a median age of 33.7 years and a majority of them were of Caucasian self-reported ancestry (84.7%). Of the participants, 49.5% reached master's degree or higher education level, 69% had a normal weight (median BMI of 22.4 kg/m^2), more than half (54.2%) were nulliparous at the time of conception, and half of the infants were females (49.0%).

Regarding phenols, the median urinary concentrations were higher in BiSC than in SEPAGES, except for bisphenol A and triclosan, for which it was the opposite (Figure 1; Table S2).

Hormone detection rates were higher in the BiSC than in the SEPAGES cohort (Table S3). In both cohorts, the highest median hair concentrations were observed for progesterone followed by cortisone and its metabolites (Figure 2; Table S3). Cortisol and cortisone were highly correlated with their respective metabolites (Spearman's rho >0.6) (Figure S4). ∑cortisol and ∑cortisone were also highly correlated (rho >0.8).

Associations between Phenols and Hormone Concentrations

Uni-pollutant models. Bisphenol S was associated with higher ∑cortisol [PC = 12.3%; 95% confidence interval (CI): 0.2, 25.9 for detected bisphenol S concentrations compared to nondetects], 11-dehydrocorticosterone (PC = 19.5%; 95% CI: 2.2, 39.8), and ∑cortisone (PC = 12.8%; 95% CI: −0.1, 27.5). We also observed an isolated negative association between bisphenol A and ∑cortisone (PC = −2.56%; 95% CI: −5.06, −0.01 for a two-fold increase in bisphenol A) (Figure 3; Table S4).

Parabens were also associated with hair hormones. Propylparaben was associated with lower ∑cortisol (PC = −1.55%; 95% CI: −2.96, −0.11), ∑cortisone (PC = −2.08%; 95% CI: −3.58, −0.56), 11-dehydrocorticosterone (PC = −2.23%; 95% CI: −4.15, −0.26), and to some extent, with lower progesterone (PC = −1.75%; 95% CI: −3.54, 0.07) and higher ∑cortisol/∑cortisone ratio (PC = 0.57%; 95% CI: −0.1, 1.24). Methylparaben was associated with lower ∑cortisol (PC = −3.22%; 95% CI: −5.98, −0.38) and ∑cortisone (PC = −2.77%; 95% CI: −5.72, 0.27) (Figure 3; Table S4).

Finally, triclosan was associated with a higher ∑cortisol/∑cortisone ratio (PC = 1.02%; 95% CI: 0.2, 1.86).

Several *p*-values for phenols–sex interaction were below 0.1 (Table S5). After stratification for child's sex, associations were only observed among women carrying female fetuses (Table 2; Figure S5). Propylparaben and methylparaben were negatively associated with ∑cortisol (PC = −0.04; 95% CI: −0.07, −0.01 and PC = −0.08; 95% CI: −0.14, −0.02, respectively), while propylparaben was negatively associated with ∑cortisone (PC = −0.05; 95% CI: −0.09, −0.02).

Mixture models. We did not observe clear association between the phenol mixture and hair hormones (Figure 4; Table S6).

Sensitivity Analyses

The results of the stratified analysis by center showed that the direction of associations remained consistent across centers, though confidence intervals for bisphenols were wider in SEPAGES (Table S7; Figure S6). For our main association, no significant interactions were found between center and exposure, indicating consistent associations across BiSC and SEPAGES (Table S7).

Table 1. Participant characteristics of the 928 women included in the study from two cohorts BiSC (Barcelona, Spain, 2018–2021) and SEPAGES (Grenoble, France, 2014–2017).

	BiSC ($n = 546$)	SEPAGES ($n = 382$)	Pooled ($n = 928$)	p-Value[a]
Child's phenotypic sex	—	—	—	0.15
Male	267 (49%)	206 (54%)	473 (51%)	—
Female	279 (51%)	176 (46%)	455 (49%)	—
Year of delivery	—	—	—	<0.001
2015	0 (0%)	98 (25.7%)	98 (10.6%)	—
2016	0 (0%)	180 (47.1%)	180 (19.4%)	—
2017	0 (0%)	100 (26.2%)	100 (10.8%)	—
2018	0 (0%)	4 (1.0%)	4 (0.4%)	—
2019	119 (21.8%)	0 (0%)	119 (12.8%)	—
2020	186 (34.1%)	0 (0%)	186 (20.0%)	—
2021	241 (44.1%)	0 (0%)	241 (26.0%)	—
Parity	—	—	—	<0.001
Multiparous	210 (38.5%)	215 (56.3%)	425 (45.8%)	—
Nulliparous	336 (61.5%)	167 (43.7%)	503 (54.2%)	—
Hair collection season	—	—	—	0.18
Winter	142 (26.0%)	84 (22.0%)	226 (24.4%)	—
Other seasons	404 (74.0%)	298 (78.0%)	702 (75.6%)	—
Maternal hair dyeing	—	—	—	—
No	266 (51%)	—	266 (51%)	—
Yes	254 (49%)	—	254 (49%)	—
Missing	26	382	408	—
Maternal active smoking[b]	—	—	—	0.62
No	493 (92%)	327 (93%)	820 (93%)	—
Yes	41 (7.7%)	23 (6.6%)	64 (7.2%)	—
Missing	12	32	44	—
Maternal passive tobacco exposure[c]	—	—	—	<0.001
No	309 (60%)	295 (82%)	604 (69%)	—
Yes	210 (40%)	64 (18%)	274 (31%)	—
Missing	27	23	50	—
Maternal alcohol consumption[d]	—	—	—	0.002
No	338 (67%)	288 (77%)	626 (71%)	—
Yes	164 (33%)	87 (23%)	251 (29%)	—
Missing	44	7	51	—
Maternal education	—	—	—	<0.001
Below university	158 (29%)	24 (6.3%)	182 (20%)	—
University below master's	147 (27%)	138 (36%)	285 (31%)	—
Master's and above	241 (44%)	218 (57%)	459 (50%)	—
Missing	0	2	2	—
Self-reported maternal ancestry	—	—	—	<0.001
Caucasian	422 (77.3%)	364 (96.6%)	786 (85.1%)	—
Latin American	117 (21.4%)	0 (0%)	117 (12.7%)	—
Other[e]	7 (1.3%)	13 (3.4%)	20 (2.2%)	—
Missing	0	5	5	—
Maternal BMI[f] (kg/m^2)	—	—	—	<0.001
Median (Q1, Q3)	23.2 (21.3, 26.0)	21.5 (19.7, 23.8)	22.4 (20.5, 25.2)	—
Missing	41	2	43	—
Maternal age (y)	—	—	—	<0.001
Median (Q1, Q3)	35.0 (32.0, 37.5)	32.2 (29.9, 35.2)	33.7 (30.7, 36.6)	—
Time elapsed from hair collection to analysis (d)	—	—	—	<0.001
Median (Q1, Q3)	162 (100, 271)	1,550 (1,380; 1,780)	352 (141; 1,480)	—
Gestational age at urine collection in T2 (wk)	—	—	—	<0.001
Median (Q1, Q3)	18.4 (16.4, 21.0)	17.6 (16.3, 18.7)	18.0 (16.4, 19.6)	—
Missing	0	28	28	—
Number of urine samples in T2	—	—	—	<0.001
Median (Q1, Q3)	12.0 (12.0, 12.0)	21.0 (20.0, 21.0)	12.0 (12.0, 21.0)	—

Note: Categorical variables are described as n (%) and continuous variables are described as median (Q1: 25th centile; Q3: 75th centile). —, no data; ANOVA, analysis of variance; BiSC, Barcelona Life Study Cohort; BMI, body mass index; Q, quantile; SEPAGES, Assessment of Air Pollution exposure during Pregnancy and Effect on Health; T, trimester.

[a]p-Values obtained for a chi-squared test (categorical variables) or ANOVA (numerical variables).

[b]For the BiSC cohort, "yes" category included women who declared any number of cigarettes smoked anytime during pregnancy, while for the SEPAGES cohort, it included women who declared smoking of ≥1 cigarette/day anytime during pregnancy.

[c]For the BiSC cohort, "yes" category included women who declared being exposed to tobacco smoke 3 months before and during pregnancy, while for the SEPAGES cohort, it included women who declared being exposed to indoor tobacco smoke ≥1 h/wk during pregnancy only.

[d]For the BiSC cohort, "yes" category included women who declared consumption of any alcoholic beverages during any trimester of pregnancy, while for the SEPAGES cohort, it included women who declared drinking of any quantity of alcohol per month when they knew they were pregnant.

[e]For the BiSC cohort, no details available; for SEPAGES cohort, five Africans, one Asian, two Oriental Mediterraneans, two South-East Asians, and no details for three women.

[f]For the BiSC cohort, maternal BMI was assessed at inclusion around 12 wk of gestation, while for SEPAGES, it was assessed before pregnancy.

When adjusting for maternal hair dyeing, all observed associations were preserved except for propylparaben and cortisol, and propylparaben and 11-dehydrocorticosterone, which remained negative but became nonsignificant (PC = −1.01; 95% CI: −2.36, 0.36 and PC = −0.78; 95% CI: −2.32, 0.79) (Table S8). This is likely due to the reduced sample size, as this analysis was conducted on the BiSC cohort only. Adding specific gravity to our models had minimal impact, and all associations were preserved (Table S9).

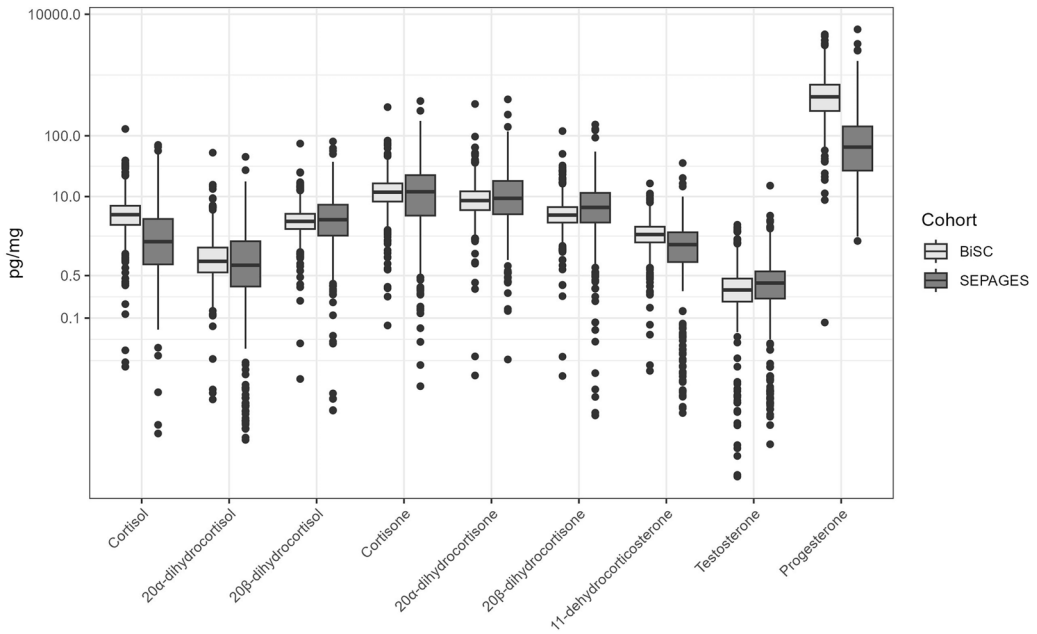

Figure 1. Standardized concentrations of phenols assessed in the urine samples, expressed as micrograms of phenol per liter of urine (BiSC, Barcelona, Spain, 2018–2021, $n = 546$; SEPAGES, Grenoble, France, 2014–2017, $n = 382$). Numerical values are displayed in Table S2. Note: BiSC, Barcelona Life Study Cohort; SEPAGES, Assessment of Air Pollution exposure during Pregnancy and Effect on Health.

In the models relying on winsorized phenol and hormone concentrations, the association between triclosan and the $\sum$cortisol/$\sum$cortisone ratio remained positive but was attenuated (PC = 0.58%; 95% CI: −0.15, 1.32 compared to PC = 1.02%; 95% CI: 0.2, 1.86 in the main analysis) (Table S10).

Finally, in the mixed effect model, the results were consistent with the main analysis (Table S11).

Discussion

This large study ($n = 928$ compared to $n \leq 174$ in previous studies) is the first to rely on many urine samples [between 12 (BiSC) and 21 (SEPAGES)] to assess phenol exposure in relation to steroid hormones production. All associations were observed for adrenal stress hormones ($\sum$cortisol, $\sum$cortisone, and 11-dehydrocorticosterone) and were overall negative with the exception of the association observed for bisphenol S that was positive. In the sex-stratified analysis, all associations were observed for women carrying female fetuses. The lack of association with progesterone and testosterone aligns with the existing studies that measured these hormones in maternal blood and which also generally reported null results for synthetic phenols.[2]

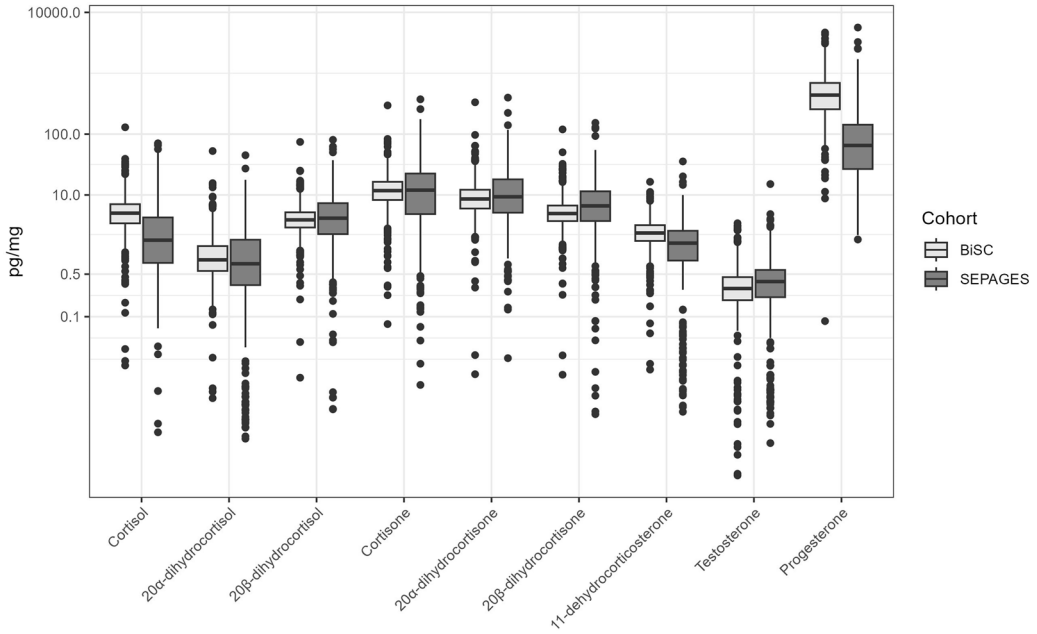

Figure 2. Standardized concentrations of hormones assessed in hair, expressed as picograms of hormone per milligram of hair (BiSC, Barcelona, Spain, 2018–2021, $n = 546$; SEPAGES, Grenoble, France, 2014–2017, $n = 382$). Numerical values are displayed in Table S3. Note: BiSC, Barcelona Life Study Cohort; SEPAGES, Assessment of Air Pollution exposure during Pregnancy and Effect on Health.

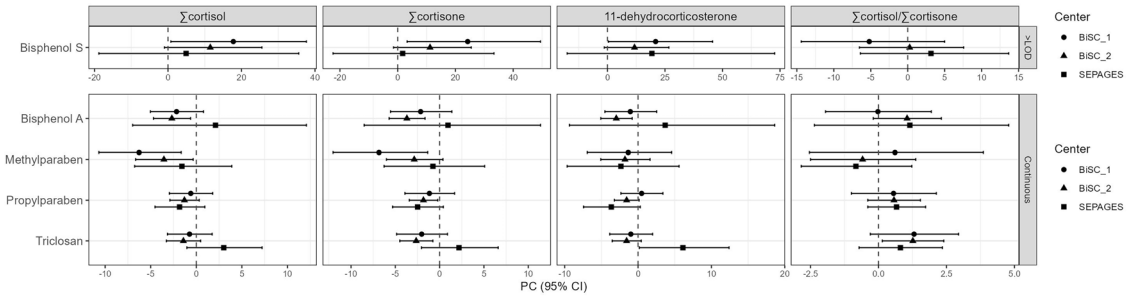

Figure 3. Adjusted associations between pregnancy phenol concentrations assessed in pooled urine samples during pregnancy and steroid hormone concentrations assessed in hair [$n = 928$ from BiSC (Barcelona, Spain, 2018–2021) and SEPAGES (Grenoble, France, 2014–2017)]. Regression models were adjusted for center, maternal age, BMI, education, active and passive smoking, alcohol consumption, ancestry, child's sex, parity, and season of hair collection. For continuous phenol biomarker concentrations (all except for bisphenol S and butylparaben), β regression estimates were expressed as PC for each two-fold increase in standardized urinary phenol biomarkers using the following formula: $(2^{\beta}-1)\times 100$. For dichotomized phenol biomarker concentrations (bisphenol S and butylparaben), β regression estimates were expressed as PC for urinary phenol biomarker concentrations ≥LOD vs. <LOD (reference category) using the following formula: $(e^{\beta}-1)\times 100$. Numerical values are displayed in Table S4. Note: BiSC, Barcelona Life Study Cohort; BMI, body mass index; CI, confidence interval; LOD, limit of detection; PC, percent change; SEPAGES, Assessment of Air Pollution exposure during Pregnancy and Effect on Health.

Bisphenols

In this study, bisphenol S was associated with higher ∑cortisol and 11-dehydrocorticosterone and tended to be positively associated with ∑cortisone levels. On the contrary, bisphenol A was associated with lower ∑cortisone. These associations were mostly driven by the BiSC cohort, which may be due to different exposure detection rates for bisphenol S (58% vs. 26% for BiSC and SEPAGES, respectively).

One study relying on cortisol measured in saliva reported lower cortisol associated with higher bisphenol A urinary concentrations in pregnant women,[16] while two epidemiological studies analyzing associations between pregnancy bisphenol A,[46,47] bisphenol S,[46] and maternal or cord blood cortisone did not reveal any associations. However, these studies assessed bisphenols in single blood draws[47] or spot urine,[46] which is not optimal for short half-life chemicals.[18] Combined with limited sample sizes ($n < 60$), these may explain the null results observed.

Our results on the associations between bisphenol S and cortisol are partially supported by toxicological studies showing that, in zebrafish, long- but not short-term exposure led to higher body cortisol levels.[48,49] Regarding potential mechanisms of action, *in silico* and *in vitro* studies revealed ability of bisphenol A but not S to interfere with glucocorticoid receptors,[11,41,50,51] as well as glucocorticoid biosynthesis and metabolism.[52,53] Moreover, bisphenol A showed inhibiting properties on human and rat 11β-HSD1 and 11β-HSD2, two enzymes necessary for the interconversion between biologically inactive cortisone and the active cortisol. Compared to bisphenol A, bisphenol S showed a lack of[54] or weak inhibiting activity on 11β-HSD1.[55]

Parabens

Propylparaben was associated with lower ∑cortisol, ∑cortisone, 11-dehydrocorticosterone, and to some extent, the ∑cortisol/∑cortisone ratio and progesterone. Methylparaben was associated with lower cortisol. After stratification for fetal sex, most of these associations were observed in women carrying female fetuses. While the mechanisms underlying the observed sex differences remain unclear, we hypothesize that they may arise from difference in the hormonal activity of the fetus and the placenta.[56] Fetal hormone production may differently affect maternal steroid hormone levels depending on fetal sex.[38,39] The placenta itself produce some steroid hormones (e.g., progesterone), has the capability to metabolize some fetal hormones (e.g., converting testosterone into oestradiol), and is known to exhibit sexual dimorphism in various aspects.[56–59] Thus, the modification of effects by sex could be attributed to factors related to the fetal sex, the placenta, or a combination of both.

Two previous epidemiological studies with a limited sample size ($n < 60$) did not report associations between propyl- or methylparaben and cortisol or cortisone, either when sex of the offspring was[46] or was not considered.[46,47] As for toxicological research, early stage exposure of zebrafish larvae to propyl- and/or methylparaben was linked to an increase of cortisol levels[60,61] and mostly down-regulated transcription of target genes reflecting the hypothalamic–pituitary–interrenal (HPI) axis function.[60] Our results differed from those of the zebrafish study, as we observed a decrease in cortisol levels with parabens exposure rather than an increase. Regarding potential biological pathways involved, further investigation into initial molecular events suggested that parabens might

Table 2. Associations between maternal prenatal urinary phenol concentrations and hair steroid hormones, stratified by child's sex ($n = 455$ females, $n = 473$ males). Study population from two cohorts, BiSC (Bacelona, Spain, 2018–2021) and SEPAGES (Grenoble, France, 2014–2017).

		∑Cortisol				∑Cortisone			
Phenol biomarker	Child's sex	*n*	PC (95% CI)	*p*-Value	Int. *p*-value	*n*	PC (95% CI)	*p*-Value	Int. *p*-value
Methylparaben	Male	473	−0.02 (−0.08, 0.04)	0.52	0.05	—	—	—	—
	Female	454	−0.08 (−0.14, −0.02)	0.01	—	—	—	—	—
Propylparaben	Male	473	−0.01 (−0.04, 0.02)	0.69	0.07	473	−0.01 (−0.04, 0.02)	0.68	0.02
	Female	454	−0.04 (−0.07, −0.01)	0.01		455	−0.05 (−0.09, −0.02)	0.00	—

Note: Stratified analysis was performed if the *p*-value for the interaction term between exposure biomarker and child's sex was below 0.1. Regression models were adjusted for center, maternal age, BMI, education, active and passive smoking, alcohol consumption, ancestry, parity, and season of hair collection. β regression estimates were expressed as PC for each two-fold increase in standardized urinary phenol biomarkers using the following formula: $(2^{\beta}-1)\times 100$. —, no data; BiSC, Barcelona Life Study Cohort; BMI, body mass index; CI, confidence interval; Int., interaction; PC, percent change; SEPAGES, Assessment of Air Pollution exposure during Pregnancy and Effect on Health.

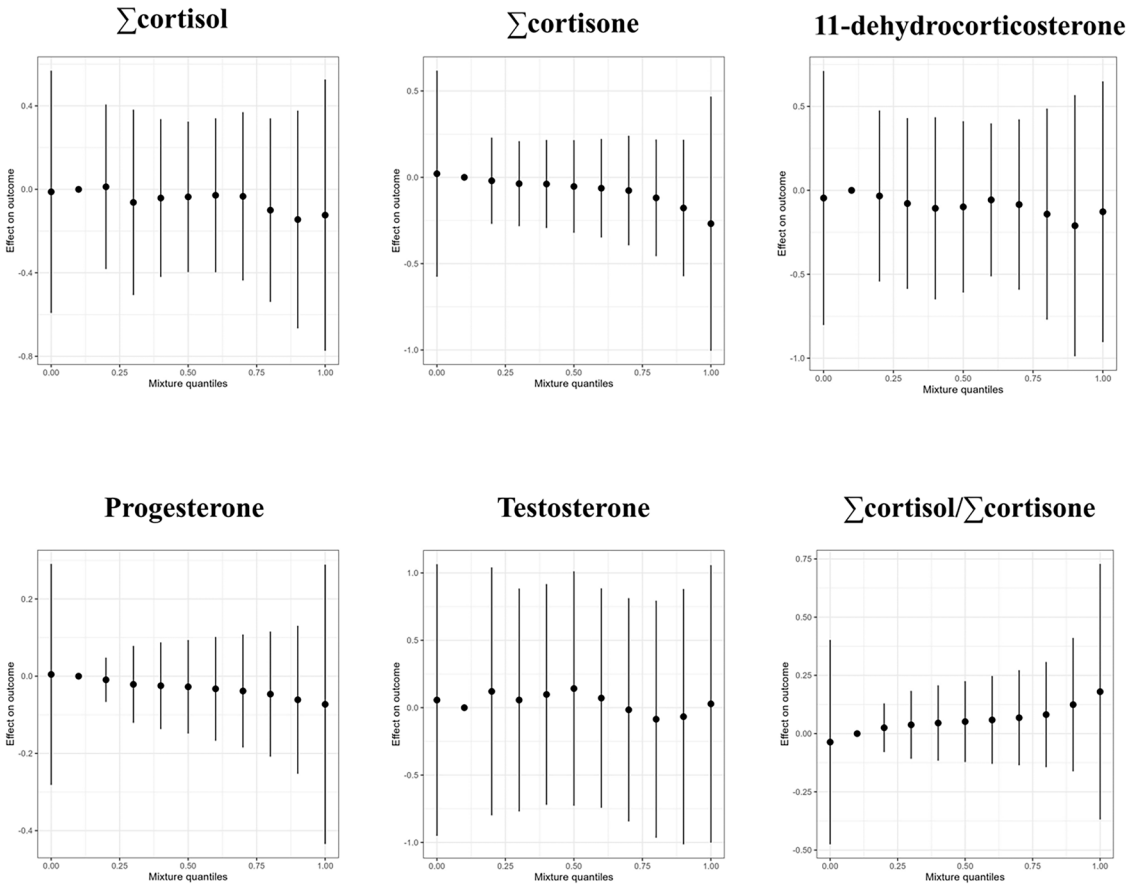

Figure 4. Change in maternal steroid hormone levels for each 10% increase in pregnancy exposure to the mixture of phenols in the BiSC ($n = 546$; Barcelona, Spain, 2018–2021) and SEPAGES ($n = 382$; Grenoble, France, 2014–2017) cohorts. BKMR models were adjusted for center, maternal age, BMI, education, active and passive smoking, alcohol consumption, ancestry, child's sex, parity, and season of hair collection. Numerical values are displayed in Table S6. Note: BiSC, Barcelona Life Study Cohort; BKMR, Bayesian kernel machine regression; BMI, body mass index; SEPAGES, Assessment of Air Pollution exposure during Pregnancy and Effect on Health.

activate the glucocorticoid receptor *in vitro* and *in silico*.[60] Such an agonistic effect, if present in humans, could trigger the HPA axis negative feedback loop, potentially explaining the reduction in circulating cortisol levels observed in our study.

Mixture of Phenols

We did not observe associations between the phenols mixture and hair hormone concentrations, which may suggest that individual phenols (bisphenol A and S and methyl- and propylparaben) rather than a "cocktail," drove the changes observed in hormone levels. Null results in a mixture model may also occur if the chemicals included in the mixture have opposing effects that could cancel each other. For example, as shown by the uni-pollutant model, bisphenol S was positively associated with cortisol while negative association was observed for parabens.

Strengths and Limitations

In our study, we intended to limit misclassification in exposure by including up to 12 urine samples in BiSC and 21 in SEPAGES. This, along with the large sample size ($n = 928$), was expected to increase statistical power compared to previous studies relying on a lower number of individuals ($n \leq 174$) and spot urine samples.[18]

Hormones measured in hair provide an indication of systemic cortisol exposure over longer time periods compared to saliva or blood.[24,62] In pregnancy, measuring cortisol in hair allowed us to identify the rise in cortisol production during the third trimester and its subsequent decline postpartum,[24] demonstrating the ability of hair cortisol concentrations to be tracked over time. However, the exact time frame that a 3-cm-long hair segment represents likely varies between individuals due to significant interindividual variations in hair growth rates and life stage, since pregnancy can influence both hair growth and thickness.[62] Additionally, hair cortisol concentration does not allow for capturing of the circadian variations, which may also be influenced by phenol exposure.

Although we measured several hormones, we were unable to assess estrogen, which would have provided a more comprehensive view of the potential effects of phenols on the HPG axis.

Relying on two cohorts with different confounding structure increased robustness of our findings. In such a setting, associations that are consistent in both cohorts (as for our main results) are less likely to result from unmeasured confounders than associations observed in one cohort only.[63] However, the women in the BiSC and SEPAGES studies were generally highly educated and of Caucasian ancestry, which may limit the generalizability of our results.

Finally, given the relatively large number of associations tested (five hormones and eight exposures) and the lack of formal correction for multiple comparisons,[43] some of the observed associations may be due to chance and should therefore be interpreted with caution.

Relevance for Public Health

The equilibrium between gestational steroid hormones is key for healthy pregnancy and fetal development.[3] Dysregulation of the

HPA axis may lead to overexposure of the developing fetus to glucocorticoids and affect its development[4,64] and health later in life.[65,66] Our study is one of the first ones to assess the associations between phenol exposure and glucocorticoid production during pregnancy.

Conclusions

Our results suggest associations between pregnancy exposure and bisphenols (A and S), as well as methyl- and propylparaben and maternal production of cortisol, cortisone, and 11-dehydrocorticosterone. Dysregulation of the HPA axis during this critical period may affect pregnancy outcomes and fetal development.

Acknowledgments

Conceptualization: C. Philippat; Data curation: S. Lyon-Caen, P. Jedynak, and M. Rolland; Formal analysis: P. Jedynak, V. Mustieles, M. Rolland, C. Philippat, M. Bustamante, and P. Dadvand; Resources: M. Bustamante, P. Dadvand, J. Sunyer, S. Bayat, R. Slama, C. Philippat, C. Thomsen, A. K. Sakhi, A. Sabaredzovic, and O. J. Pozo; Funding acquisition: C. Philippat, P. Dadvand, and M. Foraster; Investigation: P. Jedynak, C. Philippat, M. Bustamante, M. Rolland, R. Slama, and S. Lyon-Caen; Methodology: P. Jedynak, C. Philippat, M. Rolland, M. Bustamante, P. Dadvand, O. J. Pozo, J. Sunyer, R. Slama, C. Thomsen, A. K. Sakhi, and A. Sabaredzovic; Software: P. Jedynak and M. Rolland; Supervision: C. Philippat; Writing – original draft: P. Jedynak and C. Philippat; Writing – reviewing & editing: S. Bayat, M. Bustamante, P. Dadvand, M. Foraster, M. Gascon, D. Gómez-Roig, E. Llurba, S. Lyon-Caen, V. Mustieles, I. Ouellet-Morin, O. J. Pozo, I. Rivas, M. Rolland, A. Sabaredzovic, A. K. Sakhi, R. Slama, J. Sunyer, C. Thomsen, and M. Vrijheid.

We would like to thank all the BiSC and SEPAGES participants and their families for their generous collaboration. We are also very grateful to all the former and current BiSC team members (full list: https://projectebisc.org/en/team) for their tremendous contributions to this cohort. SEPAGES biospecimens are stored at Grenoble University Hospital (CHU-GA) biobank (ISO 9001 195 standard, Grenoble University Hospital, bb-0033-00069); we would like to thank the CRB team for their work of biospecimen processing and pooling as well as the SEPAGES study group along with clinical research assistants, fieldworkers, neuropsychologists, and the staff from Grenoble Center for Clinical Investigation (CIC). The SEPAGES study group includes: E. Eyriey, A. Licinia, A. Vellement (Groupe Hospitalier Mutualiste, Grenoble), I. Pin, S. Bayat, P. Hoffmann, E. Hullo, C. Llerena (Grenoble Alpes University Hospital, La Tronche), X. Morin (Clinique des Cédres, Echirolles), A. Morlot (Clinique Belledonne, Saint-Martin d'Hères), J. Lepeule, S. Lyon-Caen, C. Philippat, I. Pin, J. Quentin, V. Siroux, R. Slama (Grenoble Alpes University, Inserm, CNRS, IAB). We acknowledge K. Supernant and A. Boudier for data management. We would also like to thank M. Ouidir and N. Jovanovic for their help on running the BKMR analyses and M. Rolland for the code review. SEPAGES data are stored thanks to Inserm RE-CO-NAI platform funded by Commissariat Général à l'Investissement.

This project was supported by the French Agency for Food, Environmental and Occupational Health & Safety (ANSES, HyPAxE project number EST2019/1/039), the French National Agency for Research (ANR, EDeN project number ANR-19-CE36-000301), and the European Union's Horizon 2020 research and innovation program under grant agreement 874583 [ATHLETE]. The SEPAGES cohort was supported by the ANR (MEMORI project number ANR-21-CE34-0022, SYMER project number ANR-15-IDEX-02) and the ANSES (PNREST PENDORE 2018/1/264). Paulina Jedynak was supported by grants from ANR SYMER project number ANR-15-IDEX-02 and ANSES (HyPAxE and PENDORE project numbers EST-2019/1/039 and 2018/1/264). The SEPAGES cohort was supported by the European Research Council (number 311765-E-DOHaD), the European Community's Seventh Framework Programme (FP7/2007-206, number 308333-892 HELIX), the European Union's Horizon 2020 Research and Innovation Programme (number 874583 ATHLETE Project, number 825712 OBERON Project), ANR (PAPER project number ANR-12-PDOC-0029-01; SHALCOH project number ANR-14-CE21-0007, ANR-15-IDEX-02, and ANR-15-IDEX5; GUMME project number ANR-18CE36-005; ETAPE project ANR – EDeN project number ANR-19-CE36-000301; and ORANDANI project number ANR-22-CE36-0018), ANSES (CNAP project number EST-2016-121, HyPAxE project number EST2019/1/039), the Plan Cancer (Canc'Air project), the French Cancer Research Foundation Association de Recherche sur le Cancer (ARC), the French Endowment Fund AGIR for chronic diseases (APMC, projects PRENAPAR, LCI-FOT, DysCard), the French Endowment Fund for Respiratory Health, the French Fund – Fondation de France (CLIMATHES 00081169, SEPAGES 5 – 00099903, ELEMENTUM - 00124527). The BiSC cohort was supported by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (785994 – AirNB project) and the Health Effects Institute (HEI), an organization jointly funded by the United States Environmental Protection Agency (EPA) (Assistance Award number R-82811201) and certain motor vehicle and engine manufacturers. The contents of this article do not necessarily reflect the views of HEI or its sponsors nor do they necessarily reflect the views and policies of the EPA or motor vehicle and engine manufacturers. A full list of the funding sources that supported specific parts of the project can be found at https://projectebisc.org/en/funding-sources. Mireia Gascon holds a Miguel Servet fellowship (grant CP19/00183) funded by Acción Estratégica de Salud – Instituto de Salud Carlos III, co-funded by European Social Fund "Investing in your future." Ioar Rivas received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement number 886121 and Ramón y Cajal fellowship (RYC2021-032781-I), funded by the MCIN/AEI/10.13039/501100011033 and the European Union «NextGenerationEU»/PRTR. ISGlobal acknowledges support from the grant CEX2018-000806-S funded by MCIN/AEI/10.13039/501100011033 and support from the Generalitat de Catalunya through the CERCA Program. Vicente Mustieles was supported by a Sara Borrell postdoctoral research contract (CD22/00176), granted by Instituto de Salud Carlos III (Spain) and «NextGenerationEU» funds. Isabelle Ouellet-Morin is the Canadian Research Chair in the Developmental Origins of Vulnerability and Resilience.

The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; decision to submit the manuscript for publication. The authors have no relevant financial or nonfinancial interests to disclose.

The manuscript does not contain any individual person's data in any form (including any individual details, images, or videos).

References

1. Dunlavey CJ. 2018. Introduction to the hypothalamic–pituitary–adrenal axis: healthy and dysregulated stress responses, developmental stress and neurodegeneration. J Undergrad Neurosci Educ 16(2):R59–R60, PMID: 30057514.
2. Hansel MC, Rosenberg AM, Kinkade CW, Capurro C, Rivera-Núñez Z, Barrett ES, et al. 2024. Exposure to synthetic endocrine-disrupting chemicals in relation to maternal and fetal sex steroid hormones: a scoping review. Curr Environ Health Rep 11(3):356–379, PMID: 39037689, https://doi.org/10.1007/s40572-024-00455-6.

3. Solano ME, Arck PC. 2019. Steroids, pregnancy and fetal development. Front Immunol 10:3017, PMID: 32038609, https://doi.org/10.3389/fimmu.2019.03017.
4. Monticone S, Auchus RJ, Rainey WE. 2012. Adrenal disorders in pregnancy. Nat Rev Endocrinol 8(11):668–678, PMID: 22965163, https://doi.org/10.1038/nrendo.2012.155.
5. Cherak SJ, Giesbrecht GF, Metcalfe A, Ronksley PE, Malebranche ME. 2018. The effect of gestational period on the association between maternal prenatal salivary cortisol and birth weight: a systematic review and meta-analysis. Psychoneuroendocrinology 94:49–62, PMID: 29754005, https://doi.org/10.1016/j.psyneuen.2018.04.023.
6. Crowther CA, Doyle LW, Haslam RR, Hiller JE, Harding JE, Robinson JS, et al. 2007. Outcomes at 2 years of age after repeat doses of antenatal corticosteroids. N Engl J Med 357(12):1179–1189, PMID: 17881750, https://doi.org/10.1056/NEJMoa071152.
7. French NP, Hagan R, Evans SF, Mullan A, Newnham JP. 2004. Repeated antenatal corticosteroids: effects on cerebral palsy and childhood behavior. Am J Obstet Gynecol 190(3):588–595, PMID: 15041985, https://doi.org/10.1016/j.ajog.2003.12.016.
8. Wetherill YB, Akingbemi BT, Kanno J, McLachlan JA, Nadal A, Sonnenschein C, et al. 2007. In vitro molecular mechanisms of bisphenol A action. Reprod Toxicol 24(2):178–198, PMID: 17628395, https://doi.org/10.1016/j.reprotox.2007.05.010.
9. The International Panel on Chemical Pollution. 2017. *Overview Report II: An Overview of Current Scientific Knowledge on the Life Cycles, Environmental Exposures, and Environmental Effects of Select Endocrine Disrupting Chemicals (EDCs) and Potential EDCs*. Nairobi, Kenya: United Nations Environment Programme.
10. La Merrill MA, Vandenberg LN, Smith MT, Goodson W, Browne P, Patisaul HB, et al. 2020. Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification. Nat Rev Endocrinol 16(1):45–57, PMID: 31719706, https://doi.org/10.1038/s41574-019-0273-8.
11. Prasanth GK, Divya LM, Sadasivan C. 2010. Bisphenol-A can bind to human glucocorticoid receptor as an agonist: an in silico study. J Appl Toxicol 30(8):769–774, PMID: 20669259, https://doi.org/10.1002/jat.1570.
12. Rebuli ME, Cao J, Sluzas E, Delclos KB, Camacho L, Lewis SM, et al. 2014. Investigation of the effects of subchronic low dose oral exposure to bisphenol A (BPA) and ethinyl estradiol (EE) on estrogen receptor expression in the juvenile and adult female rat hypothalamus. Toxicol Sci 140(1):190–203, PMID: 24752507, https://doi.org/10.1093/toxsci/kfu074.
13. Cao J, Rebuli ME, Rogers J, Todd KL, Leyrer SM, Ferguson SA, et al. 2013. Prenatal bisphenol a exposure alters sex-specific estrogen receptor expression in the neonatal rat hypothalamus and amygdala. Toxicol Sci 133(1):157–173, PMID: 23457122, https://doi.org/10.1093/toxsci/kft035.
14. Panagiotidou E, Zerva S, Mitsiou DJ, Alexis MN, Kitraki E. 2014. Perinatal exposure to low-dose bisphenol a affects the neuroendocrine stress response in rats. J Endocrinol 220(3):207–218, PMID: 24323913, https://doi.org/10.1530/JOE-13-0416.
15. Chen F, Zhou L, Bai Y, Zhou R, Chen L. 2015. Hypothalamic-pituitary-adrenal axis hyperactivity accounts for anxiety- and depression-like behaviors in rats perinatally exposed to bisphenol A. J Biomed Res 29(3):250–258, PMID: 26060449, https://doi.org/10.7555/JBR.29.20140058.
16. Giesbrecht GF, Liu J, Ejaredar M, Dewey D, Letourneau N, Campbell T, et al. 2016. Urinary bisphenol A is associated with dysregulation of HPA-axis function in pregnant women: findings from the APrON cohort study. Environ Res 151:689–697, PMID: 27640068, https://doi.org/10.1016/j.envres.2016.09.007.
17. Giesbrecht GF, Ejaredar M, Liu J, Thomas J, Letourneau N, Campbell T, et al. 2017. Prenatal bisphenol A exposure and dysregulation of infant hypothalamic-pituitary-adrenal axis function: findings from the APrON cohort study. Environ Health 16(1):47, PMID: 28526030, https://doi.org/10.1186/s12940-017-0259-8.
18. Perrier F, Giorgis-Allemand L, Slama R, Philippat C. 2016. Within-subject pooling of biological samples to reduce exposure misclassification in biomarker-based studies. Epidemiology 27(3):378–388, PMID: 27035688, https://doi.org/10.1097/EDE.0000000000000460.
19. Dadvand P, Gascon M, Bustamante M, Rivas I, Foraster M, Basagaña X, et al. 2024. Cohort profile: Barcelona Life Study Cohort (BiSC). Int J Epidemiol 53(3):dyae063, PMID: 38725298, https://doi.org/10.1093/ije/dyae063.
20. Lyon-Caen S, Siroux V, Lepeule J, Lorimier P, Hainaut P, Mossuz P, et al. 2019. Deciphering the impact of early-life exposures to highly variable environmental factors on foetal and child health: design of SEPAGES couple-child cohort. Int J Environ Res Public Health 16(20):3888, PMID: 31615055, https://doi.org/10.3390/ijerph16203888.
21. Sauvé B, Koren G, Walsh G, Tokmakejian S, Van Uum SHM. 2007. Measurement of cortisol in human hair as a biomarker of systemic exposure. Clin Invest Med 30(5):E183–E191, PMID: 17892760, https://doi.org/10.25011/cim.v30i5.2894.
22. Cooper GAA, Kronstrand R, Kintz P. 2012. Society of hair testing guidelines for drug testing in hair. Forensic Sci Int 218(1–3):20–24, PMID: 22088946, https://doi.org/10.1016/j.forsciint.2011.10.024.
23. Gomez-Gomez A, Pozo OJ. 2020. Determination of steroid profile in hair by liquid chromatography tandem mass spectrometry. J Chromatogr A 1624:461179, PMID: 32540057, https://doi.org/10.1016/j.chroma.2020.461179.
24. Kirschbaum C, Tietze A, Skoluda N, Dettenborn L. 2009. Hair as a retrospective calendar of cortisol production—increased cortisol incorporation into hair in the third trimester of pregnancy. Psychoneuroendocrinology 34(1):32–37, PMID: 18947933, https://doi.org/10.1016/j.psyneuen.2008.08.024.
25. Wang W, Moody SN, Kiesner J, Tonon Appiani A, Robertson OC, Shirtcliff EA, et al. 2019. Assay validation of hair androgens across the menstrual cycle. Psychoneuroendocrinology 101:175–181, PMID: 30469084, https://doi.org/10.1016/j.psyneuen.2018.10.029.
26. Philippat C, Calafat AM. 2021. Comparison of strategies to efficiently combine repeated urine samples in biomarker-based studies. Environ Res 192:110275, PMID: 33022216, https://doi.org/10.1016/j.envres.2020.110275.
27. Vernet C, Philippat C, Agier L, Calafat AM, Ye X, Lyon-Caen S, et al. 2019. An empirical validation of the within-subject biospecimens pooling approach to minimize exposure misclassification in biomarker-based studies. Epidemiology 30(5):756–767, PMID: 31373935, https://doi.org/10.1097/EDE.0000000000001056.
28. Sakhi AK, Sabaredzovic A, Papadopoulou E, Cequier E, Thomsen C. 2018. Levels, variability and determinants of environmental phenols in pairs of Norwegian mothers and children. Environ Int 114:242–251, PMID: 29524920, https://doi.org/10.1016/j.envint.2018.02.037.
29. Lee L. 2020. NADA: Nondetects and Data Analysis for Environmental Data. https://cran.r-project.org/web/packages/NADA/index.html [accessed 5 May 2025].
30. Jackson C. 2011. Multi-state models for panel data: the msm package for R. J Stat Soft 38(8):1–28, https://doi.org/10.18637/jss.v038.i08.
31. Jackson C. 2022. Msm: Multi-State Markov and Hidden Markov Models in Continuous Time. https://cran.r-project.org/web/packages/msm/index.html [accessed 5 May 2025].
32. Helsel DR. 1990. Less than obvious - statistical treatment of data below the detection limit. Environ Sci Technol 24(12):1766–1774, https://doi.org/10.1021/es00082a001.
33. Lubin JH, Colt JS, Camann D, Davis S, Cerhan JR, Severson RK, et al. 2004. Epidemiologic evaluation of measurement data in the presence of detection limits. Environ Health Perspect 112(17):1691–1696, PMID: 15579415, https://doi.org/10.1289/ehp.7199.
34. Guilbert A, Rolland M, Pin I, Thomsen C, Sakhi AK, Sabaredzovic A, et al. 2021. Associations between a mixture of phenols and phthalates and child behaviour in a French mother–child cohort with repeated assessment of exposure. Environ Int 156:106697, PMID: 34147998, https://doi.org/10.1016/j.envint.2021.106697.
35. Mortamais M, Chevrier C, Philippat C, Petit C, Calafat AM, Ye X, et al. 2012. Correcting for the influence of sampling conditions on biomarkers of exposure to phenols and phthalates: a 2-step standardization method based on regression residuals. Environ Health 11(1):29, PMID: 22537080, https://doi.org/10.1186/1476-069X-11-29.
36. Mustieles V, Lascouts A, Pozo OJ, Haro N, Lyon-Caen S, Jedynak P, et al. 2023. Longitudinal associations between prenatal exposure to phthalates and steroid hormones in maternal hair samples from the SEPAGES cohort. Environ Sci Technol 57(48):19202–19213, PMID: 37931007, https://doi.org/10.1021/acs.est.3c03401.
37. Textor J, van der Zander B, Gilthorpe MS, Liskiewicz M, Ellison GT. 2016. Robust causal inference using directed acyclic graphs: the R package 'dagitty'. Int J Epidemiol 45(6):1887–1894, PMID: 28089956, https://doi.org/10.1093/ije/dyw341.
38. Meulenberg PMM, Hofman JA. 1991. Maternal testosterone and fetal sex. J Steroid Biochem Mol Biol 39(1):51–54, PMID: 2069866, https://doi.org/10.1016/0960-0760(91)90012-t.
39. Vrijkotte T, de Rooij SR, Roseboom TJ, Twickler TB. 2023. Maternal serum cortisol levels during pregnancy differ by fetal sex. Psychoneuroendocrinology 149:105999, PMID: 36543024, https://doi.org/10.1016/j.psyneuen.2022.105999.
40. Chen F, Zhou L, Bai Y, Zhou R, Chen L. 2014. Sex differences in the adult HPA axis and affective behaviors are altered by perinatal exposure to a low dose of bisphenol A. Brain Res 1571:12–24, PMID: 24857958, https://doi.org/10.1016/j.brainres.2014.05.010.
41. Poimenova A, Markaki E, Rahiotis C, Kitraki E. 2010. Corticosterone-regulated actions in the rat brain are affected by perinatal exposure to low dose of bisphenol A. Neuroscience 167(3):741–749, PMID: 20219646, https://doi.org/10.1016/j.neuroscience.2010.02.051.
42. Amrhein V, Greenland S, McShane B. 2019. Scientists rise up against statistical significance. Nature 567(7748):305–307, PMID: 30894741, https://doi.org/10.1038/d41586-019-00857-9.
43. Rothman KJ. 1990. No adjustments are needed for multiple comparisons. Epidemiology 1(1):43–46, PMID: 2081237, https://doi.org/10.1097/00001648-199001000-00010.
44. Bobb JF, Valeri L, Claus Henn B, Christiani DC, Wright RO, Mazumdar M, et al. 2015. Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. Biostatistics 16(3):493–508, PMID: 25532525, https://doi.org/10.1093/biostatistics/kxu058.

45. Bobb JF, Claus Henn B, Valeri L, Coull BA. 2018. Statistical software for analyzing the health effects of multiple concurrent exposures via Bayesian kernel machine regression. Environ Health 17(1):67, PMID: 30126431, https://doi.org/10.1186/s12940-018-0413-y.
46. Banker M, Puttabyatappa M, O'Day P, Goodrich JM, Kelley AS, Domino SE, et al. 2021. Association of maternal-neonatal steroids with early pregnancy endocrine disrupting chemicals and pregnancy outcomes. J Clin Endocrinol Metab 106(3):665–687, PMID: 33280001, https://doi.org/10.1210/clinem/dgaa909.
47. Kolatorova L, Vitku J, Hampl R, Adamcova K, Skodova T, Simkova M, et al. 2018. Exposure to bisphenols and parabens during pregnancy and relations to steroid changes. Environ Res 163:115–122, PMID: 29433019, https://doi.org/10.1016/j.envres.2018.01.031.
48. Park C-B, Kim G-E, On J, Pyo H, Park J-W, Cho S-H, et al. 2022. Sex-specific effects of bisphenol S with tissue-specific responsiveness in adult zebrafish: the antiandrogenic and antiestrogenic effects. Ecotoxicol Environ Saf 229:113102, PMID: 34942420, https://doi.org/10.1016/j.ecoenv.2021.113102.
49. Wei P, Zhao F, Zhang X, Ru S. 2020. Long-term exposure of zebrafish to bisphenol S impairs stress function of hypothalamic-pituitary-interrenal axis and causes anxiety-like behavioral responses to novelty. Sci Total Environ 716:137092, PMID: 32044495, https://doi.org/10.1016/j.scitotenv.2020.137092.
50. Ma M, Zhao W, Tan T, Hitabatuma A, Wang P, Wang R, et al. 2022. Study of eighteen typical bisphenol analogues as agonist or antagonist for androgen and glucocorticoid at sub-micromolar concentrations in vitro. Sci Total Environ 822:153439, PMID: 35093365, https://doi.org/10.1016/j.scitotenv.2022.153439.
51. Sargis RM, Johnson DN, Choudhury RA, Brady MJ. 2010. Environmental endocrine disruptors promote adipogenesis in the 3T3-L1 cell line through glucocorticoid receptor activation. Obesity (Silver Spring) 18(7):1283–1288, PMID: 19927138, https://doi.org/10.1038/oby.2009.419.
52. Guo J, Yuan X, Qiu L, Zhu W, Wang C, Hu G, et al. 2012. Inhibition of human and rat 11β-hydroxysteroid dehydrogenases activities by bisphenol A. Toxicol Lett 215(2):126–130, PMID: 23069880, https://doi.org/10.1016/j.toxlet.2012.10.002.
53. Zhang B, Wang S, Tang Y, Hu Z, Shi L, Lu J, et al. 2023. Direct inhibition of bisphenols on human and rat 11β-hydroxysteroid dehydrogenase 2: structure-activity relationship and docking analysis. Ecotoxicol Environ Saf 254:114715, PMID: 36871355, https://doi.org/10.1016/j.ecoenv.2023.114715.
54. Lu H, Wang S, Zheng J, Zhu Y, Wang Y, Li H, et al. 2023. Distinct inhibitory strength of bisphenol a analogues on human and rat 11β-hydroxysteroid dehydrogenase 1: 3D quantitative structure-activity relationship and in silico molecular docking analysis. Ecotoxicol Environ Saf 267:115638, PMID: 37918333, https://doi.org/10.1016/j.ecoenv.2023.115638.
55. Wang H, Sang J, Ji Z, Yu Y, Wang S, Zhu Y, et al. 2024. Halogenated bisphenol a derivatives potently inhibit human and rat 11β-hydroxysteroid dehydrogenase 1: structure–activity relationship and molecular docking. Environ Toxicol 39(5):2560–2571, PMID: 38189224, https://doi.org/10.1002/tox.24124.
56. Pasqualini JR. 2005. Enzymes involved in the formation and transformation of steroid hormones in the fetal and placental compartments. J Steroid Biochem Mol Biol 97(5):401–415, PMID: 16202579, https://doi.org/10.1016/j.jsbmb.2005.08.004.
57. Gonzalez TL, Sun T, Koeppel AF, Lee B, Wang ET, Farber CR, et al. 2018. Sex differences in the late first trimester human placenta transcriptome. Biol Sex Differ 9(1):4, PMID: 29335024, https://doi.org/10.1186/s13293-018-0165-y.
58. Gonzalez TL, Willson BE, Wang ET, Taylor KD, Novoa A, Swarna A, et al. 2024. Sexually dimorphic DNA methylation and gene expression patterns in human first trimester placenta. Biol Sex Differ 15(1):63, PMID: 39152463, https://doi.org/10.1186/s13293-024-00629-9.
59. Yu P, Chen Y, Ge C, Wang H. 2021. Sexual dimorphism in placental development and its contribution to health and diseases. Crit Rev Toxicol 51(6):555–570, PMID: 34666604, https://doi.org/10.1080/10408444.2021.1977237.
60. Liang J, Yang X, Xiang T, Chen X, Ren Z, Wang X, et al. 2023. The perturbation of parabens on the neuroendocrine system in zebrafish larvae. Sci Total Environ 882:163593, PMID: 37087015, https://doi.org/10.1016/j.scitotenv.2023.163593.
61. Luzeena Raja G, Divya Subhashree K, Lite C, Santosh W, Barathi S. 2019. Transient exposure of methylparaben to zebrafish (Danio rerio) embryos altered cortisol level, acetylcholinesterase activity and induced anxiety-like behaviour. Gen Comp Endocrinol 279:53–59, PMID: 30395803, https://doi.org/10.1016/j.ygcen.2018.11.001.
62. Marceau K, Wang W, Robertson O, Shirtcliff EA. 2020. A systematic review of hair cortisol during pregnancy: reference ranges and methodological considerations. Psychoneuroendocrinology 122:104904, PMID: 33080521, https://doi.org/10.1016/j.psyneuen.2020.104904.
63. Richmond RC, Al-Amin A, Smith GD, Relton CL. 2014. Approaches for drawing causal inferences from epidemiological birth cohorts: a review. Early Hum Dev 90(11):769–780, PMID: 25260961, https://doi.org/10.1016/j.earlhumdev.2014.08.023.
64. Mina TH, Reynolds RM. 2014. Mechanisms linking in utero stress to altered offspring behaviour. Curr Top Behav Neurosci 18:93–122, PMID: 24577734, https://doi.org/10.1007/7854_2014_291.
65. Dickens MJ, Pawluski JL. 2018. The HPA axis during the perinatal period: implications for perinatal depression. Endocrinology 159(11):3737–3746, PMID: 30256957, https://doi.org/10.1210/en.2018-00677.
66. Duthie L, Reynolds RM. 2013. Changes in the maternal hypothalamic-pituitary-adrenal axis in pregnancy and postpartum: influences on maternal and fetal outcomes. Neuroendocrinology 98(2):106–115, PMID: 23969897, https://doi.org/10.1159/000354702.