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Organophosphate Flame Retardants, Highly Fluorinated Chemicals, and Biomarkers of Placental Development and Disease During Mid-Gestation

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ABSTRACT

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) and organophosphate flame retardants (OPFRs) are chemicals that may contribute to placenta-mediated complications and adverse maternal-fetal health risks. Few studies have investigated these chemicals in relation to biomarkers of effect during pregnancy. We measured 12 PFASs and four urinary OPFR metabolites in 132 healthy pregnant women during mid-gestation and examined a subset with biomarkers of placental development and disease ($n = 62$). Molecular biomarkers included integrin alpha-1 (ITGA1), vascular endothelial-cadherin (CDH5), and matrix metalloproteinase-1 (MMP1). Morphological endpoints included potential indicators of placental stress and the extent of cytotrophoblast (CTB)-mediated uterine artery remodeling. Serum PFASs and urinary OPFR metabolites were detected in ~50%–100% of samples. The most prevalent PFASs were perfluorononanoic acid (PFNA), perfluorooctanoic acid (PFOA), and perfluorooctane sulfonic acid (PFOS), with geometric mean (GM) levels of ~1.3–2.8 (95% confidence limits from 1.2–3.1) ng/ml compared to ≤0.5 ng/ml for other PFASs. Diphenyl phosphate (DPhP) and bis(1,3-dichloro-2-propyl) phosphate (BDCIPP) were the most prevalent OPFR metabolites, with GMs of 2.9 (95% CI: 2.5–3.4) and 3.6 (95% CI: 2.2–3.1) ng/ml, respectively, compared to <1 ng/ml for bis(2-chloroethyl) phosphate (BCEP) and bis(1-chloro-2-propyl) phosphate (BCIPP). We found inverse associations of PFASs or OPFRs with ITGA1 or CDH5 immunoreactivity and positive associations with indicators of placental stress in multiple basal plate regions, indicating these chemicals may contribute to abnormal

placentation and future health risks. Associations with blood pressure and lipid concentrations warrant further examination. This is the first study of these chemicals with placental biomarkers measured directly in human tissues and suggests specific biomarkers are sensitive indicators of exposure during a vulnerable developmental period.

Impact Statement: This is the first investigation of maternal PFAS and OPFR levels in relation to biomarkers of placental development and disease. Findings from this study signal potential associations of PFAS and OPFR exposures with specific biomarkers related to placenta-mediated pregnancy complications.

Key words: flame retardants; perfluoroalkyl and polyfluoroalkyl substances; biomonitoring; developmental/reproductive health effects; endocrine disruption; preeclampsia; pregnancy complications; maternal health; birth outcomes; cytotrophoblast differentiation.

Perfluoroalkyl and polyfluoroalkyl substances (PFASs) and organophosphate flame retardants (OPFRs) are ubiquitous chemicals of concern that may contribute to a range of adverse maternal and fetal health outcomes (Blake and Fenton, 2020; Philbrook *et al.*, 2018; Rock *et al.*, 2018, 2020; Varshavsky *et al.*, 2020a). PFASs are widely used in numerous industrial and consumer products, including food contact materials, dental floss, clothing, furniture, and other textiles to make them nonstick, water-proof, and stain-resistant (ATSDR, 2018). These "forever chemicals" are highly persistent, with strong carbon-fluorine bonds that do not readily break down in the environment (Prevedouros *et al.*, 2006) and are detected in 98% of the U.S. population (Calafat *et al.*, 2007). OPFRs are also in widespread use and may be of particular concern for pregnant women in California, due to the State's historical flammability standards (Blum *et al.*, 2019). OPFRs have replaced polybrominated diphenyl ethers (PBDEs) in many applications since the mid-2000s (Blum *et al.*, 2019; Dodson *et al.*, 2014), when PBDEs were phased out of U.S. production (Blum *et al.*, 2019; Dodson *et al.*, 2014) prior to banning all flame retardants used in furniture and children's products within the State of California in 2018 (Assembly Bill 2998: California State Assembly, 2018).

PFASs and OPFRs have the potential to alter human health and development through multiple biological pathways. Evidence in human population-based studies and rodent models suggest that PFASs disrupt thyroid (Bloom *et al.*, 2010; Gutshall *et al.*, 1989; Lopez-Espinosa *et al.*, 2012; Wen *et al.*, 2013), immune system (Frawley *et al.*, 2018; Huang *et al.*, 2020; Rockwell *et al.*, 2017; Shane *et al.*, 2020), and placental function (Blake and Fenton, 2020; Blake *et al.*, 2020; Jiang *et al.*, 2020). They have been associated with a wide variety of health impacts including bladder cancer (Alexander and Olsen, 2007), congenital hypothyroidism (Kim *et al.*, 2016), and placenta-mediated adverse pregnancy and birth outcomes, including preeclampsia, gestational diabetes, and low birth weight, in human studies (Blake and Fenton, 2020; Varshavsky *et al.*, 2020a). Maternal PFAS exposures have also been associated with biomarkers of effect during pregnancy, including altered serum lipid, triglyceride, and cholesterol levels (Matilla-Santander *et al.*, 2017; Starling *et al.*, 2014), suggesting these compounds can disrupt critical metabolic pathways essential for pregnancy. OPFRs bioaccumulate in the placenta (Ding *et al.*, 2016) and can also induce placental toxicity in rodents (Baldwin *et al.*, 2017; Rock *et al.*, 2018, 2020) as well as in a human placental choriocarcinoma cell line (Hu *et al.*, 2017). Like their PBDE counterparts, OPFRs may further be neurotoxic and cause reproductive and developmental harm, and thus have been identified as regrettable replacements for PBDEs (AbouDonia *et al.*, 2016; Blum *et al.*, 2019).

Previously, we have shown that PBDEs are associated with altered expression of molecules on RNA (Robinson *et al.*, 2019; *in vitro*) and protein (Varshavsky *et al.*, 2020b; *in vivo*) levels that are essential for placental development and function. For example, we found that PBDE levels, measured in placenta and maternal sera during mid-gestation, were correlated with altered expression profiles of integrin alpha-1 (ITGA1) and vascular endothelial-cadherin (VE-cadherin or CDH5) in uterine-invading cytotrophoblast (CTB) cells (Varshavsky *et al.*, 2020b). These observations are corroborated by experimental *in vitro* studies demonstrating BDE-47 to inhibit the ability of primary 2nd trimester human CTBs to migrate and invade (Robinson *et al.*, 2019). Here, we expanded those studies to examine the relationship between levels of PFASs and OPFRs during pregnancy and biomarkers of placental-mediated disease, employing a strategy to measure biomarkers in placental tissues *in situ* during a vulnerable time in placental development. We measured 12 PFASs in maternal serum and four urinary OPFR metabolites among a diverse population of pregnant women during a sensitive period of placentation for maternal-fetal health (early/mid-2nd trimester). We examined PFAS and OPFR exposures in relation to our panel of molecular and morphological biomarkers of placental development and disease during this important period of gestation. We further examined chemical associations with physiological indicators of maternal-fetal health, including maternal blood pressure and total serum lipid concentrations.

MATERIALS AND METHODS

Study Recruitment and Sample Collection

We collected maternal serum, placenta, and urine samples from 138 healthy nonsmoking pregnant women during mid-gestation (gestational week [GW] 15-24) at the Women's Options Center (WOC) in the San Francisco Bay Area from 2014 to 2016 (Figure 1). The WOC is a women's health clinic that provides elective termination procedures to a racially/ethnically diverse low-income population of pregnant women in Northern and Central California. Written and verbal consents to participate were obtained during the procedure visit, followed by administration of survey questionnaires to collect sociodemographic data on race/ethnicity, educational attainment, employment status, geographical region of residence, and type of medical insurance (public vs. private). Physiological parameters, including maternal systolic blood pressure (SBP),

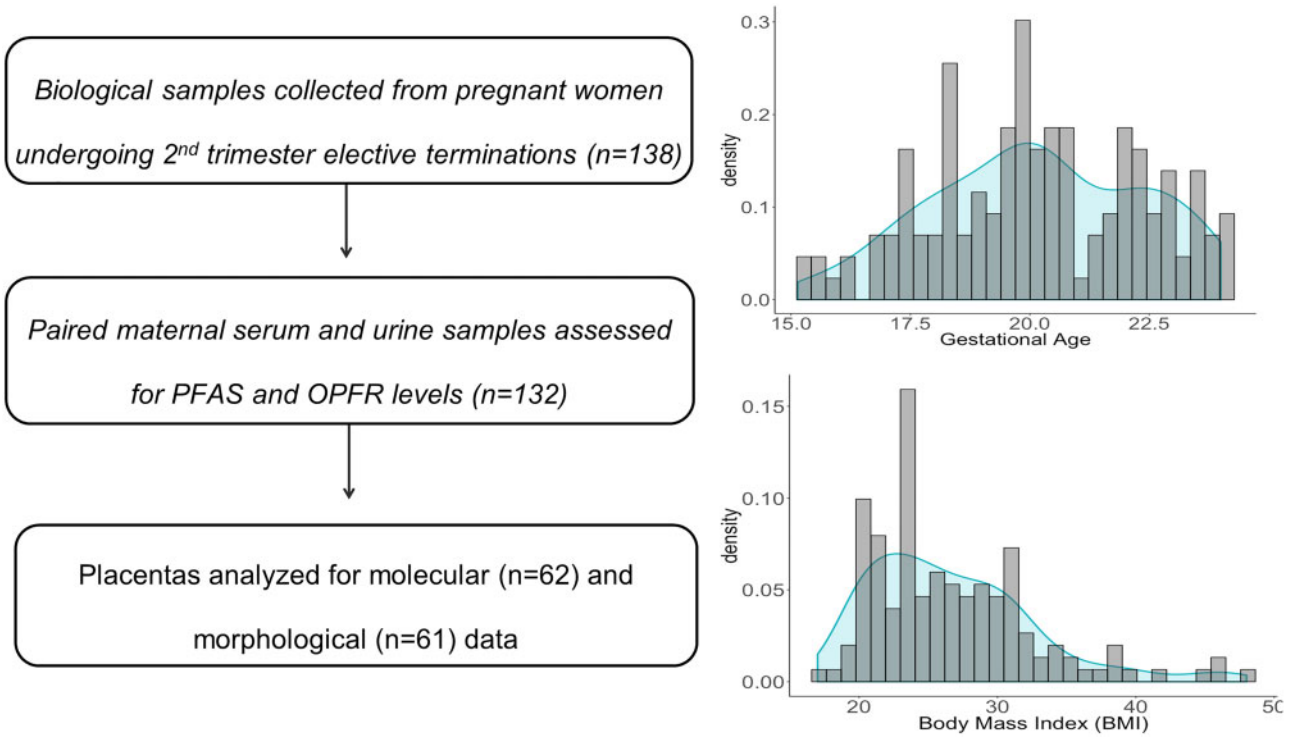

Figure 1. Study population and biological sample collection at the Women's Options Center (WOC) in Northern California from 2014 to 2016. Tissue samples and questionnaire data were obtained from 138 pregnant women undergoing elective terminations during mid-gestation. Perfluoroalkyl and polyfluoroalkyl substances and organophosphate flame retardants metabolite levels were measured in 132 matched serum and urine samples, respectively. We then evaluated a subset of matched placental samples for molecular ($n = 62$) and morphological ($n = 61$) biomarkers of placental development and disease.

diastolic blood pressure (DBP), and body mass index (BMI), were also measured during the clinical visit and later abstracted from medical records. Fetal sex was determined through gonad examination after the medical procedure. Study protocols were approved by the UCSF Institutional Review Board prior to recruitment.

For chemical analyses, maternal serum samples were centrifuged at 3000 RPM for 10 min at 4°C prior to aliquoting and transfer of the serum with glass pipettes into pre-screened (to confirm absence of PFAS) sterilized 2 ml cryovials (storage at −80°C). Urine samples were collected in 60 ml polypropylene collection cups and immediately aliquoted into 5 ml cryovials and stored at −80°C until analysis. Wet-weight serum PFAS and urinary OPFR metabolite concentrations were then measured in $n = 132$ samples (ng/ml). A subset of placental samples ($n = 62$) was further processed and evaluated for molecular and morphological features (Figure 1). As described (Varshavsky *et al.*, 2020b), placental biopsies were fixed with 3% paraformaldehyde (PFA), embedded in a cryomold containing optimum cutting temperature (OCT) compound (Sakura Finetek, SA62550-01), and stored at −80°C. Biopsies were later dissected into sections (5 μm thickness) using a cryostat (Leica Biosystems), mounted on positively charged glass slides, and stored at −80°C for experimental assessments (see below).

Chemical Laboratory Analysis

Twelve PFASs, including perfluorobutane sulfonic acid (PFBS), perfluorohexane sulfonic acid (PFHxS), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorooctane sulfonamide (PFOSA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoA), perfluorobutanoic acid (PFBA), and perfluorohexanoic acid (PFHxA), were analyzed in maternal serum samples (0.5 ml) at UCSF using a liquid chromatography- tandem mass spectrometry (LC-MS/MS) platform- Agilent LC1260 (Sta. Clara, CA)-AB Sciex API 5500 (Foster City, CA). Samples were prepared for LC-MS/MS injection by solid phase extraction using a Waters Oasis HLB cartridge (10 mg, 1 cc). Extracted aliquots (25uL) were run in duplicate; analytes were separated by elution gradient chromatography using Phenomenex Kinetex C18 column (100 × 4.6 mm, 2.6 μ) at 40°C. Electrospray ionization (negative mode) was used as method of ionization for individual analytes.

Analytes were detected in each sample by multiple reaction monitoring using two transitions per analyte. To determine the presence of each analyte retention time matching (within 0.15 min) along with the peak area ratio between its qualifier and quantifier ions (within 20%) were used. Quantification of each detected analyte was done by isotope dilution method using a 10-point calibration curve (0.02–50 ng/ml) and employing two C13-labeled PFAS isotopologues as internal standards. Procedural quality control materials and procedural blanks were run along with the calibration curve at the start, middle, and end of each run. Two QC materials were used at low and high concentrations. To accept the results of a batch run, QC materials measurements must have been within 20% of target values. The limits of detection (LOD) and quantification (LLOQ) for the twelve analytes ranged from 0.01 to 0.1 ng/ml and 0.05 to 0.1 ng/ml, respectively. Analyte identification from total ion chromatograms was evaluated using AB Sciex Analyst v2.1 software while quantification of each analyte was processed using AB Sciex MultiQuant v2.02 software.

Table 1. Organophosphate Flame Retardant (OPFR) Urinary Metabolites and Parent Compounds

OPFR Urinary Metabolites	OPFR Parent Compounds
bis(2-chloroethyl) phosphate (BCEP)	tris(2-chloroethyl) phosphate (TCEP)
bis(1,3-dichloro-2-propyl) phosphate (BDCIPP)	tris(1,3-dichloro-2-propyl) phosphate (TDCIPP)[a]
bis(1-chloro-2-propyl) phosphate (BCIPP)	tris(1-chloro-2-propyl) phosphate (TCIPP)
diphenyl phosphate (DPhP)	triphenyl phosphate (TPhP)
	ethylhexyl diphenyl phosphate (EHDPP)

[a]Also known as chlorinated "tris."

In addition, four OPFR metabolites, including bis(1-chloro-2-propyl) phosphate (BCIPP), bis(2-chloroethyl) phosphate (BCEP), diphenyl phosphate (DPhP), and bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), which are metabolites of tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), tris(2-chloroethyl) phosphate (TCEP), triphenyl phosphate (TPhP) and ethylhexyl diphenyl phosphate (EHDPP), and tris(1-chloro-2-propyl) phosphate (TCIPP), respectively (Table 1), were measured in maternal urine (1 ml) by the Environmental Chemistry Laboratory at California's Department of Toxic Substances Control in Berkeley, CA, using liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a coupled system of a Shimadzu Prominence High Performance Liquid Chromatography (HPLC) system (Shimadzu Scientific Instruments, Pleasanton, CA) and a Sciex QTRAP 5500 Mass Spectrometer (Sciex, Redwood City, CA). Samples were prepared for LC-MS/MS injection by solid phase extraction using Phenomenex Strata-X-AW Polymeric Weak Anion 60 mg/3 ml SPE cartridges (Phenomenex, Torrance, CA), extracted aliquots (100 uL) and separated on a Phenomenex Luna C18 (2) column (150 × 2.1 mm, 3 μm) at 45°C with an ion paired reverse phase chromatography gradient program using 80:20 v/v water: methanol and methanol: water 95:5 v/v, both containing 2.5 mM tributylamine and 2.5 mM acetic acid as mobile phase (Petropoulou *et al.*, 2016). Analytes were detected using multiple reaction monitoring with negative electrospray ionization mode. Quantification of each detected analyte was done by isotope dilution method using a 12-point calibration curve (0.05–25 ng/ml) for BDCIPP and BCIPP and (0.2–100 ng/ml) for DPhP and BCEP. Method detection limits (MDLs) ranged from 0.04 to 0.2 ng/ml, with some measurements "not reportable" due to interference of the internal standard from the sample matrix after repeat analysis. Specific gravity was measured at the time of urine analysis and used to adjust wet-weight OPFR metabolite concentrations for urine dilution with the following equation: $Pc = P \times [(1.024–1)/(SG—1)]$, where Pc = specific-gravity–corrected OPFR metabolite concentration (ng/ml); P = measured urinary OPFR metabolite concentration (ng/ml); and SG = specific gravity of individual urine sample (Hauser *et al.*, 2004).

Finally, total serum cholesterol and triglyceride levels (mg/dl) were measured enzymatically by Boston Children's Hospital (Boston, MA) and used to calculate total serum lipid levels (mg/ml) with the Phillips formula (Phillips *et al.*, 1989).

Placental Biomarker Analysis

As described (Varshavsky *et al.*, 2020b), we evaluated for immunoreactivity of ITGA1, CDH5 or matrix metalloproteinase-1 (MMP1) in CTBs within five regions, ie, "zones," of the placenta ($n = 62$): Zone (I) CTBs resident in floating villi (FV); Zone (II) CTBs in the proximal (p) regions of anchoring villi (AV) cell columns; Zone (III) CTBs within the distal (d) regions of AV cell columns; Zone (IV) interstitial invasive/extravillous CTBs (iCTB); and Zone (V) endovascular CTBs (eCTB) (Figure 2). ITGA1 and CDH5 were selected based on *in vitro* and *in vivo* studies demonstrating the importance of these adhesion receptor molecules in placental development (eg, CTB invasion and endovascular remodeling) as well as their associations with placenta-mediated pregnancy complications (Damsky *et al.*, 1994; Zhou *et al.*, 1993, 1997a, 1997b). MMP1 is a metalloproteinase that facilitates CTB migration/invasion by degrading the extracellular matrix (Vettraino *et al.*, 1996) and also is implicated in preeclampsia pathogenesis (Espino Y Sosa *et al.*, 2017). *In vitro*, *MMP1* expression is altered by exposure to environmental chemicals (ie, BDE-47) in human primary CTBs (2nd trimester) (Robinson *et al.*, 2019) (RNA) and transformed extravillous trophoblasts (Park *et al.*, 2020) (RNA and protein).

We immunolocalized for proteins using methods previously established in our group with modifications (Zhou *et al.*, 2003). In brief, after washing with PBS, we submerged slides in cold 100% histoglogical grade methanol (Millipore Corporation) for 2 min then washed 3 times with PBS to remove the residual methanol. Next, we incubated slides in blocking solution containing 3% Bovine Serum Albumin (Sigma) and 0.1% Tween® 20 (Fisher Scientific) in PBS for 1 h. The blocking solution was gently aspirated from each slide and incubated with a species-specific primary antibodies: anti-ITGA1 (Lifespan Biosciences, LS-C36920, mouse monoclonal, 1:100), anti-CDH5 (BD Biosciences, mouse IgG1 monoclonal, 1:100), or anti-MMP1 (Abcam, ab38929, rabbit polyclonal, 1:100), diluted in blocking buffer at 37°C for 1 h. We identified CTBs by co-immunolocalizing for cytokeratin 7 (anti-CK7, 7Δ3, Fisher_001-clone7D3, RRID: AB_2631235, rat monoclonal, 1:100), an established marker of human placental tropohblasts (Damsky *et al.*, 1992). Detection of primary antibodies was performed by incubating slides in the dark for 1 h at room temperature with species-specific secondary antibodies: donkey anti-rat IgG (JacksonImmuno Research, 712-025-153, 1:100); donkey anti-rabbit IgG (JacksonImmuno Research, 715-095-152, 1:100); or donkey anti-mouse IgG (JacksonImmuno Research, 715-095-151, 1:100) diluted in blocking buffer. The slides were washed three times with PBS prior to adding 4′6-diamidino-2-phenylindole (DAPI) (Vector Laboratories, H-1200) to identify cell nuclei. Slides were imaged using an upright Leica DFC450 digital microscope camera, and the Leica Advanced Fluorescence Application Suite Ver. 3.2 (Leica Microsystems). No specific immunoreactivity was detected staining with the primary or secondary antibody alone. Batch effects which influence immunofluorescence (IF) intensity were continuously monitored by including serial sections from the same two placental samples in each assessment. Two blinded independent investigators scored molecular immunoreactivity into three groups based on visual inspection of the percent of CTBs that stained for each antibody: (1) <25% (−); (2) 25%–75% (±); and (3) >75% (+), with sample sizes varying in

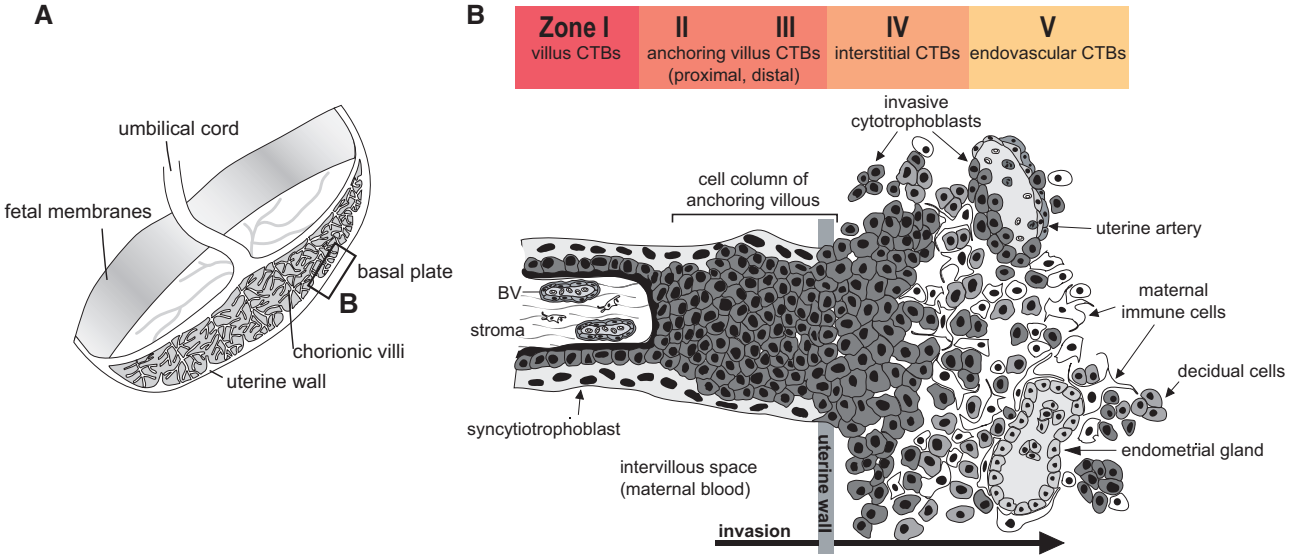

Figure 2. Human placental villous cytotrophoblast (CTB) differentiation at the maternal-fetal interface. A, Anatomy of the human placenta. Chorionic villi are the functional units. The histology of the boxed area is shown in the panel to the right. B, Depiction of the maternal-fetal interface at the cellular level. The mononuclear cytotrophoblasts (CTB) of the (early gestation) chorionic villi fuse to become multinuclear syncytiotrophoblasts (STBs), which form the surface of the placenta. Floating villi are perfused by maternal blood. Anchoring villi give rise to invasive interstitial CTBs (CTBi) that emigrate from the chorionic villi via cell columns that attach the placenta to the maternal unit and infiltrate the uterine wall. Maternal cells in this region include the decidua, remodeled uterine blood vessels, which are lined by cytotrophoblasts (CTBe), and immune cells. During vascular invasion, the cells breach both veins and arteries, but they have more extensive interactions with the arterial portion of the uterine vasculature. Here, they replace the endothelial lining and intercalate within the smooth muscle walls of the spiral arteries, producing hybrid vessels that are composed of both embryonic/fetal and maternal cells. Vascular invasion connects the uterine circulation to the intervillous space where maternal blood perfuses the chorionic villi. Immunoreactivity of molecular biomarkers was evaluated in five zones (I–V) corresponding to different stages of CTB differentiation: (I) CTB progenitors in floating villi (FV); (II) CTBs of the proximal (AVp) and (III) distal (AVd) anchoring villi; (IV) invading interstitial CTBs (CTBi); and (V) endovascular CTBs (CTBe) that remodel the uterine vasculature. Image and text reproduced from Varshavsky *et al.* (2020b) (Environmental Health); originally modified from Damsky *et al.* (1992) and Maltepe and Fisher (2015).

each placental region depending on whether tissue sections contained required structures.

We assessed morphological parameters previously associated with placental stress, dysfunction, and disease via Hematoxylin and Eosin (H&E) staining. As described (Varshavsky *et al.*, 2020b), placenta sections were washed with 1x PBS to remove OCT then dehydrated two times in 100% ethanol (Decon Labs Inc.) for 5 min and twice in 95% ethanol for 5 min. Next, samples were stained in Modified Harris Hematoxylin (Thermo Scientific) for 6 min. After rinsing with water, samples were placed in a solution containing: 1% acid alcohol; 70% ethanol, distilled water, 1% hydrocholoric acid (Fisher Scientific). A second water rinse was performed and samples were submerged in Scott's Water followed by another rinse before being dipped in 1% Eosin Y counterstain (VWR) for two min. Samples were subsequently placed in graded alcohol concentrations of 70%, 70% 95%, 95%, 100%, and 100%. Slides were dried in histological grade xylenes (Sigma-Aldrich) then mounted onto slides with Cytoseal 60 (Thermo Scientific).

Two blinded independent investigators scored the following features: (1) average total number of white blood cells (WBC) present in basal plate (BP) per 10X field; (2) percent of FV with perivillous fibrinoid deposits (~ 60 villi evaluated per field); 3) fibrinoid deposition at the utero-placental junction; and (4) total number of modulated uterine spiral arteries which contained CTBs (defined as presence of >50% endovascular CTBs in the lining of uterine spiral arteries). All values were based on examination of the entire placental section at 10X resolution (~6–10 independent fields). One sample was lost while processing morphological assessments, leaving a total of $n = 61$ placental biopsies for our morphological assessments. Images were acquired using a bright field Leica DFC450 microscope equipped with a camera.

Statistical Analysis

To account for left-censored chemical concentrations below the laboratory MDL, we assumed a log-normal distribution and used maximum likelihood estimation (MLE) to calculate descriptive statistics for serum PFAS (wet-weight) and urinary OPFR metabolite (wet-weight and SG-adjusted) concentrations.

To examine the association between continuous chemical levels and categorical molecular immunoreactivity groups (as scored in the laboratory), we used censored ANOVA/MLE regression models to test group mean differences on the log scale of PFAS and OPFR metabolite levels between three immunoreactivity groups (< 25%, 25–75%, and > 75%) while accounting for left-censored PFAS and OPFR metabolite data (Helsel, 2005). From these bivariate censored regression/ANOVA models, we calculated the pairwise percent (%) differences in chemical concentrations as $(e^{\beta} - 1) \times 100$ and the 95% confidence interval (95% CI) as $(e^{\beta \pm 1.96 \times SE} - 1) \times 100$, where the referent group comprised placental samples in the <25% (–) immunoreactivity group.

We then assessed the correlation of all chemicals using censored Kendall's Tau Correlation Coefficient, a nonparametric measure of rank order correlation that can account for left-censored chemical data with < MDL values. We also used the censored Kendall's Tau method to estimate the correlation between chemical concentrations and placental biomarkers (molecular and morphological). We corrected for multiple comparisons using the Benjamini and Hochberg method to estimate false discovery rate (Benjamini and Hochberg, 1995). The

percent of modulated uterine spiral arteries was calculated by dividing the number of spiral arteries with >50% endovascular CTB modulation by the total number of maternal spiral arteries observed. If the total number (ie, denominator) was less than two, the percent modulation was recoded as missing. We modeled molecular immunoreactivity categorically rather than continuously based on the three pre-defined categories that were scored in the laboratory.

In addition, we used bivariate censored ANOVA/MLE regression models to examine PFAS and OPFR metabolite levels with biological and sociodemographic variables, including fetal sex, maternal and gestational age (in years and weeks, respectively), body mass index (BMI, in kg/m3), geographical region of residence (SF Bay Area, S. Central Valley/Fresno, N. Central Valley/Sierras/Coast), and race/ethnicity (Latina/Hispanic, NonHispanic Black, NonHispanic White, and Asian/Pacific Islander).

We also examined associations of PFAS and OPFR metabolite levels with maternal blood pressure, which is a clinical indicator of placenta-mediated pregnancy complications (eg, preeclampsia and other pregnancy-induced hypertensive disorders), as well as with serum lipid levels, which have been associated with serum PFAS concentrations (Matilla-Santander *et al.*, 2017; Starling *et al.*, 2014) and are potential indicators of metabolic function and maternal health during pregnancy. We used regular linear regression for this analysis because the outcomes of interest (maternal blood pressure and serum lipid levels) were not left-censored variables. For the blood pressure analysis, we removed two outliers with extreme blood pressure measurements that were considered impossibly high (>800 mmHg SBP) or low (<11 mmHg DBP). Two blood pressure endpoints, including SBP and DBP, were examined separately as continuous dependent variables in bivariate linear regression models that assessed chemical concentrations and covariates as continuous and categorical independent variables. We estimated the unadjusted associations between blood pressure endpoints (SBP and DBP), total serum lipid levels, and individual population characteristics in order to test whether blood pressure endpoints and lipid levels varied by covariates. Unadjusted associations were estimated from bivariate linear regression models using the following equation: $y = \beta_1 * \ln(x) + \beta_o$. We then performed multiple linear regression to assess the relationship of PFAS and OPFR metabolite levels with each blood pressure endpoint and with total serum lipid levels (in separate adjusted models). We selected covariates for inclusion in multivariable models based on whether they were associated with the exposure and outcome and/or whether they changed the effect estimate for the exposure-outcome association by more than 10%.

RESULTS

Our study population was diverse, with the majority of women identifying as either Latina/Hispanic or NonHispanic Black (56% combined) and about half reporting they were currently employed and had at least some college education (Table 2). Most women were 20–24 years old, relied on public insurance, and had at least one prior birth. We did not observe differences in PFAS levels by region of residence, fetal sex, gestational age, maternal age, or BMI. However, we observed higher levels among white women ($p < .0001$), women with some college education or more compared to women with a high school degree or equivalent, women with private rather than public health insurance, as well as women who had never given birth before compared to women with at least one prior live birth. Urinary OPFR metabolite levels generally did not vary by population characteristics, although higher levels were associated with increased BMI (Table 2).

Five of 12 PFASs were detected in more than 80% of maternal serum samples, with detection frequencies ranging from 49.2% for PFBS to 100% for PFNA, PFOA, and PFOS, which were also the PFASs with the highest levels (Table 3). The geometric means (GMs) for PFNA, PFOA, and PFOS of 1.3 ng/ml (95% CI: 1.2–1.4 ng/ml), 2.6 (95% CI: 2.4–2.9 ng/ml), and to 2.8 ng/ml (95% CI: 2.6–3.1 ng/ml), respectively, were higher by one of order of magnitude than the GMs for other PFASs (0.5 ng/ml or less). Maximum serum PFAS concentrations varied widely, ranging from 1.80 ng/ml for PFUnDA to 20.3 ng/ml for PFOA. All four urinary OPFR metabolites were also widely detected among pregnant women during mid-gestation, with detection frequencies ranging from 83% to 99%. DPhP and BDCIPP were the urinary OPFR metabolites with the highest levels, with wet-weight GM concentrations of 2.9 ng/ml (95% CI: 2.5–3.4 ng/ml) and 2.6 ng/ml (95% CI: 2.2–3.1 ng/ml), respectively, compared to < 1 ng/ml for BCEP and BCIPP. The SG-adjusted GMs for DPhP and BDCIPP were 4.0 ng/ml (95% CI: 3.5–4.6 ng/ml) and 3.5 (95% CI: 3.0–4.1 ng/ml), respectively. The 95th percentile values for wet-weight and SG-adjusted DPhP and BDCIPP concentrations ranged from 12 to 16 ng/ml compared to 2–8 ng/ml for BCEP and BCIPP. Maximum values for wet-weight and SG-adjusted concentrations ranged from 5.6 to 112 ng/ml and 7.0 to 85 ng/ml, respectively, for all four OPFR metabolites (Table 3).

In general, serum PFAS levels did not correlate with urinary OPFR metabolite levels (Figure 3). Correlation coefficients were generally higher among PFASs (ranging from 0.18 to 0.59 [$p < 0.05$]) than among OPFR metabolites. The correlation of wet-weight OPFR metabolite concentrations ranged from 0.17 ($p = .008$) for BDCIPP and BECP, 0.26-0.27 for BCEP and BCIPP ($p < .0001$), and 0.34 for BCEP and BCIPP ($p < .0001$) (Figure 3).

We found a pattern of modest inverse correlation (τ ranging from −0.12 to −0.20; $p < .10$) with ITGA1 immunoreactivity in CTBs within multiple placental regions (dAV, iCTB, and eCTB) for five PFASs and one OPFR metabolite (BDCIPP) (Figure 4). Similar inverse correlations were observed with CDH5 in CTBs within the same placental regions for a smaller number of PFASs and for OPFR metabolites. We also found evidence of positive correlations with morphological indicators of placental stress. For example, PFDA and PFOSA were both positively correlated with WBC count and basal plate fibrinoid deposition (τ = 0.16–19; $p < .10$), while BDCIPP was similarly correlated with fibrinoid deposition in both the basal plate and floating villi. In addition, two PFASs were modestly inversely correlated with CTB artery modulation ($\tau = -0.15$, $p < .10$) (Figure 4).

The ANOVA/MLE regression analysis revealed a similar inverse trend between placental ITGA1 or CDH5 immunoreactivity in CTBs of specific placental regions and serum PFAS concentrations (Table 4). For example, placentas with the highest levels of ITGA1 expression in CTBs of distal anchoring villi (dAV) had 77% (95% CI: −94%, −18%) and 95% (95% CI: −100, −38%) lower levels of PFHpA and PFBS, respectively, compared to the referent group. We also found inverse associations between these PFAS compounds and dAV CDH5 immunoreactivity in CTBs within multiple placental regions ($p < .05$) (Table 4).

We also found evidence of inverse relationships between PFOA, PFNA, PFDA, PFHxS, and PFOS levels (but not OPFR metabolites) with total maternal serum lipid levels (Figure 5). However, the associations were attenuated in multivariable models with adjustment for race/ethnicity, insurance type, and parity (Table 5). In addition, we observed a positive association between serum PFNA levels and DBP and between PFOA, PFNA,

Table 2. Population Characteristics and Wet-Weight Chemical Levels During Mid-Gestation Pregnancies in Northern California Women (N = 132; 2014–2016)

		Serum PFOA (ng/ml)		Urinary BDCIPP (ng/ml)	
Characteristic[a]	N (%)	GM (95% CI)	p	GM (95% CI)	p
Maternal Age					
<20 years	17 (13)	2.5 (1.9, 3.2)	.56	2.6 (1.7, 3.9)	.94
20–24 years	56 (43)	2.7 (2.0, 3.6)		2.6 (1.6, 4.2)	
25–29 years	29 (22)	2.8 (2.0, 3.8)		2.7 (1.6, 4.7)	
≥30 years	28 (22)	2.4 (1.7, 3.3)		2.5 (1.5, 4.3)	
Body Mass Index (BMI)					
Normal/Under (<25 kg/m^2)	58 (45)	2.9 (2.5, 3.3)	.22	**2.0 (1.6, 2.6)**	**.03**
Overweight (25–30 kg/m^2)	34 (26)	2.5 (2.0, 3.1)		**3.1 (2.1, 4.6)**	
Obese (≥30 kg/m^2)	38 (29)	2.4 (1.9, 3.0)		**3.2 (2.2, 4.7)**	
Diastolic Blood Pressure					
Normal (<80 mmHg)	113 (87)	2.6 (2.4, 2.9)	.95	2.5 (2.2, 3.1)	.97
Elevated (≥80 mmHg)	15 (12)	2.7 (2.0, 3.6)		2.7 (1.6, 4.6)	
Systolic Blood Pressure					
Normal (<120 mmHg)	93 (72)	2.6 (2.3, 2.9)	.63	2.7 (2.2, 3.3)	.84
Elevated (≥120 mmHg)	35 (27)	2.8 (2.3, 3.5)		2.4 (1.7, 3.5)	
Birth Country					
U.S. born	67 (52)	2.5 (2.2, 2.8)	.48	2.7 (2.2, 3.4)	.96
Foreign born	9 (6.9)	2.0 (1.4, 2.8)		2.5 (1.3, 4.7)	
Education					
≤High School	64 (49)	2.3 (2.1, 2.7)	.06	2.7 (2.1, 3.4)	.95
>High School	65 (50)	2.9 (2.4, 3.5)		2.5 (1.8, 3.5)	
Employment Status					
Employed	63 (49)	2.8 (2.5, 3.2)	.19	2.8 (2.2, 3.5)	.73
Unemployed	63 (49)	2.4 (2.0, 2.9)		2.4 (1.7, 3.4)	
Gestational Age					
<19 weeks	35 (27)	2.6 (2.2, 3.2)	.86	2.4 (1.7, 3.3)	.74
19–21 weeks	50 (39)	2.7 (2.1, 3.4)		2.6 (1.7, 3.9)	
>21 weeks	45 (35)	2.5 (2.0, 3.2)		2.8 (1.9, 4.3)	
Insurance Type					
Public	95 (73)	**2.4 (2.1, 2.6)**	**.001**	2.8 (2.3, 3.4)	.30
Private/Self-pay	34 (26)	**3.5 (2.9, 4.3)**		2.1 (1.5, 3.0)	
Parity					
0 live births	52 (40)	**3.3 (2.8, 3.7)**	**.001**	2.4 (1.9, 3.1)	.77
≥1 live birth	78 (60)	**2.3 (1.9, 2.7)**		2.7 (2.0, 3.8)	
Race/ethnicity					
Latina/Hispanic	44 (34)	**2.2 (1.9, 2.6)**	**<.001**	3.0 (2.3, 4.1)	.66
NonHispanic Black	29 (22)	**2.5 (2.0, 3.2)**		2.6 (1.6, 4.1)	
NonHispanic White	18 (14)	**3.9 (3.0, 5.2)**		2.5 (1.5, 4.1)	
Asian/Pacific Islander	16 (12)	**2.5 (1.8, 3.4)**		2.7 (1.5, 4.9)	
Region of Residence					
SF Bay Area	73 (56)	2.6 (2.29, 2.94)	.43	2.4 (1.9, 3.0)	.64
S. Central Valley/Fresno	21 (16)	2.3 (1.80, 3.02)		2.6 (1.6, 4.1)	
N. Central Valley/Coast/Sierras	34 (26)	2.8 (2.27, 3.52)		2.9 (2.0, 4.3)	
Fetal Sex					
Male	68 (52)	2.5 (2.17, 2.80)	.43	2.8 (2.3, 3.5)	.58
Female	62 (48)	2.8 (2.32, 3.36)		2.4 (1.7, 3.3)	
Year of Collection					
2014	31 (24)	2.7 (2.23, 3.24)	.19	2.8 (2.0, 3.8)	.89
2015	91 (70)	2.7 (2.15, 3.31)		2.5 (1.7, 3.7)	
2016	8 (6.2)	1.9 (1.25, 2.83)		2.7 (1.4, 5.2)	

[a]Missing values for blood pressure ($n = 2$), birth country ($n = 54$), education ($n = 1$), employment ($n = 4$), insurance ($n = 1$), race/ethnicity ($n = 23$), and region of residence ($n = 2$). Significant results bolded ($p < .05$).

and PFHxA levels with SBP in multivariable regression models that were adjusted for race/ethnicity, insurance type, and parity. Of the urinary OPFR metabolites, BCIPP was also positively associated with DBP and SBP (marginally significant) in multivariable models, but the association was attenuated with additional adjustment for BMI (Table 5).

DISCUSSION

Building on our previous proof of principle with PBDEs (Varshavsky *et al.*, 2020b), we measured maternal exposure to PFASs and OPFRs—environmental chemicals of concern that we focused on due to widespread human exposure and

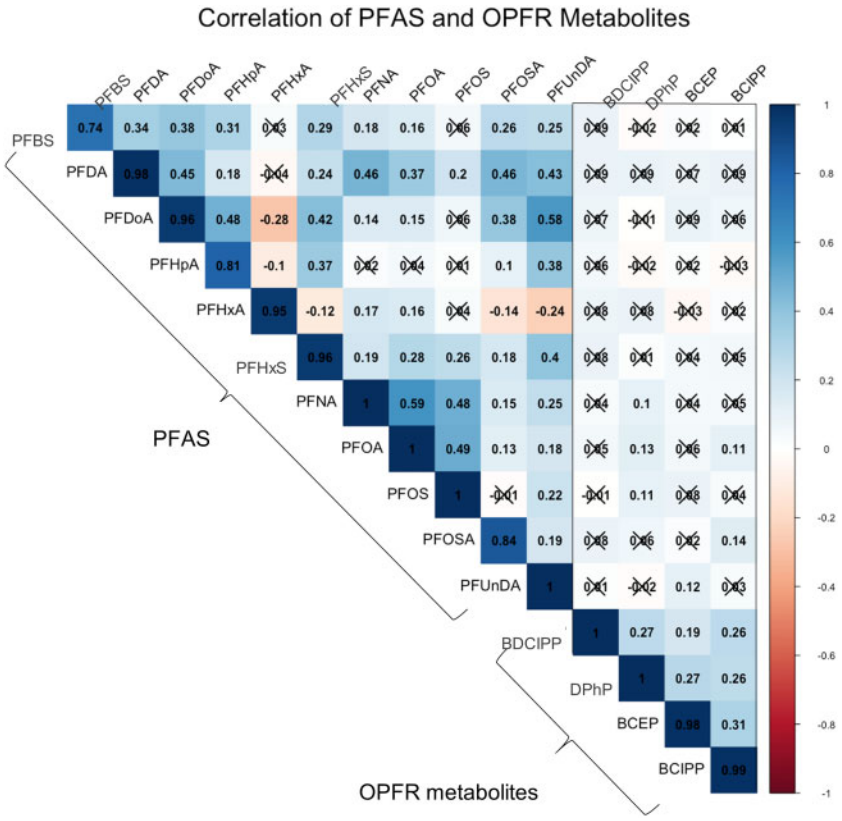

Figure 3. Correlation of serum perfluoroalkyl and polyfluoroalkyl substances and urinary organophosphate flame retardants metabolite levels among pregnant women during mid-gestation (N = 132; 2014–2016). Rank order correlation assessed using censored Kendall's Tau Correlation Coefficients which accounts for bivariate left-censored chemical data (below detection limit). Values represent magnitude and shading denotes direction of correlation (red = decreasing; blue = increasing), while "X" symbol denotes lack of statistical significance ($p > .05$).

Table 3. Descriptive Statistics*a* on Wet-Weight Maternal Serum PFAS and Urinary OPFR Metabolite Levels (ng/ml) Among Pregnant Women During Mid-Gestation ($n = 132$; 2014–2016)

Chemical	N	MDL	% Detection	GM (95% CI)	95th Percentile (95% CI)	Min.	Max.
PFAS Compounds							
PFNA	132	0.10	100	1.3 (1.2, 1.4)	3.2 (2.8, 3.7)	0.28	12
PFOA	132	0.02	100	2.6 (2.4, 2.9)	6.3 (5.5, 7.3)	0.76	20
PFOS	132	0.02	100	2.8 (2.6, 3.1)	6.8 (5.6, 7.8)	0.67	9.8
PFUnDA	132	0.05	94	0.3 (0.3, 0.4)	1.4 (1.1, 1.7)	0.04	1.8
PFDA	132	0.05	87	0.5 (0.4, 0.6)	3.5 (2.5, 4.8)	0.04	6.5
PFDoA	132	0.10	79	0.2 (0.2, 0.3)	1.1 (0.9, 1.4)	0.07	6.1
PFHxA	132	0.05	79	0.1 (0.1, 0.1)	0.3 (0.2, 0.3)	0.04	0.4
PFHxS	132	0.10	79	0.3 (0.3, 0.4)	2.3 (1.7, 3.3)	0.07	6.3
PFOSA	132	0.01	61	0.0 (0.0, 0.1)	1.6 (0.8, 3.2)	0.01	1.3
PFHpa	132	0.10	56	0.1 (0.1, 0.2)	0.8 (0.6, 1.2)	0.07	6.4
PFBS	132	0.01	49	0.0 (0.0, 0.0)	0.5 (0.3, 1.1)	0.01	9.4
OPFR Metabolites							
DPhP	132*b*	0.20	99	2.9 (2.5, 3.4)	13 (10, 16)	0.20	112
BDCIPP	130	0.04	98	2.6 (2.2, 3.1)	12 (9.6, 16)	0.30	22
BCIPP	130*b*	0.04	95	0.4 (0.3, 0.4)	1.9 (1.5, 2.5)	0.04	5.6
BCEP	126*b*	0.20	83	0.8 (0.7, 1.0)	6.3 (4.5, 8.8)	0.20	29
sg-ADJUSTED opfr Metabolites							
DPhP	132*b*	0.20	99	4.0 (3.5, 4.6)	15 (12, 19)	0.20	85
BDCIPP	130	0.04	98	3.5 (3.0, 4.1)	16 (12, 20)	0.26	34
BCIPP	130*b*	0.04	95	0.5 (0.4, 0.6)	2.3 (1.8, 3.0)	0.04	7.0
BCEP	126*b*	0.20	83	1.1 (0.9, 1.4)	8.0 (5.8, 11)	0.20	34

Abbreviations: GM, geometric mean; MDL, method detection limit; SG, specific gravity.

[a]Descriptive statistics calculated using censored maximum likelihood estimation (MLE) to account for chemical concentrations below MDL (assuming a log-normal chemical distribution).

[b]Sample size decreased due to nonreportable laboratory measurements (internal standard interference from sample matrix after repeat analysis).

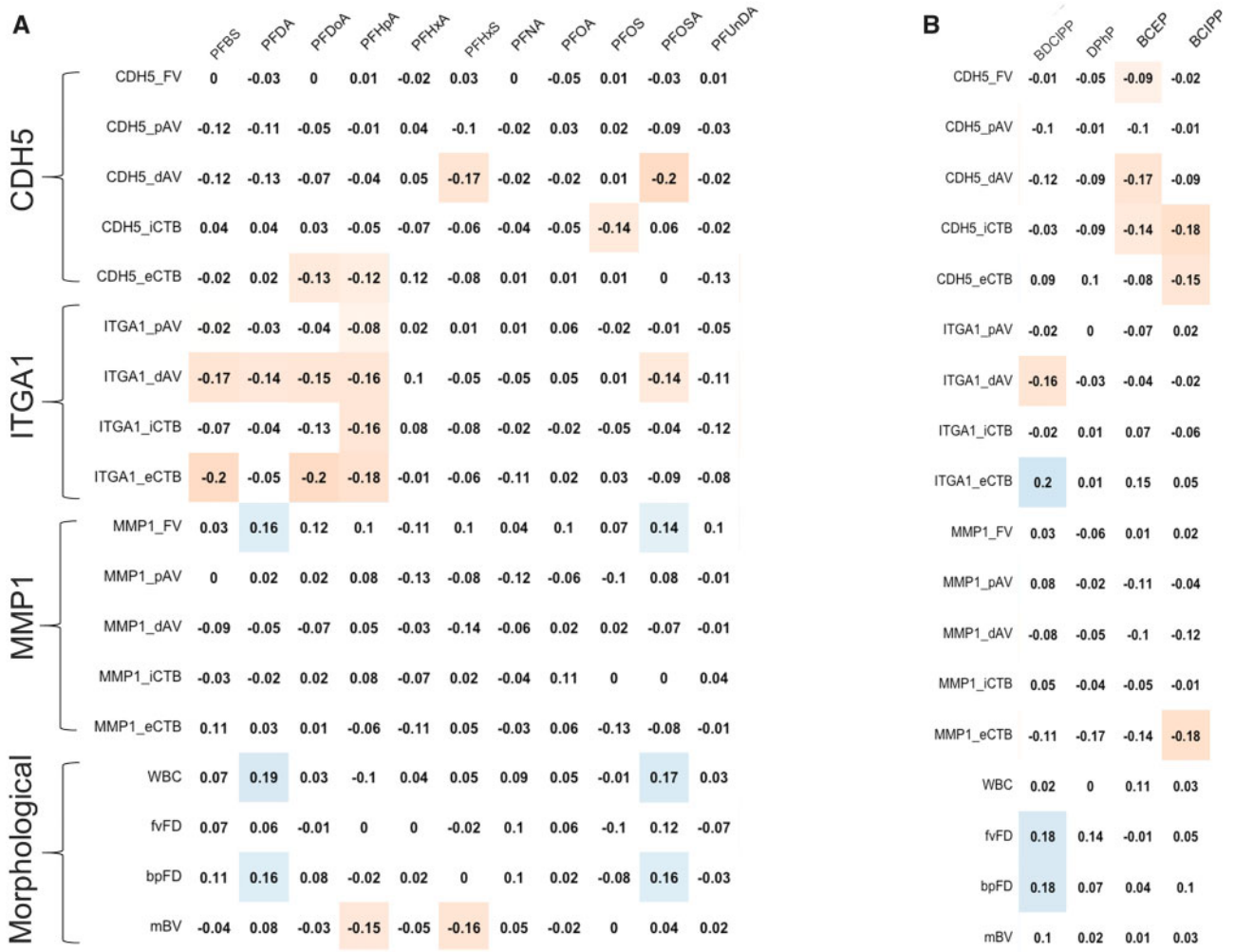

Figure 4. Correlation of (A) serum perfluoroalkyl and polyfluoroalkyl substances and (B) urinary organophosphate flame retardants metabolite levels with placental biomarkers among pregnant women during mid-gestation ($N = 62$; 2014–2016). Rank order correlation assessed using censored Kendall's Tau Correlation Coefficients which accounts for left-censored chemical data (below detection limit). Color denotes significance ($p < .05$). dAV, Anchoring villi (distal); pAV, Anchoring villi (proximal); eCTB, Endovascular CTB; iCTB, Interstitial CTB; FV, Floating villi (bilayer); bpFD, % Fibrinoid deposition in basal plate; fvFD, % Floating villi with fibrinoid deposition; mBV, % CTB-modulated blood vessels; WBC, White blood cell count.

developmental toxicity—and investigated associations with biomarkers of placental development and disease during a critical period of placentation for maternal-fetal health (early/mid-2nd trimester). We found that serum PFASs and urinary OPFR metabolites were widely detected across maternal samples during mid-gestation. We also observed associations with molecular and morphological biomarkers of placental development and function, which may underlie disease, as well as associations with physiological indicators of maternal health during pregnancy, including blood pressure and serum lipid concentrations.

Pregnant women in our study population had ~2–26-fold higher PFAS and OPFR metabolite levels compared to women ≥20 years old in the general U.S. population sampled in 2013–2014 (OPFR metabolites) and 2015–2016 (PFASs) by the National Health and Nutritional Examination Survey (NHANES), a nationally representative questionnaire and physical survey of the U.S. civilian, noninstitutionalized population (CDC [Centers for Disease Control and Prevention], 2015). These differences could be due to different exposure sources in California, differences in the underlying study populations (eg, regional, demographic, and/or behavioral), and/or methodological differences between laboratories. With the exception of PFOS and PFHxS, PFAS levels in our study population were similar or higher than those reported previously among fire fighters and office workers sampled in California during the same time period (2014–2015) and for which serum PFAS chemical analysis was performed by the same laboratory (Trowbridge *et al.*, 2020). Urinary OPFR metabolite levels were also detected at similar or slightly higher levels than wet-weight concentrations measured among 16 nonsmoking adults sampled in California in 2011 (Dodson *et al.*, 2014). Maximum OPFR metabolite levels in our investigation were ~5–17 times higher than in the previous study, which could potentially be due to the small sample size of that study or to temporal trends reflecting increasing use of OPFRs as replacements for PBDEs (Blum *et al.*, 2019; Dodson *et al.*, 2014). The OPFR metabolites, DPhP and BDCIPP, were measured at the highest concentrations (>2.5 ng/ml) in this study, which may reflect greater exposure among this study population to their parent compounds, TPhP and EHDPP (for DPhP) and TDCIPP (for BDCIPP). The OPFR, TDCIPP, is also referred to as "chlorinated tris" and is listed along with TCEP as a carcinogen under CA's proposition

Table 4. Percent (%) Difference in Serum PFAS (PFHpA, PFDoA, and PFBS) or Urinary OPFR Metabolite (BCEP) Levels and Immunoreactivity of ITGA1, CDH5, or MMP1 in Region-Specific Placental Cytotrophoblasts During Mid-Gestation ($n = 62$)

		PFHpA (ng/ml)		PFDoA (ng/ml)		PFBS (ng/ml)		BCEP (ng/ml)	
Immunoreactivity	*n*	% diff (95% CI)[a]	*p*-value[b]	% diff (95% CI)[a]	*p*-value[b]	% diff (95% CI)[a]	*p*-value[b]	% diff (95% CI)[a]	*p*-value[b]
Anchoring Villi (proximal)									
ITGA1 < 25% (−)	46	Referent	**.053**	Referent	.435	Referent	.922	Referent	**.094**
25%–75% (±)	2	−100 (−100, Inf)		−17 (−84, 328)		−28 (−99, 6432)		4 (−80, 439)	
>75% (+)	2	−100 (−100, Inf)		−67 (−94, 86)		−59 (−100, 3908)		**−87 (−98, −14)****	
CHD5 < 25% (−)	10	Referent	.141	Referent	.158	Referent	.356	Referent	.360
25%–75% (±)	20	−66 (−89, 5)*		**−56 (−80, −1)****		−71 (−97, 145)		−42 (−78, 52)	
>75% (+)	21	−33 (−77, 95)		−42 (−74, 29)		−79 (−98, 85)		−50 (−81, 29)	
MMP1 < 25% (−)	1	Referent	.357	Referent	.256	Referent	.482	Referent	.561
25%–75% (±)	30	76451 (−100, Inf)		45128 (−100, Inf)		33039604 (−100, Inf)		15 (−89, 1133)	
>75% (+)	19	99719 (−100, Inf)		43528 (−100, Inf)		25447013 (−100, Inf)		−22 (−93, 752)	
Anchoring Villi (distal)									
ITGA1 < 25% (−)	36	Referent	**.042**	Referent	**.077**	Referent	**.037**	Referent	.810
25%–75% (±)	3	−61 (−95, 187)		−26 (−80, 165)		−82 (−100, 624)		−5 (−77, 296)	
>75% (+)	11	**−77 (−94, −18)****		**−59 (−81, −11)****		**−95 (−100, −38)****		−24 (−67, 74)	
CHD5 < 25% (−)	9	Referent	**.038**	Referent	**.034**	Referent	.193	Referent	**.051**
25%–75% (±)	22	**−75 (−92, −23)****		**−66 (−85, −25)*****		−83 (−98, 44)		**−66 (−87, −9)****	
>75% (+)	20	−45 (−81, 63)		**−49 (−77, 13)***		**−85 (−98, 34)***		**−72 (−90, −23)****	
MMP1 < 25% (−)	0	Referent	.891	Referent	.676	Referent	.359	Referent	.499
25%–75% (±)	24	−16 (−60, 74)		29 (−27, 129)		178 (−32, 1030)		52 (−24, 201)	
>75% (+)	26	0 (0, 0)		0 (0, 0)		0 (0, 0)		0 (0, 0)	
Interstitial CTB									
ITGA1 < 25% (−)	6	Referent	**.076**	Referent	.245	Referent	.390	Referent	.125
25%–75% (±)	24	−20 (−77, 177)		9 (−55, 168)		205 (−77, 3897)		**−60 (−86, 13)***	
>75% (+)	29	**−65 (−90, 22)***		−31 (−71, 67)		15 (−91, 1367)		−31 (−75, 90)	
CHD5 < 25% (−)	8	Referent	.760	Referent	.236	Referent	.485	Referent	.204
25%–75% (±)	14	−32 (−82, 155)		−50 (−79, 21)		−67 (−97, 281)		−52 (−83, 38)	
>75% (+)	37	−35 (−79, 104)		−20 (−63, 71)		−5 (−88, 659)		−58 (−83, 8)*	
MMP1 < 25% (−)	2	Referent	.580	Referent	.739	Referent	.973	Referent	.408
25%–75% (±)	23	23 (−87, 1095)		86 (−62, 804)		1 (−98, 5660)		123 (−59, 1104)	
>75% (+)	34	84 (−80, 1625)		80 (−62, 758)		−15 (−98, 4531)		53 (−71, 708)	
Endovascular CTB									
ITGA1 < 25% (−)	6	Referent	.159	Referent	.190	Referent	**.072**	Referent	.263
25%–75% (±)	5	−39 (−91, 338)		−23 (−71, 110)		−73 (−98, 341)		−46 (−85, 88)	
>75% (+)	18	**−78 (−96, 9)***		**−51 (−78, 9)***		**−93 (−99, −33)****		32 (−51, 257)	
CHD5 < 25% (−)	8	Referent	**.059**	Referent	.188	Referent	.780	Referent	.495
25%–75% (±)	3	230 (−48, 1990)		−33 (−81, 135)		94 (−93, 4948)		−31 (−81, 155)	
>75% (+)	31	−53 (−85, 46)		**−49 (−76, 5)***		−28 (−89, 376)		−38 (−71, 35)	
MMP1 < 25% (−)	11	Referent	.465	Referent	.934	Referent	.281	Referent	.174
25 − 75% (±)	19	−47 (−86, 106)		9 (−50, 137)		257 (−75, 4981)		**−48 (−75, 5)***	
>75% (+)	5	42 (−78, 796)		−10 (−71, 184)		1542 (−48, 51881)		−49 (−81, 40)	

*$p < .10$.
**$p < .05$.
***$p < .01$. Significant and marginally significant results bolded.
[a]Calculated from bivariate censored regression models, where referent group included placental samples with trophoblast cells (identified as cytokeratin+ cells) that did not stain (−) or stained only minimally (0%−25%) for antigen specific antibodies corresponding to each molecular biomarker.
[b]P-value from ANOVA test of mean difference in Ln PFAS or OPFR metabolite level across three immunoreactivity groups.

65 (California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, 2013, 65). A comparison of our study population to reproductive-aged and/or pregnant women in California and the U.S. population would be more ideal, and we were unable to delineate potential differences in exposure sources using our questionnaire data. Further work is needed to examine the possibility of (and potential reasons for) disproportionate PFAS and OPFR exposures among pregnant women. This is especially critical in California, given the presumed replacement of brominated flame retardants with emerging chemicals of concern, such as OPFRs (Zhang *et al.*, 2018), and the similar toxicity profiles of these chemical classes (Behl *et al.*, 2016; Blum *et al.*, 2019).

Interestingly, we also found evidence of lower PFAS concentrations among pregnant women who had given birth before, which is consistent with prior literature suggesting that PFAS levels may be lower among parous compared to nonparous women (Brantsæter *et al.*, 2013; Singer *et al.*, 2018). Factors that could explain this difference include: (1) increased elimination through placental transfer and accumulation (expelled during labor), (2) increased excretion (through increased glomerular filtration rate during pregnancy), and (3) increased elimination during breastfeeding (Brantsæter *et al.*, 2013; Singer *et al.*, 2018).

Our placental biomarker findings suggest that exposures to PFASs, and to a lesser extent, OPFRs, are inversely correlated with ITGA1 immunoreactivity of CTBs across multiple placental

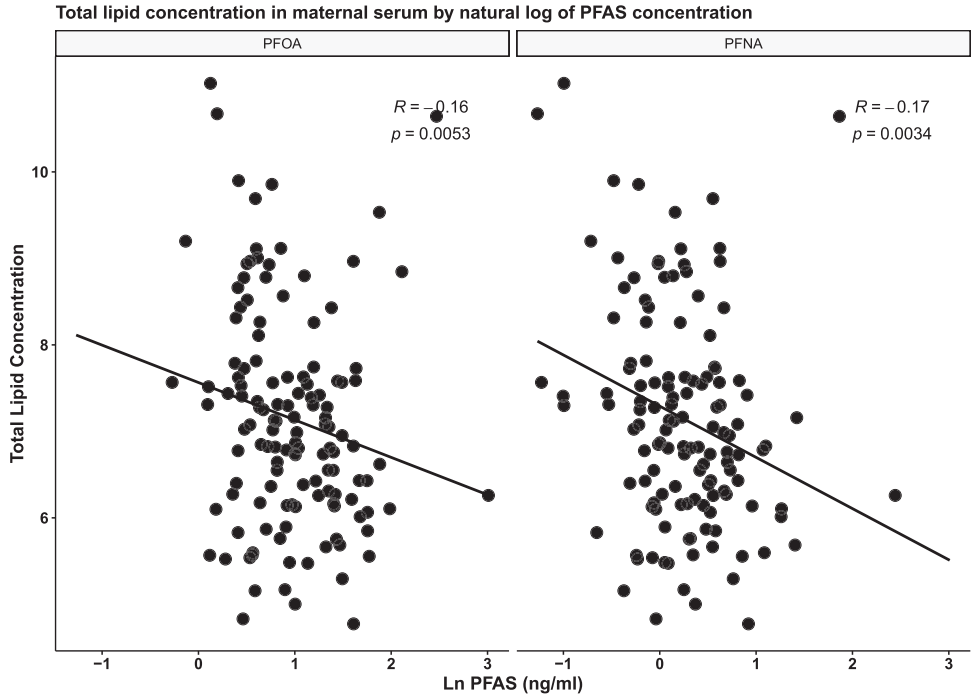

Figure 5. Correlation of PFOA and PFNA levels with total lipid levels in maternal serum among pregnant women during mid-gestation ($N = 132$; 2014–2016). Rank order correlation assessed using Kendall's Tau Correlation.

regions. This is consistent with our previous publication, in which we reported a decreasing relationship between maternal and fetal PBDE levels and ITGA1 in the same study population (Varshavsky *et al.*, 2020b). Unlike the previous study, we also found an inverse correlation between PFAS/OPFR levels and CDH5 immunoreactivity in CTBs within more than one placental region. As ITGA1 and CDH5 are both critical molecules for the proper differentiation of placental cells into invasive phenotypes that contribute to healthy placentation during mid-gestation, our results indicate the possibility that PFASs and OPFRs may contribute to abnormal placental/CTB development and associated risks of pregnancy complications through interference with these molecules. We also found evidence that PFASs may influence CTB-mediated endovascular remodeling during this critical period of placental development, which is consistent with prior literature that has found PFAS exposures can increase risk of low birth weight, preeclampsia, and other pregnancy complications (Blake and Fenton, 2020; Varshavsky *et al.*, 2020a). Future research should examine the effect of PFASs and OPFRs in combination with PBDEs and other environmental chemicals on the biomarkers of placental development and function that we measured and additional relevant biomarkers. Epidemiologic studies assessing these associations in a larger cohort of pregnant women are also warranted.

The positive associations we found between PFNA and maternal blood pressure may indicate potential SBP and DBP changes (increases) in relation to PFAS exposures during pregnancy. Whereas this is the first study to our knowledge which investigates blood pressure associations with maternal PFAS exposures, previous research has reported significant blood pressure associations (both positive and inverse) with other EC exposures during pregnancy (Farzan *et al.*, 2015; Liu *et al.*, 2019; Osorio-Yañez *et al.*, 2016; Werner *et al.*, 2015), indicating these relationships warrant further examination. We also found an inverse association between maternal PFAS exposure and total serum lipid concentrations (although attenuated in multivariable models), which may indicate potential lipid disturbances in relation to 2nd trimester PFAS exposures. Previous studies have demonstrated significant associations between changes in maternal serum lipid concentrations and pregnancy complications such as preeclampsia (Starling *et al.*, 2014). For example, increased levels of triglycerides, total cholesterol, and low density lipid (LDL) cholesterol, and decreased levels of high density lipid (HDL) cholesterol, have been associated with increased risk of preeclampsia (Starling *et al.*, 2014). However, we were unable to differentiate between cholesterol types in this study. Further research should expand on this analysis to include additional specific metrics of lipid endpoints that have been associated with adverse outcomes in prior research.

Despite its limitations, our study applied advanced analytical techniques by measuring biomarkers of effect directly in placental tissues and examined their associations with biomarkers of environmental chemical exposures that are associated with human pregnancy complications. Furthermore, we were able to achieve this during a critical period of placental development for maternal and fetal health outcomes.

CONCLUSIONS

Findings from this study signal potential associations of PFAS and OPFR exposures with specific biomarkers related to placenta-mediated pregnancy complications. This research builds on our earlier work in which we demonstrated the utility of assessing environmental chemical exposures (ie, PBDEs) with molecular and morphological biomarkers of placental development and disease. We expanded the analysis in this study by including additional chemicals of concern (PFAS and OPFRs) and physiological measures of placental function and maternal health (blood pressure and serum lipid concentrations). Our results suggest that PFASs, OPFRs, and other environmental

Table 5. Associations Between Chemical Levels and Maternal Blood Pressure or Serum Lipids During Mid-Gestation ($N = 132$; 2014–2016)

Compound	Model	Estimate (95% CI)[b]	*p*-value	Estimate (95% CI)[c]	*p*-value	Estimate (95% CI)[d]	*p*-value
PFAS Compound							
PFNA	U	**3.9 (0.8, 6.9)**	**.015**	**5.1 (1.2, 9)**	**.011**	**−0.6 (−1, −0.2)**	**.002**
	A	**3.6 (0.4, 6.7)**	**.028**	**5.4 (1.3, 9.5)**	**.010**	−0.4 (−0.8, 0)	.065
PFOA	U	2.3 (−1, 5.5)	.183	**5.1 (1, 9.2)**	**.016**	**−0.4 (−0.8, 0)**	**.038**
	A	2.3 (−1.3, 5.9)	.218	**6.6 (2, 11.2)**	**.005**	−0.2 (−0.7, 0.2)	.283
PFOS	U	1.7 (−1.6, 5)	.323	0.8 (−3.4, 5.1)	.704	**−0.5 (−0.9, −0.1)**	**.026**
	A	2.2 (−1.3, 5.6)	.216	1.3 (−3.2, 5.9)	.566	−0.2 (−0.7, 0.2)	.303
PFUnDA	U	1.5 (−0.6, 3.5)	.170	0.7 (−2, 3.4)	.608	**−0.3 (−0.5, 0)**	**.035**
	A	0.8 (−1.3, 2.9)	.446	−0.3 (−3.1, 2.5)	.843	−0.1 (−0.4, 0.1)	.306
PFDA	U	1.5 (−0.2, 3.1)	.085	**2.2 (0.1, 4.3)**	**.041**	**−0.3 (−0.5, −0.1)**	**.009**
	A	1 (−0.6, 2.6)	.234	1.7 (−0.4, 3.8)	.122	**−0.2 (−0.4, 0)**	**.057**
PFDoA	U	0.5 (−1.8, 2.7)	.674	−0.5 (−3.4, 2.3)	.713	−0.2 (−0.4, 0.1)	.289
	A	0.4 (−1.8, 2.5)	.740	−0.8 (−3.6, 1.9)	.554	−0.1 (−0.4, 0.1)	.289
PFHxA	U	1.8 (−1.6, 5.2)	.299	**4.9 (0.7, 9.1)**	**.023**	−0.2 (−0.6, 0.2)	.411
	A	0.7 (−2.5, 3.9)	.675	**4.4 (0.3, 8.5)**	**.039**	−0.1 (−0.5, 0.3)	.603
PFHxS	U	−0.1 (−1.8, 1.7)	.944	−0.6 (−2.8, 1.6)	.602	**−0.3 (−0.5, 0)**	**.019**
	A	0.2 (−1.6, 1.9)	.862	−0.8 (−3.1, 1.5)	.501	−0.2 (−0.4, 0)	.104
PFOSA	U	0.4 (−0.7, 1.6)	.462	0.6 (−0.9, 2)	.453	−0.1 (−0.3, 0)	.125
	A	0.4 (−0.7, 1.5)	.437	0.6 (−0.9, 2)	.434	−0.1 (−0.2, 0)	.175
PFHpA	U	0.9 (−1.5, 3.2)	.472	0.1 (−2.9, 3.1)	.945	−0.1 (−0.4, 0.2)	.466
	A	0.8 (−1.5, 3)	.511	−0.3 (−3.3, 2.6)	.835	0 (−0.3, 0.3)	.903
PFBS	U	0.9 (−0.4, 2.2)	.176	0.3 (−1.4, 1.9)	.747	−0.1 (−0.3, 0)	.155
	A	0.7 (−0.5, 1.9)	.231	0.1 (−1.5, 1.7)	.873	−0.1 (−0.3, 0)	.152
OPFR Metabolite							
DPhP	U	1.1 (−0.9, 3.1)	.278	1.8 (−0.7, 4.3)	0.167	0 (−0.2, 0.3)	.893
	A	1.2 (−0.6, 3.1)	.202	1.9 (−0.6, 4.3)	0.136	0 (−0.2, 0.3)	.854
BDCIPP	U	0.2 (−1.7, 2)	.862	0.4 (−1.9, 2.8)	0.717	0.1 (−0.1, 0.3)	.396
	A	0.5 (−1.2, 2.3)	.554	1 (−1.3, 3.3)	0.411	0.1 (−0.2, 0.3)	.556
BCIPP	U	**1.9 (0.1, 3.6)**	**.035**	2 (−0.2, 4.3)	0.076	0 (−0.2, 0.2)	.819
	A	**2 (0.3, 3.6)**	**.019**	1.9 (−0.3, 4.1)	0.086	0 (−0.2, 0.2)	.995
BCEP	U	1.4 (−0.2, 3)	.100	1.8 (−0.3, 3.9)	0.090	0.1 (−0.1, 0.3)	.205
	A	1.3 (−0.3, 2.8)	.109	1.4 (−0.6, 3.5)	0.175	**0.2 (0, 0.4)**	**.021**

[a]Unadjusted (U) or adjusted (A) for race/ethnicity, insurance type, and parity.
[b]Diastolic blood pressure model.
[c]Systolic blood pressure model.
[d]Serum lipid level model.
Significant results bolded ($p < .05$).

chemicals that target similar pathways may contribute to perturbations in placental development and function during vulnerable periods in pregnancy.

DECLARATION OF CONFLICTING INTERESTS

The authors have no competing interests to declare.

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HUMAN SUBJECTS

Written and verbal consent for this study was obtained according to the Declaration of Helsinki and has been approved by UCSF's institutional review board.

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