

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

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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 (Σ cortisol, Σ 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 birth⁵ 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 agonist¹¹ 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.

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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, Spain¹⁹ 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 (20 α - and 20 β -dihydrocortisol), cortisone and its metabolites (20 α - and 20 β -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 μ L of K₂CO₃ (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 μ L of water:methanol (1:1), and 10 μ L 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 woman (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 ($<\text{LOD}$, $\geq\text{LOD}$). 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 metabolites³⁶ 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 $\geq$ 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/m²), 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). $\sum$ cortisol and $\sum$ cortisone were also highly correlated (rho > 0.8).

Associations between Phenols and Hormone Concentrations

Uni-pollutant models. Bisphenol S was associated with higher $\sum$ 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 $\sum$ cortisone (PC = 12.8%; 95% CI: -0.1, 27.5). We also observed an isolated negative association between bisphenol A and $\sum$ 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 $\sum$ cortisol (PC = -1.55%; 95% CI: -2.96, -0.11), $\sum$ 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 $\sum$ cortisol/ $\sum$ cortisone ratio (PC = 0.57%; 95% CI: -0.1, 1.24). Methylparaben was associated with lower $\sum$ cortisol (PC = -3.22%; 95% CI: -5.98, -0.38) and $\sum$ cortisone (PC = -2.77%; 95% CI: -5.72, 0.27) (Figure 3; Table S4).

Finally, triclosan was associated with a higher $\sum$ cortisol/ $\sum$ 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 $\sum$ 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 $\sum$ 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.

^ap-Values obtained for a chi-squared test (categorical variables) or ANOVA (numerical variables).

^bFor 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.

^cFor 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.

^dFor 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.

^eFor 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.

^fFor 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).

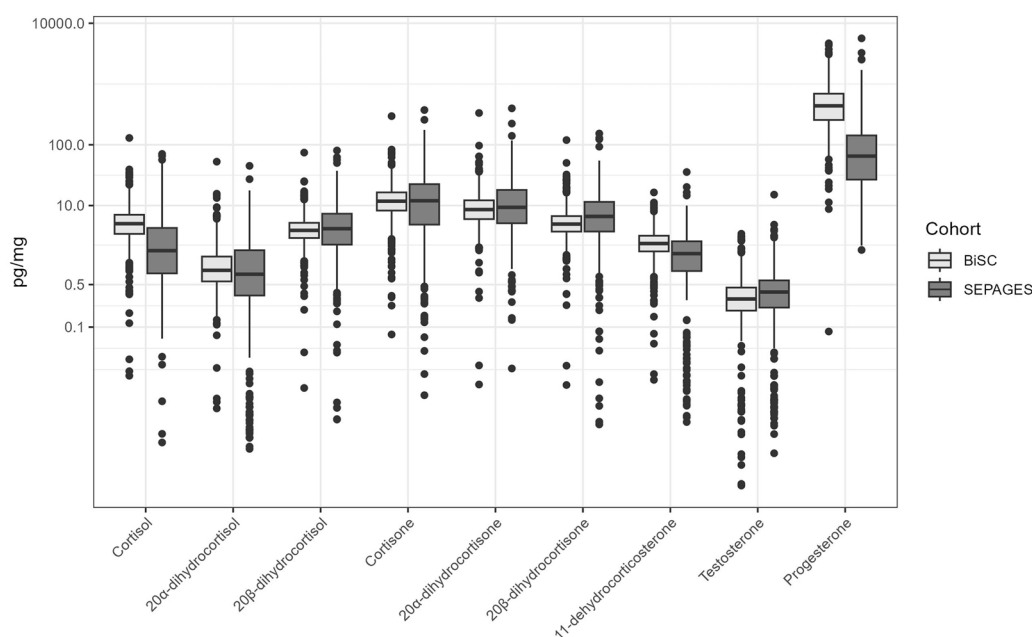


Figure 1. Standardized concentrations of phenols assessed in the urine samples, expressed as micrograms of phenol per liter of urine (BiSC, Barcelona, Spain, 2018–2021, $n = 546$; SEPAGES, Grenoble, France, 2014–2017, $n = 382$). Numerical values are displayed in Table S2. Note: BiSC, Barcelona Life 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

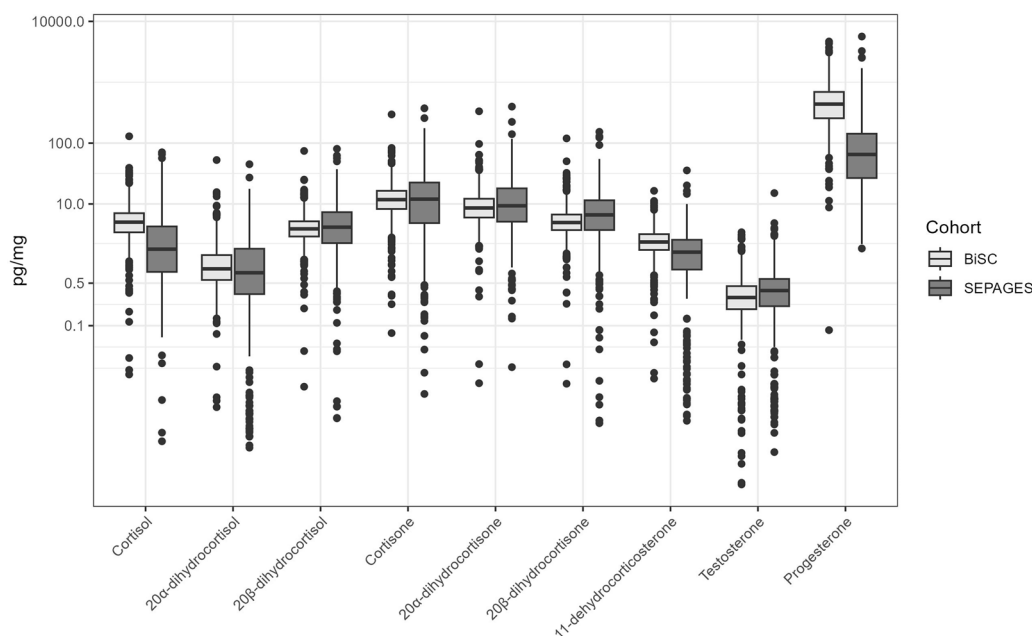


Figure 2. Standardized concentrations of hormones assessed in hair, expressed as picograms of hormone per milligram of hair (BiSC, Barcelona, Spain, 2018–2021, $n = 546$; SEPAGES, Grenoble, France, 2014–2017, $n = 382$). Numerical values are displayed in Table S3. Note: BiSC, Barcelona Life Study Cohort;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

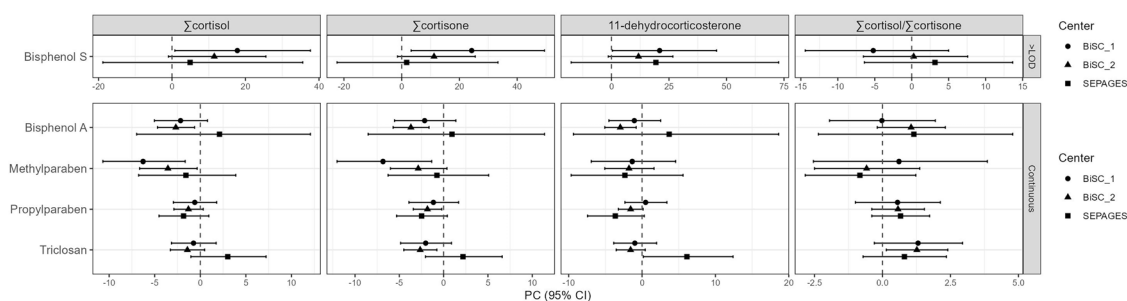


Figure 3. Adjusted associations between pregnancy phenol concentrations assessed in pooled urine samples during pregnancy and steroid hormone concentrations assessed in hair [$n=928$ from BiSC (Barcelona, Spain, 2018–2021) and SEPAGES (Grenoble, France, 2014–2017)]. Regression models were adjusted for 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 $\geq$ 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 Σ cortisol and 11-dehydrocorticosterone and tended to be positively associated with Σ cortisone levels. On the contrary, bisphenol A was associated with lower Σ 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 draws⁴⁷ 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 11 β -HSD1 and 11 β -HSD2, two enzymes necessary for the interconversion between biologically inactive cortisone and the active cortisol. Compared to bisphenol A, bisphenol S showed a lack of⁵⁴ or weak inhibiting activity on 11 β -HSD1.⁵⁵

Parabens

Propylparaben was associated with lower Σ cortisol, Σ cortisone, 11-dehydrocorticosterone, and to some extent, the Σ cortisol/ Σ 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 was⁴⁶ 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 (Barcelona, Spain, 2018–2021) and SEPAGES (Grenoble, France, 2014–2017).

Phenol biomarker	Child's sex	Σ Cortisol				Σ Cortisone			
		<i>n</i>	PC (95% CI)	<i>p</i> -Value	Int. <i>p</i> -value	<i>n</i>	PC (95% CI)	<i>p</i> -Value	Int. <i>p</i> -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.

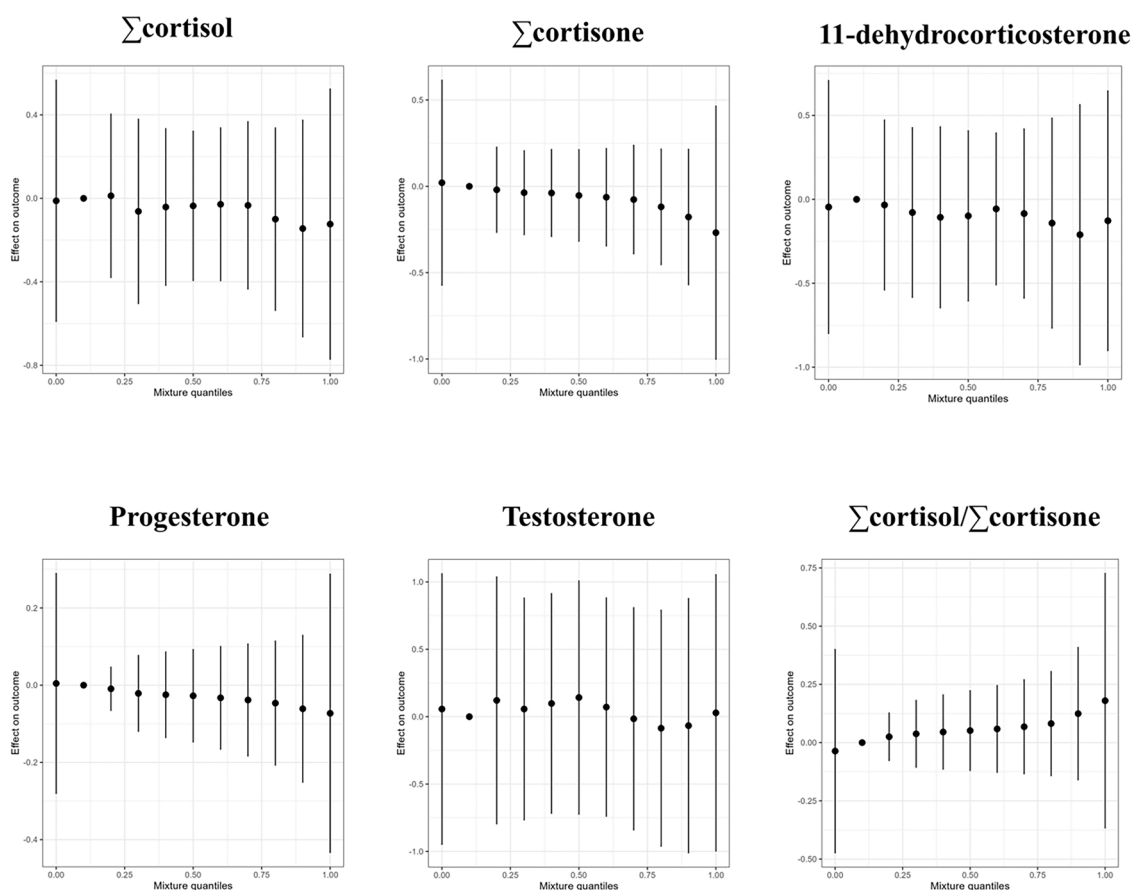


Figure 4. Change in maternal steroid hormone levels for each 10% increase in pregnancy exposure to the mixture of phenols in the BiSC ($n = 546$; Barcelona, Spain, 2018–2021) and SEPAGES ($n = 382$; Grenoble, France, 2014–2017) cohorts. BKMR models were adjusted for center, maternal age, BMI, education, 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.

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

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