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A Suspect Screening Method for Characterizing Multiple Chemical Exposures among a Demographically Diverse Population of Pregnant Women in San Francisco

Aolin Wang,[1,2] Roy R. Gerona,[3*] Jackie M. Schwartz,[1] Thomas Lin,[3] Marina Sirota,[2,4] Rachel Morello-Frosch,[5] and Tracey J. Woodruff[1,6]*

[1]Program on Reproductive Health and the Environment, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, California, USA
[2]Bakar Computational Health Sciences Institute, University of California, San Francisco, California, USA
[3]Clinical Toxicology and Environmental Biomonitoring Lab, University of California, San Francisco, California, USA
[4]Department of Pediatrics, University of California, San Francisco, California, USA
[5]School of Public Health and Department of Environmental Science, Policy and Management, University of California, Berkeley, California, USA
[6]Philip R Lee Institute for Health Policy Studies, University of California, San Francisco, California, USA

Background: *In utero* exposure to environmental chemicals can adversely impact pregnancy outcomes and childhood health, but minimal biomonitoring data exist on the majority of chemicals used in commerce.

Objectives: We aimed to profile exposure to multiple environmental organic acids (EOAs) and identify novel chemicals that have not been previously biomonitored in a diverse population of pregnant women.

Methods: We used liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-QTOF/MS) to perform a suspect screen for 696 EOAs, (e.g., phenols and phthalate metabolites) on the maternal serum collected at delivery from 75 pregnant women delivering at two large San Francisco Hospitals. We examined demographic differences in peak areas and detection frequency (DF) of suspect EOAs using a Kruskal-Wallis Rank Sum test or Fisher's exact test. We confirmed selected suspects by comparison with their respective reference standards.

Results: We detected, on average, 56 [standard deviation (SD)]: 8) suspect EOAs in each sample (range: 32–73). Twelve suspect EOAs with DF $\geq$ 60 were matched to 21 candidate compounds in our EOA database, two-thirds of which are novel chemicals. We found demographic differences in DF for 13 suspect EOAs and confirmed the presence of 6 priority novel chemicals: 2,4-Di-tert-butylphenol, Pyrocatechol, 2,4-Dinitrophenol, 3,5-Di-tert-butylsalicylic acid, 4-Hydroxycoumarin, and 2′-Hydroxyacetophenone (or 3′-Hydroxyacetophenone). The first two are high-production-volume chemicals in the United States.

Conclusion: Suspect screening in human biomonitoring provides a viable method to characterize a broad spectrum of environmental chemicals to prioritize for targeted method development and quantification. https://doi.org/10.1289/EHP2920

Introduction

Scientific evidence demonstrates that *in utero* exposure to multiple environmental chemicals can adversely impact pregnancy outcomes and lead to adverse health effects throughout the lifespan (Diamanti-Kandarakis et al. 2009; National Cancer Institute 2010; The American College of Obstetricians and Gynecologists 2013; Wang et al. 2016). Over 30,000 pounds of industrial chemicals were produced for every American in 2012 alone (Di Renzo et al. 2015; U.S. EPA 2013). Further, the U.S. chemical industry is expecting faster growth in production volume in 2017 and 2018 in comparison with 2016 (an overall growth of 3.6% in 2017 and 4.8% in 2018 in comparison with 1.6% in 2016) (The American Chemistry Council 2016). The ubiquitous use of industrial chemicals results in measurable levels found in pregnant women as a result of their contact with contaminated food, water, air, soil, dust, and consumer products. Furthermore, women of color and low-income women in the United States can experience a higher frequency and magnitude of exposure to environmental chemicals and other stressors (Committee on Environmental Justice 1999; Morello-Frosch and Shenassa 2006). The combination of both chemical and nonchemical stressors can lead to adverse developmental outcomes (Morello-Frosch et al. 2011; Vesterinen et al. 2017; Vishnevetsky et al. 2015). Based on an analysis of the nationally representative National Health and Nutrition Examination Survey (NHANES) data, we found previously that virtually all pregnant women in the United States are exposed to at least 43 different chemicals (Woodruff et al. 2011). We have also found, in a smaller study of 65 pregnant women in San Francisco, a median of $\sim$25 chemicals measured above detectable levels in maternal serum (out of 59 compounds tested), and we detected the vast majority of these chemicals ($\sim$80%) in matched umbilical cord serum samples (Morello-Frosch et al. 2016).

Existing biomonitoring research mainly relies on targeted analytical chemistry methods, which measure only those chemicals selected *a priori* for analysis (Dennis et al. 2017). Only a few hundred chemicals are routinely measured in humans through targeted methods (U.S. CDC 2017; Wang et al. 2016), which are resource and time intensive to develop, whereas the potential number of chemicals to which we are exposed is much higher, as 8,000 chemicals in commerce are manufactured or imported in >25,000 lbs/year (U.S. EPA 2016a). However, the lack of publicly available data on chemical ingredients in industrial or commercial products in the United States hinders our ability to prioritize chemicals for targeted biomonitoring and exposure analysis. A more holistic approach that measures exposures via biomonitoring in a high-throughput fashion is critical to understanding the breadth of human exposure to the thousands of chemicals used in commerce and the subsequent health consequences of those exposures.

Advances in high-resolution mass spectrometry offer an opportunity to rapidly screen biological and environmental specimens for a large array of chemicals (Andra et al. 2017; Dennis et al. 2017). This screening allows better characterization of the chemical components

*These authors contributed equally to this work.

Address correspondence to T. J. Woodruff, UCSF Program on Reproductive Health and the Environment, 550 16th St., 7th floor, San Francisco, CA 94158, Telephone: +1 (415)-476-3198. Email: Tracey.Woodruff@ucsf.edu

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of the "exposome," defined as the totality of human environmental exposures from conception onwards (Wild 2005). Both "suspect screening" [e.g., (Chiaia-Hernandez et al. 2013; Plassmann et al. 2015; Rager et al. 2016)] and "nontargeted" [e.g., (Dennis et al. 2017; Schymanski et al. 2015; Sobus et al. 2017)] methods have been used to analyze high-resolution mass spectrometry data to complement targeted biomonitoring approaches. Though both methods acquire data in a nontargeted fashion, suspect screening differs from the truly nontargeted analysis in that it searches for analytes against a user-defined chemical database or existing chemical inventories, paired with software-matching algorithms that make use of accurate mass and isotope patterns. Nontargeted analysis does not search for candidate compounds using such pre-specified lists of suspected or targeted chemicals; hence, both data acquisition and analysis are agnostic (Krauss et al. 2010; Rager et al. 2016; Schymanski et al. 2015). We have developed a suspect screening method that combines nontargeted data acquisition using high-resolution mass spectrometry and targeted data analysis to screen for a subset of environmental chemicals in human biological samples that we call environmental organic acids (EOAs) (Gerona et al. 2017). EOAs are environmental organic compounds with at least one ionizable proton; many EOAs are widely used in consumer products (Dodson et al. 2012; Rudel et al. 2011). Some EOAs (such as bisphenol-A (BPA), methylparaben, and triclosan) have chemical structures that are similar to hormones and thus have the potential to cause endocrine disruption, which can negatively affect development (Diamanti-Kandarakis et al. 2009; WHO/UNEP 2012).

For this study, we apply our suspect screening approach to identify novel EOA exposures during pregnancy in a racially and economically diverse population. Our goals are to: 1) identify potential multiple chemical exposures, 2) determine whether these exposures differ by race/ethnicity and socioeconomic status in our study population, and 3) facilitate chemical prioritization for confirmation and targeted method development. Ultimately, we seek to develop a computational pipeline to identify high-priority chemicals for future targeted biomonitoring among pregnant women and to better assess environmental health disparities by race and income, and thereby improve efficiencies in the discovery of novel environmental chemicals for confirmation and measurement in human biomonitoring studies.

Methods

Study Population

We analyzed serum samples from pregnant women participating in the Chemicals in Our Bodies 2 Study (CiOB2), as part of the UCSF Pregnancy Exposures to Environmental Chemicals Children's Center (Morello-Frosch et al. 2016). We recruited pregnant women from the Zuckerberg San Francisco General Hospital (ZSFGH), which serves predominantly low-income women of color who do not have health insurance, and UCSF Mission Bay Medical Center (MB), which serves an economically and ethnically diverse population, including women of higher socioeconomic status. Eligibility criteria included: English- or Spanish-speaking, age 18 through 40 y old, and singleton pregnancies that were between 13 to 27 wk gestation (second trimester) at the time of recruitment. Eligible patients were recruited at a routine second trimester prenatal appointment, which included a request for permission to access Personal Health Information from maternal and infant medical records. Participants were interviewed, and biospecimens (urine and blood) were collected at the clinic upon enrollment or during a follow-up appointment. Between 1 March 2014, and 31 March 2016, 220 women enrolled in our study and 166 had delivered. Seventy-seven out of the 166 women agreed to have their samples banked and included in supplemental studies. In the current study, we analyzed maternal serum collected at delivery from 75 women whose banked maternal serum was available at the time of analysis.

CiOB2 study protocols were approved by the Institutional Review Boards of the University of California, San Francisco and Berkeley (13-12160).

Suspect Screening Summary

Our suspect screen workflow comprised four steps: chemical analysis, data processing, data analysis, and compound confirmation (Figure 1). Briefly, we first performed a high-resolution mass spectrometry analysis of maternal serum (see Chemical Analysis) and characterized the potential presence of 696 suspect candidates in our EOA database (based on suspect features that are successfully matched, by accurate mass, to candidate chemicals in our EOA database, but not yet confirmed) (see Data Processing). Then, we selected a subset of suspect candidates for confirmation based on a set of *a priori* criteria (see Data Analysis) and confirmed the presence of a suspect EOA if the LC-QTOF/MS results matched those of its corresponding reference standard (see Compound Confirmation).

Chemical analysis. **Sample preparation.** We analyzed 75 banked (−80°C) serum samples from pregnant women participating in the CiOB2 Study. We thawed 250 μL of each serum sample, spiked it with 2.5 μL of 1 μg/ml internal standards [2.5 ng BPA-d16, 2.5 ng C-13 Monobenzyl phthalate, 2.5 ng C-13 Mono-(2-ethylhexyl) phthalate], and centrifuged it at 3,000 rpm for 10 min before preparing it for LC–QTOF/MS by solid phase extraction (SPE) using Waters Oasis HLB cartridge (10 mg, 1 mL). We washed each SPE cartridge with five column volumes of methanol to eliminate possible environmental chemical contamination and then activated the cartridge with water before loading 250 μL of serum. We then washed the column with 5% methanol before eluting each analyte by methanol. We evaporated the methanol eluates under a stream of nitrogen gas, then reconstituted them in 250 μL of 10% methanol for column injection.

LC–QTOF/MS Instrumental Analysis. Separation of analytes in each sample was achieved by LC using an Agilent LC 1260 (Agilent Technologies). A 50 μL aliquot of the extract was used for each of the duplicate injections of the sample into an Agilent Poroshell 120 C18 column (2.1 Å 100 mm, 2.7 μm) maintained at 55°C. Chromatographic separation of the analytes was achieved by gradient elution using water with 0.05% ammonium acetetate (pH = 7.8) as mobile phase A and methanol with 0.05% ammonium acetate (pH = 7.8) as mobile phase B. The use of higher pH aids in further ionizing acidic compounds, thus enhancing the sensitivity of the assay. The elution gradient employed was: 0–0.5 min, 5% B; 1.5 min, 30% B; 4.5 min, 70% B; 7.5–10 min, 100% B; 10.01– 14 min, 5% B.

The LC system was connected to an Agilent QTOF/MS 6550, which collects both accurate mass precursor ion and product ion scans using an Agilent Jetstream electrospray ionization source operated in the negative polarity, a mode that facilitates better ionization of acidic compounds such as environmental organic acids. The QTOF/MS was run under the following conditions: gas temperature at 255°C; sheath gas temperature at 350°C; drying gas flow at 14 L/min; sheath gas flow at 11 L/min; nebulizer pressure at 14 psi; voltage cap at −2,500V; and, nozzle voltage at −1,500V. Data acquisition was run at 2 GHz in extended dynamic range mode. A TOF-MS scan across the range of 80–600 m/z was collected at high resolution for eluates coming out of the LC from 0.5–12 min. Using the Auto MS/MS mode (information-dependent acquisition), a product ion scan (MS/MS) of the three most abundant peaks at high resolution was triggered each time a precursor ion with an intensity of ≥500 counts per second was generated in the TOF-MS scan; active exclusion of previously selected peak was held for 0.1 min. The LC–QTOF/MS run produces a total ion chromatogram for each sample, which includes the following: the

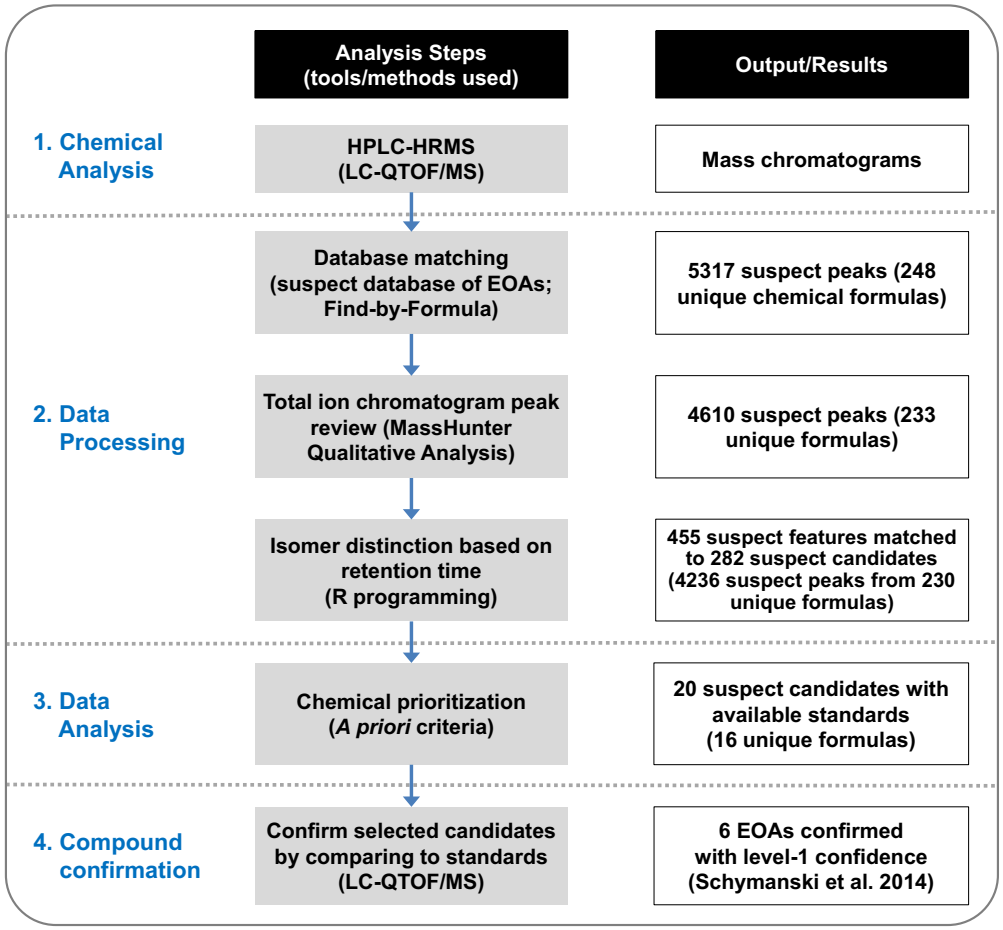


Figure 1. Suspect screening workflow and corresponding output/results using LC-QTOF/MS for detecting environmental organic acids (EOAs) in 75 serum samples collected at delivery.

accurate mass of each unique compound (expressed as m/z of their corresponding anion), peak area, and retention time (RT) and spectral data on the parent ion (compounds) and fragment ions, including isotopic pattern.

Blank samples. Along with the samples, we included two sets of blanks: solvent blank (consisted of only the mobile phase solution, also called double blank) and matrix blank (synthetic human serum that has undergone the same analytical process as the samples). Each batch runs seven solvent blanks and six matrix blanks. The analytic chemist in our team performed visual inspections of chromatogram peaks and excluded mass features that appeared in either blank. Because nontargeted data acquisition collects all accurate masses in a given sample, the matrix blank provided the indication of potential contamination in the analytical method.

Quality assurance. We spiked several labeled internal standards in each sample and into the matrix blank to normalize each run. Two of these internal standards consistently showed up in each sample: C13-Monobenzyl phthalate and C13- Mono 2-ethylhexyl phthalate. Because 50 uL of serum extract was used for sample analysis, there were RT shifts for some mass features across batch runs as the column aged. Each column was retired when more than 0.3 min in RT shift was observed in the internal standard. Because this is a screening method that collects all accurate masses in a sample and not a targeted method, the analysts have no basis for the appropriate compounds and their levels to use as quality control (QC) samples. Accordingly, no QC samples were used in this particular batch of sample analysis.

Data processing. **Suspect database of EOAs.** Our suspect database of EOAs (referred to EOA database throughout) had 696 entries and included chemicals from the following classes: phenols, such as parabens; phenolic and acidic pesticides and their predicted acidic and phenolic metabolites (referred to as "predicted pesticide metabolites" throughout the remaining of this paper); per- and polyfluoroalkyl substances (PFAS); phthalate metabolites; phenolic metabolites of polybrominated diphenyl ethers (OH-BDEs) and polychlorinated biphenyls (OH-PCBs). We generated predicted pesticide metabolites from relevant parent compounds by applying two common biological transformations that occur from metabolism: (1) hydroxylation of aromatic ring leading to the formation of phenolic metabolite, and (2) hydrolysis of carboxylic, phosphonic and sulfonic esters leading to the formation of their corresponding acids (Parkinson and Ogilvie 2010). Each entry in the EOA database included the molecular formula, chemical name, and class. We compiled the EOA database using the following data sources: the U.S. EPA's (U.S. Environmental Protection Agency) Toxic Substances Control Act Inventory (U.S. EPA 2016b), ToxCast Chemicals (U.S. EPA 2010), and High Production Volume (U.S. EPA 2012b), U.S. EPA Inventory Update Reporting (U.S. EPA 2006) and Chemical Data Reporting (U.S. EPA 2012a) chemical lists; the NHANES 2009 biomonitoring chemicals list (U.S. CDC 2009); the TEDX List of Potential Endocrine Disruptors (The Endocrine Disruption Exchange 2011); the California Environmental Protection Agency's Proposition 65 List of Chemicals (Office of Environmental Health Hazard Assessment 2010) and Pesticide Use Reporting (PUR) database (California Department of Pesticide Regulation); and the Agilent Pesticide Personal Compound Database and Library (Agilent Technologies). We performed additional PubMed literature

searches of environmental chemical biomonitoring studies published between 2000 and 2012 using specific terms such as "bisphenol," "environmental phenol," "phthalate metabolite," and "perfluorinated compound." The EOA database is available in the supplementary file (Excel Table S1) and in the EPA's CompTox Chemistry Dashboard (https://comptox.epa.gov/dashboard/chemical_lists).

Find-by-formula algorithm. We used the Agilent MassHunter Qualitative Analysis software (version B.06.00) Find-by-Formula (FBF) algorithm to analyze output data from the LC- QTOF/MS, using a set of optimized parameters previously reported (Gerona et al. 2017) (Table 1). We generated a list of suspect peaks —— chromatogram peaks whose accurate masses (acquired in the LC-QTOF/MS chemical analysis) matched the exact masses of candidate chemicals (based on chemical formulas) in the EOA database, accounting for the ionization mode (i.e., formation of M-H) with target score of 70 as the threshold. Because our LC-QTOF/MS analysis can detect several chromatogram features with the same mass (isomers) in maternal serum, one unique chemical formula from our EOA database might be matched to either one or many features in the total ion chromatogram generated from the LC-QTOF/MS analysis. For the chemicals that are isomers in our EOA database, we assigned equal probabilities to the mass feature being any one of the matched candidate chemicals in the database. We called a feature a "mass match" when it was successfully matched to a chemical formula from the EOA database. We did not include acetate adducts in our analysis to reduce the number of false positives.

Total ion chromatogram (TIC) peak review. We performed visual reviews of total ion chromatogram (TIC) peaks to remove 707 (13%) suspect peaks that (1) had poor peak shape (e.g., very broad peaks, peaks with multiple shoulders, peaks with signal-to-noise (S/N) <3), or (2) had peak areas ≤1.10 times the maximum observed peak area in the solvent or the double blanks.

Isomer distinction. As noted earlier, isomers are compounds with the same chemical formula but differ in chemical structure. Because RTs depend on chemical structure, isomers detected in the LC-QTOF/MS will have the same accurate mass but different RTs. Thus, we used a custom R script to distinguish between isomers from the chromatogram peaks of all individual samples. The R script clustered mass matches into isomer groups based on RT, i.e., realigning suspect features (available in the supplementary file, Isomer_distinction_R_script.txt). Briefly, we first ranked all suspect peaks by chemical formula, then RT. We considered a suspect peak to be from a different isomer if its RT differed from the RT of the same chemical formula in the previous row by more than 0.16 min.

Table 1. Agilent Find-by-Formula algorithm parameters used in suspect screening.

Tab	Criteria used
Options	
Formula Source	Database/Library: *Environmental Organic Acid suspect database*
	Maximum number of matches per formula: *1*
	Check "*Automatically increase for isomeric compounds*"
	Values to match: *Mass*
Formula Matching	Masses: $+/-10.00\,ppm$
Negative Ions	Check only "*-H*"
	Charge state range: *1*
Scoring	Contribution to overall score
	Mass score: *100*
	Isotope abundance score: *60*
	Isotope spacing score: *50*
Results	Chromatogram and Spectra
	Check "*Extract EIC*"
	Check "*Extract cleaned spectrum*"
Result Filters	Warn if score is <75.00
	Do not match if score is <70.00

Cutoff points ranging from 0.15 to 0.20 with a 0.01 increment were tested, and 0.16 allowed the best distinction based on graphical examination. For isomers that could not be correctly separated using this method (usually isomers with similar structures and thus very similar RTs), we consulted the analytical chemists on the research team whenever there were questions on the RT shift of mass features observed across samples, visualized data in both the Agilent software and R, and assigned isomer groups manually per batch. After isomer grouping, one "suspect feature" will represent a group of suspect peaks with the same chemical formula and similar RTs. The detection frequency of a suspect feature will be the number of suspect peaks in this group. When no isomers are present, suspect feature and "suspect candidate" are equivalent. In the case of the 180 isomers in our EOA database, multiple candidate chemicals with the same formula can be matched to either one (if only one isomer was detected in maternal serum) or many suspect features (if >1 isomer was detected). Figure S1 shows an illustration of the many (suspect features)-to-many (suspect candidates) matching for the latter case.

***Statistical analysis.* Summary list of highly detected suspect EOAs.** We compiled a list of suspect features that had detection frequency (DF) ≥60 (80% of the participants) to identify those chemicals that were more likely to be present in our study population (Table 2). Based on the suspect candidates that these features were matched to, we searched the candidates against the list of chemicals currently being biomonitored by the NHANES (as of April 2016) (U.S. CDC 2015) or the California Environmental Contaminant Biomonitoring Program (also known as Biomonitoring California) (Biomonitoring California 2015), or chemicals that were of high production volume in the United States (i.e., being manufactured in or imported into the United States with an aggregate volume of 1 million pounds or more per year) (U.S. EPA 2012b).

Demographic differences. As part of prioritizing suspect features for confirmation, we evaluated which suspect features appeared to differ by demographic variables, including race/ethnicity, education, household income, and nativity (U.S.-born status). Thirty-three participants had missing values in the nativity variable; we considered them to be born in the United States if the difference between their age and the reported years lived in the United States was one year or less. Using this imputation approach, we were able to include data on an additional 115 suspect features detected in two participants in the analysis. We included observations with missing nativity information in all analysis except for when we evaluated difference by nativity (N = 44) in detection frequency or peak area.

We used the chromatogram peak area to evaluate demographic differences in concentration semiquantitatively for suspect features detected in ≥80% of participants. The chromatogram peak area, as integrated by the Agilent Qualitative Analysis software, is a function of both the absolute concentration and ionization efficiency of an individual compound. It can be roughly used to compare the concentration for the same compound across samples and across batches. As the peak area may also depend on matrix effects that could manifest in ion suppression in human sample extracts, we used the nonparametric Kruskal-Wallis Rank Sum test to compare the rank orders of the peak area values (instead of the actual values) in assessing differences by demographic variables.

For suspect features detected in <80% of participants, we used a Fisher's exact test to examine differences in detection by demographic category. We limited this analysis to compounds that were detected in at least 20% of participants with nonmissing values for a certain demographic variable. We restricted analysis by race/ethnicity to whites, Latinas, and Asians as there were only four African Americans (with a total of 240 suspect peaks) and one participant (with 55 suspect peaks) who identified herself as "nonHispanic other."

We also calculated adjusted p-values to correct for multiple hypothesis testing for each demographic comparison (separately for

Table 2. List of suspect features that were detected in greater than 80% of maternal serum samples collected at delivery (N = 75).

Suspect features identified after FBF			Information on matched suspect candidates in EOA database					
Chemical formula	RT (mean)	DF	Isomers[a]	Names	Chemical class	NHANES[b]	CA[c]	HPV[d]
C8HF17O3S	5.502	75	0	Perfluorooctane sulfonic acid (PFOS)	Per- and polyfluoroalkyl substances	√	√	
C14H22O[d]	6.719	74	4	2,4-Di-tert-butylphenol (2,4-DTBP)	Phenols			
				2,6-Di-tert-butylphenol (2,6-DTBP)	Phenols			
				4-Octylphenol	Phenols			
				4-tert-Octylphenol	Phenols	√		√
C10H14O2[e]	4.029	70	0	4-Butoxyphenol	Phenols			
C7H8O	1.999	70	2	2-Methylphenol	Phenols			√
				4-Methylphenol	Phenols			√
C8H8O3[e]	1.931	66	3	2,4-Dihydroxyphenone	Phenols			
				Methyl paraben	Phenols	√	√	√
				Phenoxyacetic acid	Acidic pesticides			
C15H22O3	5.132	64	0	3,5-Di-tert-Butylsalicylic acid	Phenols			
C9H12O2	4.553	64	2	2-Isopropoxyphenol	Phenols	√	√	
				4-Propoxyphenol	Phenols			
C11H14O2[e]	5.129	63	0	Methyl eugenol	Phenols			
C12H17NO3	3.977	63	0	Promecarb metabolite	Predicted pesticide metabolites			*
C16H22O4	4.773	63	3	Mono-2-ethylhexyl phthalate (MEHP)	Phthalate metabolites	√	√	
				Monoisooctyl phthalate	Phthalate metabolites			
				Monooctyl phthalate (MOP)	Phthalate metabolites	√		
C12H15NO4	1.119	61	0	Carbofuran metabolite	Predicted pesticide metabolites			*
C16H26O2[e]	6.153	61	0	Octylphenol monoethoxylate	Phenols			

Note: EOA, environmental organic acid; FBF, find-by-formula; DF, detection frequency; RT, retention time (in minutes); NHANES, National Health and Nutrition Examination Survey; HPV, high production volume.
[a]Number of isomers based on EOA chemical database.
[b]Currently biomonitored by NHANES.
[c]Currently biomonitored by the Biomonitoring California Designated Chemical list (October 2015), http://biomonitoring.ca.gov/sites/default/files/downloads/DesignatedChemicalsList_October2015.pdf.
[d]Chemicals being manufactured and/or imported into the U.S. with an aggregate volume of 1 million to 10 million pounds/year, according to the U.S. HPV list by the U.S. Environmental Protection Agency (2004), https://iaspub.epa.gov/sor_internet/registry/substreg/list/details.do?listId=74.
[e]Compounds that showed potential differences in peak area (semi-quantification of a chemical) by demographic variables based on raw *p*-values.
*Parent compounds promecarb and carbofuran are HPV chemicals.

peak area or detection frequency comparisons) using the Benjamini-Hochberg procedure (i.e., the false discovery rate method) at a pre-specified alpha level of 0.1 (due to the limited sample size) (Benjamini and Hochberg 1995). Isomer distinction and statistical analysis were performed using R (version 3.3.3; R Core Team).

Chemical prioritization criteria. The goal of this step was to prioritize and select suspect candidates based on a set of *a priori* criteria for compound confirmation to identify novel EOAs that have not been biomonitored. We applied three major steps. First, we ranked the suspect candidates based on the detection frequencies of their corresponding suspect features and gave a higher priority if the corresponding suspect feature differed by demographic characteristics in peak area or detection frequency. Then, we excluded suspect candidates that were phthalate metabolites because most of the matched phthalate metabolites have been biomonitored by NHANES or the California Biomonitoring program. We also excluded suspect candidates that were predicted pesticide metabolites for the current analysis because (1) their commercial standards are not available (due to the difficulties in identifying their chemical names and structure), through searching literature for some pesticide metabolites, or (2) the actual metabolite of some pesticides may not be the ones included in our EOA database. We presented information for suspect features matched to these pesticide metabolites for completeness and for the broader research community. Finally, we obtained additional information on the remaining suspect candidates of the detected suspect features according to their rank order by search the following database or resources: (1) list of chemicals currently being biomonitored in NHANES (U.S. CDC 2015) or the Biomonitoring California program (Biomonitoring California 2015); (2) U.S. EPA's CPCat (Chemical and Product Categories) database (Dionisio et al. 2015; U.S. EPA 2014) for their usage information; (3) high-production-volume chemical list in the United States (U.S. EPA 2012b); and (4) PubChem Substance and Compound database (Kim et al. 2016) for their safety and hazards and toxicity information. We did not select suspect candidates that are currently being biomonitored, not used in consumer products (e.g., endogenous human metabolites such as sex hormones), or classified as Group E (Evidence of Noncarcinogenicity for Humans) for carcinogenicity.

We purchased available chemical reference standards for further confirmation. Vender information for the reference standards is presented in Excel Table S2.

Compound confirmation. We confirmed the presence of suspect EOAs by re-running the LC-QTOF/MS analysis with their corresponding reference standard. A suspect EOA was considered confirmed (present in maternal serum) with level-1 confidence in identification (Schymanski et al. 2014) if it had the same RT, accurate mass, and MS/MS spectral pattern as the LC-QTOF/MS results for the reference standard. At least two MS/MS spectral fragments were used to confirm similarity in fragmentation pattern. In the case of isomers, we compared the RT, accurate mass, and MS/MS spectral pattern of all suspect EOAs with the same formula to the reference standard results. All the remaining suspects were assigned a level-3 confidence in identification as tentative candidate(s) (Schymanski et al. 2014).

The processed dataset used in this study is available via the ImmPort database (accession: SDY1363).

Results

Description of the EOA Database

Of the 696 EOAs included in the database, predicted pesticide metabolites (51.1%) and phenols (24.1%) made up the majority, whereas PFAS (7.0%), acidic pesticides (6.3%), phthalate metabolites (5.6%), phenolic pesticides (2.3%), OH-BDEs (1.7%), and OH-PCBs (1.7%) made up the rest (Figure 2A). Of the EOAs in the EOA database, 516 (74%) had unique molecular formulas, but 180 of the EOAs were

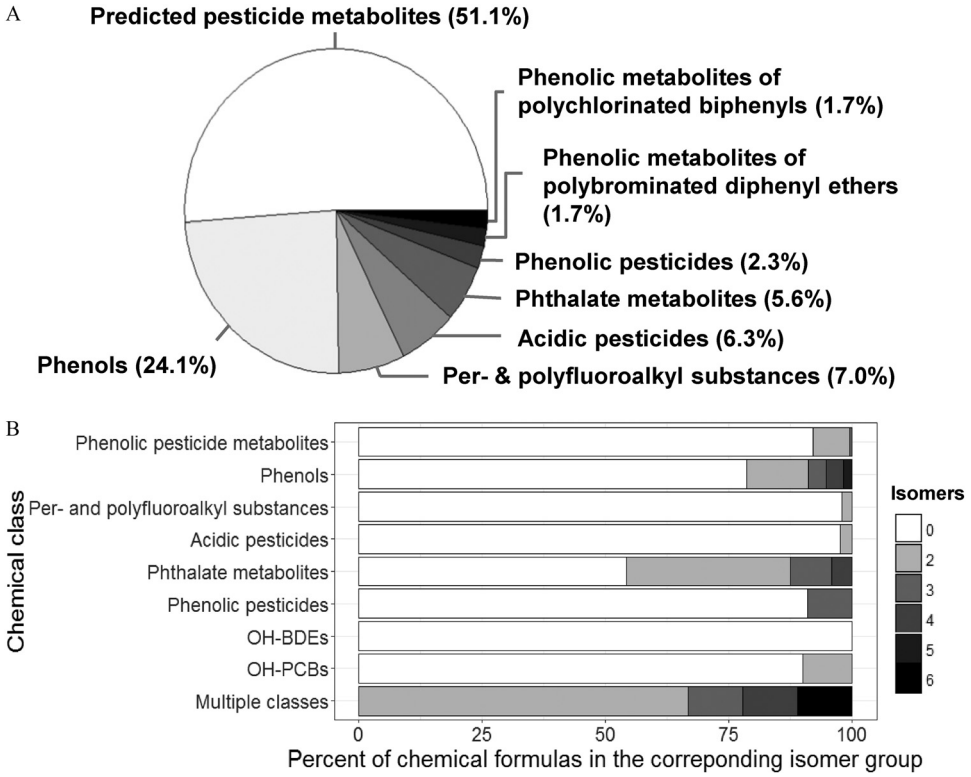


Figure 2. Composition of environmental organic acid (EOA) database: (*A*) Number of compounds (N = 696) by chemical class; (*B*) Isomer composition by chemical class (590 unique formulas).

isomers (compounds with the same molecular formula but with different chemical structures). The number of isomeric forms present in the EOA database ranged from 2 to 6 (55 EOAs have 1 other isomer in the database, 10 have 2, 6 have 3, 2 have 4 and 1 has 5). Twenty-five EOAs (corresponding to 9 unique chemical formulas) in the EOA database had an isomer that was of a different chemical class and thus were recategorized as "multiple chemicals classes" (see Table S1 for a list of chemical formulas, compound names, and chemical classes for these EOAs). Phthalate metabolites and phenols had a higher percentage of isomers (46% and 21%, respectively) than the other chemical classes (ranging from 0% for OH-BEDs to 10% for OH-PCBs) (Figure 2B).

Participant Characteristics

One-third ($n = 25$) of our participants obtained care at ZSFGH and the balance ($n = 50$) from MB. The majority of women were Latinas or nonHispanic whites and were married or cohabitating. About half of the participants had high socioeconomic status (45% having postgraduate education and 57% having an annual household income ≥$80,000). Nine participants (out of 44 who answered the question regarding nativity) indicated that they were born outside of the United States. Latina women, women with some college education or college degree (in comparison with women with high school or less education or women with a postgraduate degree) or women with lower household income (in comparison with women with an annual household income ≥$80,000), had fewer suspect EOAs detected in serum (Table 3).

Detection Frequencies of Suspect EOAs

We obtained 5,317 initial suspect peaks for 248 unique chemical formulas after using the FBF algorithm (Figure 1). Based on our isomer distinction method, we successfully assigned isomer grouping for 3,043 (66%) out of 4,610 post-TIC-peak-review suspect peaks. We used R and Agilent MassHunter Qualitative Analysis to visualize data and consulted the analytical chemists on our research team for the remaining 1,567 suspect peaks that needed further examination, and through this process assigned isomer groups for 1,193 out of the 1,567 remaining suspect peaks. The final analytical sample consisted of 4,236 (92%) suspect peaks that made up 455 suspect features. An overview of the 455 suspect features by RT and mass is included in the Supplemental Figure S2. We detected, on average, 56 (SD: 8) suspect features in each woman (range: 32–73). The majority of the detected suspect features were phenols (43.0%) and predicted pesticide metabolites (22.6%), followed by phthalate metabolites (17.0%), chemicals belonging to multiple chemical classes (6.8%), PFASs (5.2%), phenolic pesticides (2.7%), and acidic pesticides (2.6%) (Figure 3), based on chemical classes of their matched suspect candidates. We detected more suspect features that were phenols or phthalate metabolites than expected based on the chemical class composition of our EOA database, possibly because there are more isomers in these two chemical classes than in other classes (Figure 2B). Yet, we detected fewer than expected pesticide metabolites [comparing 23% (actual detection) with 51% (expected)]. We did not detect suspect features that were OH-BDEs or OH-PCBs.

The DF distribution of 455 suspect features was right-tailed, with 174 suspect features being detected in only one participant, 89 suspect EOAs with DF ≥ 15 and 32 suspect features with DF ≥ 38. There were 12 suspect features with DFs ≥ 60 that were matched to 21 candidate chemicals in the EOA database (Table 2). Based on external information from NHANES and the California Biomonitoring program, 15 out of these 21 suspect candidates have not been previously biomonitored by either of these two programs (Table 2).

Difference by Demographic Characteristics

We observed no clear visual clustering of the log-transformed (base 2) and scaled-by-compound peak area values of 89

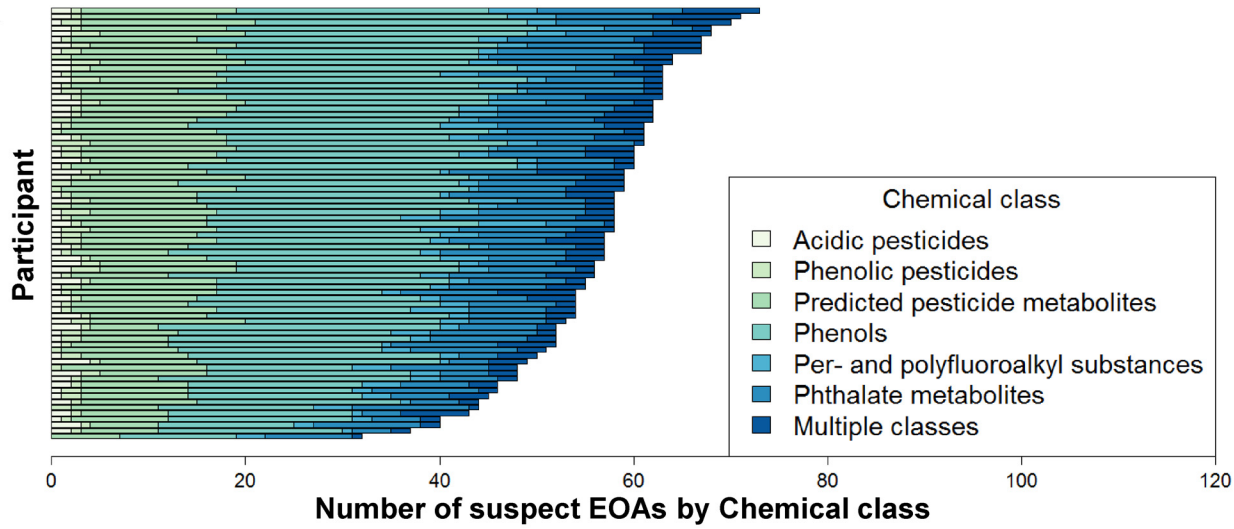
Table 3. Characteristics of pregnant participants from the Chemicals in Our Bodies 2 Study. (Morello-Frosch et al. 2016) (N = 75).

Characteristics	Descriptive statistics		Suspect EOAs detected per women, mean (SD)
	Mean (SD)	N (%)	
Age	33.3 (4.6)		
Race/ethnicity			
Latina		26 (35)	53 (7.6)
Non-Hispanic white		29 (39)	57 (7.9)
Non-Hispanic Asian		12 (16)	60 (7.7)
Non-Hispanic African Americans		4 (5)	60 (10.6)
Non-Hispanic other		1 (1)	55 (-)
Missing		3 (4)	58 (5.7)
Marital status			
Married/cohabitating		63 (84)	57 (7.6)
Separated/divorced		4 (5)	50 (9.1)
Never married		5 (7)	51 (11.5)
Missing		3 (4)	58 (5.7)
Education			
High school or less		18 (24)	57 (6.6)
Some college/college completed		19 (25)	53 (10.7)
Post-graduate		34 (45)	58 (6.6)
Missing		4 (5)	56 (7.0)
Household income			
<$20,000		15 (20)	54 (9.2)
$20,000–$79,999		14 (19)	54 (9.0)
≥$80,000		43 (57)	58 (7.1)
Missing		3 (4)	58 (5.7)
Nativity (Born in the United States)[a]			
Yes		35 (47)	57 (7.3)
No		9 (12)	57 (11.1)
Missing		31 (41)	56 (8.0)
Years lived in the US	24.0 (12.7)		

[a]Two observations with missing values were imputed based on participant's age and the reported years lived in the United States.

(DF ≥ 15) suspect EOAs by race/ethnicity, education, or household income (Figure 4). When 12 suspect EOAs with DF ≥ 60 (listed in Table 2) were examined on an individual basis, we found differences in peak areas by education for four suspect EOAs and by income for one suspect EOA, but the *p*-values were not significant after correcting for multiple testing (Figure S3). Among

A



B

	Acidic pesticides	Phenolic pesticides	Predicted pesticide metabolites	Phenols	Per- and polyfluoroalkyl substances	Phthalate metabolites	Multiple classes	Total
Min	0	0	7	12	1	4	1	**32**
Max	4	4	18	35	7	15	9	**73**
Mean (%)	1.5 (2.6)	1.5 (2.7)	12.7 (22.6)	24.3 (43.0)	2.9 (5.2)	9.6 (17.0)	3.9 (6.8)	**56.5 (100)**

Figure 3. Detections of suspect environmental organic acids (EOAs) among 75 pregnant women: (*A*) detections for each participant by chemical class, (*B*) distribution of detections by chemical class.

Figure 4. Visualization of peak area values (log transformed and scaled by compounds) of 89 suspect environmental organic acids (detection frequency ≥ 15), annotated by race/ethnicity, education, household income, and chemical class.

compounds with DF <60, we found that 13 suspect EOAs differed in DF by at least one of the three demographic variables: race/ethnicity, household income, or education, after multiple comparison adjustments (Table S2). One suspect EOA [formula: C6H4Cl2O, RT: 4.07, matched chemicals: 1,4-Dichlorobenzene metabolite, 2,3-Dichlorophenol (2,3-DCP), 2,4-DCP, 2,5-DCP, 2,6-DCP, or 3,4-DCP)] showed a difference in DF by all three demographic variables. None of the suspect EOAs differed by nativity status.

Prioritization and Confirmation of Selected Compounds

We first selected 25 suspect features (40 matched suspect candidates and 23 unique formulas) whose corresponding compounds had DF ≥ 60 (Table 2) or that differed by demographic variables (Figure S3 and Table S2). After excluding candidates that were phthalates or predicted pesticide metabolites (14 candidates), we prioritized 14 suspect candidates for chemical confirmation after evaluating information on chemical use and production, potential toxicity, and whether compounds had been measured in prior biomonitoring studies such as NHANES or the California Biomonitoring program. We persued further confirmation for 11 candidates (8 unique formulas) whose reference standards were available at the time and confirmed the presence of four novel (not previously biomonitored) EOAs: 2,4-Di-tert-butylphenol (2,4-DTBP), 3,5-Di-tert-butylsalicylic acid, 2,4-Dinitrophenol (2,4-DNP), and 4-Hydroxycoumarin. The remaining seven suspect EOAs were not confirmed, either because they did not have the same profile (same RT and MS/MS spectral pattern) or they were not detected in the confirmation QTOF/MS analysis even after spiking and running them in drug-free serum at high concentration. We then repeated this process for the remaining suspect candidates with DF ≥ 20, and we confirmed an additional two novel EOAs: Pyrocatechol and 2′-Hydroxyacetophenone (and/or its isomer 3'-Hydroxyacetophenone). Isomeric compounds 2′-Hydroxyacetophenone and 3'-Hydroxyacetophenone have very close structures and cannot be distinguished by RT solely in the current LC-QTOF/MS analysis. Compound structure and the corresponding extracted ion chromatograms for the confirmed chemicals and list of 20 suspect EOAs (16 unique formulas) selected for confirmation can be found in the Supplemental Material (Figure 5 and Table S3, respectively). A full list of 455 detected suspect features, their matched suspect candidates, and detailed information on prioritization can be found in Excel Table S2.

Discussion

We used a suspect screening approach to characterize the presence of EOAs in pregnant women's serum. We detected an average of 56 (range: 32–73) suspect features with mass matched to EOAs in maternal serum. Twelve highly detected suspect features were matched to 21 candidate chemicals in our EOA database; two-thirds of these 21 candidates have not been previously biomonitored. Thirteen suspect features differed in detection frequency by demographic characteristics. After confirmation, via

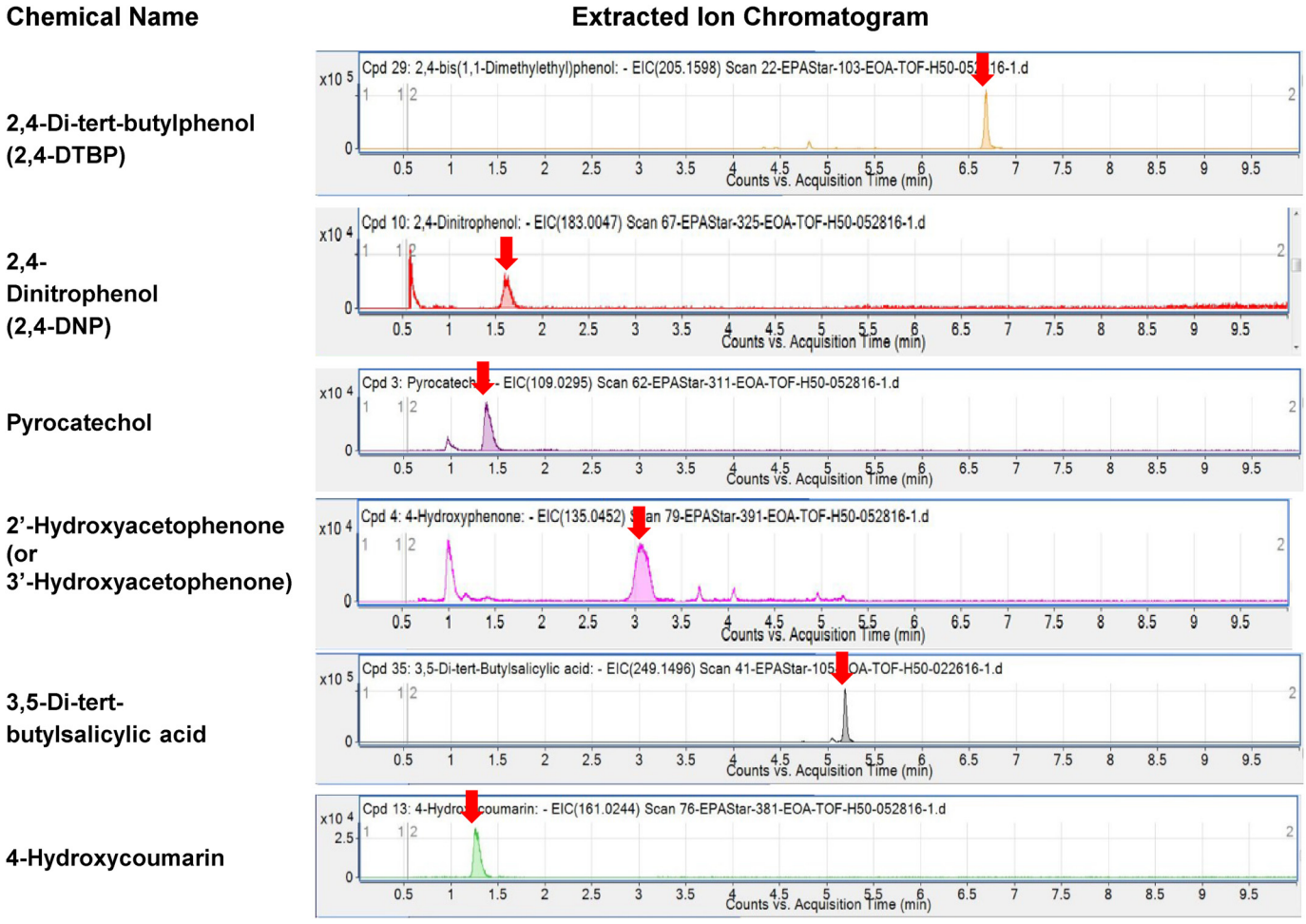


Figure 5. Extracted ion chromatograms for six confirmed compounds (with red arrow pointing to the corresponding peak).

comparison with reference standards, of 20 suspect candidates, we confirmed the presence of six novel EOAs in our sample, two of which —— 2,4-DTBP and pyrocatechol —— are of high production volume in the United States, with national aggregation of production volumes of 10 million to 50 million pounds per year (U.S. EPA 2017).

Five out of six confirmed novel EOAs have product-use information in the U.S. EPA's Chemical and Product Categories (CPCat) database (Dionisio et al. 2015; U.S. EPA 2014), and they have been used in manufacturing (e.g., chemicals and chemical products), pharmaceuticals, consumer products (e.g., cosmetics), and pesticides (Table 4). People can be exposed through eating contaminated food, drinking contaminated water, or breathing contaminated air. For example, 2,4-DTBP is an antioxidant widely used in food-related plastic products (Paquette 2004) and is found to be the most widely detected estrogenic compound (measured by estrogen equivalence concentration) leaching into drinking water from plastic pipes (Kelley et al. 2014; Liu et al. 2017; Löschner et al. 2011; Lund et al. 2011). European researchers also found 2,4-DTBP to be a major migrant in water from bottles or electric kettles made of polyolefin, polypropylene, Tritan™, or silicone, the latter three of which are substitutions for the polycarbonate baby bottles that contained the polycarbonate monomer BPA (Onghena et al. 2014; Skjevrak et al. 2005).

Evidence of the potential health risks associated with the majority of these confirmed compounds is lacking except for 2,4-DNP and pyrocatechol. Due to its harmful effect (cataract formation), the U.S. Food and Drug Administration banned 2,4-DNP from use for weight control in 1938 [ATSDR (Agency for Toxic Substances and Disease Registry) 1995]. It is also suspected of causing genetic defects and of damaging fertility and the fetus (Japan National Institute of Technology and Evaluation – Chemical Management Center) and can result in death due to occupational exposure (ATSDR 1995). Pyrocatechol, often known as catechol, has been classified as a possible human (Group 2B) carcinogen by the International Agency for Research on Cancer (IARC) (IARC 1999). However, the U.S. EPA has not classified catechol with respect to potential carcinogenicity (U.S. EPA 2000). We found limited information on chemical properties, bioactivities or health impact of 3,5-Di-tert-butylsalicylic acid, 4-Hydroxycoumarin, 2′-Hydroxyacetophenone, and 3′-Hydroxyacetophenone using the U.S. EPA's CompTox Chemistry Dashboard (U.S. EPA 2017). This finding suggests that the current suspect screening approach can indeed provide new insights regarding human exposures to the "known, unknown" chemicals —— chemicals that are unknown to an investigator but that are contained within a reference database or literature source (McEachran et al. 2017).

There are several limitations to our study. The first challenge, which is also common to nontargeted metabolomic research, is the transition from detected suspect features (mass peaks) to confident chemical (metabolite) annotations. During the "find-by-formula (mass)" or the annotation step, the matching of a detected suspect feature to a specific candidate chemical is solely based on mass (and isotopic pattern), potentially resulting in multiple candidates being matched to the same suspect feature and multiple suspect features being matched to the same candidate simultaneously, i.e., many (suspect features)-to-many (suspect candidates) match as illustrated in Supplemental Figure S1. Thus, it is possible that the confirmed compound turns out not to be the suspect feature of primary interest, but its isomer. For example, the primary suspect feature of interest (chemical formula: C8H8O2, RT: 1.04 min) has a DF of 54. However, the confirmed isomers 2′-Hydroxyacetophenone (and/or 3′-Hydroxyacetophenone) have RTs of 3.3, which were matched to another suspect feature with the same formula but low detection (DF = 1). Also, we cannot rule out the possibility that an unconfirmed suspect feature is, in fact, an isomer that is not included in our current EOA database or an endogenous metabolite that is an isomer of the candidate EOA listed in our database. Although our method is optimized for acidic compounds, a few neutral organic compounds and their metabolites may also not be ruled out among these possibilities.

Another limitation of our suspect screening method is the relatively lower sensitivity of the LC-QTOF/MS for detecting environmental chemicals with low concentrations in blood. Thus, some chemicals with relatively low DF based on the QTOF/MS could, in fact, be more widespread in our study population. To increase the sensitivity of EOA detection in our current study, we increased the injection volume of serum extract (50 uL, in comparison with typical volume of 1–2.5 uL). However, this approach can result in RT drift, i.e., peaks being broader in width with more tailing, which could affect peak identification and isomer grouping. In the present analysis, we performed visual peak review to remove features that had poor peak shapes. We also discarded some suspect peaks where there were a big RT drift and isomer grouping deemed

Table 4. Summary of the confirmed compounds and their uses from suspect screening of pregnant women (N = 75).

Name (DF in our sample)	Casrn	CPCat use categories[a]
2,4-Di-tert-butylphenol (2,4-DTBP)[b] (DF = 74)	96-76-4	Manufacturing (coke, transportation equipment, chemicals and chemical products, machinery and equipment, fabricated metal products, petrochemical, plastics material and resin); Fuel additives; Lubricants and additives; Solvents (for cleaning or degreasing); Stabilizers; Antioxidants; Consumer Products (Toys: PVCs); Personal Care Products (sanitary napkins)
3,5-Di-tert-butylsalicylic acid (DF = 64)	19715-19-6	NA
2,4-Dinitrophenol (2,4-DNP) (DF = 52)	51-28-5	Coloring agents; Uncoupling Agents; Pharmaceuticals; Cosmetics; Pesticides (biocides, inert ingredients)
Pyrocatechol[b] (DF = 39)	120-80-9	Manufacturing (chemicals and chemical products, leather and related products, luggage, metal, radio, television and communication equipment); Surface treatment; Process regulators; Coloring agents; Pharmaceuticals; Cosmetics (hair dyes, cosmetics); Food additives (flavorings, food contact substances); Pesticides; Human Metabolites
2′-Hydroxyacetophenone[c] (DF = 6)	118-93-4	Manufacturing (chemicals and chemical products, basic pharmaceutical products); Pharmaceuticals; Food additives; Consumer products (fragrances); Pesticides
4-Hydroxycoumarin (DF = 1)	1076-38-6	Pharmaceutical; Human Metabolites

Note: These listed compounds were confirmed with synthetic standards, with level-1 confidence in identification (Schymanski et al. 2014). Abbreviations: DF, detection frequency; CASRN, Chemical Abstracts Service (CAS) registry number; CPCat, Chemical and Product Categories; PVC, polyvinyl chloride.

[a]Condensed information based on the cassettes obtained from the U.S. EPA's Chemical and Product Categories (CPCat) database (Dionisio et al. 2015; U.S. EPA 2014). Underlined categories are shared categories between at least two chemicals.

[b]Chemicals being manufactured and/or imported into the United States with an aggregate volume of 10 million to 50 million pounds/year, according to the 2016 Chemical Data Reporting (CDR) data (U.S. EPA 2017).

[c]3′-Hydroxyacetophenone (CASRN: 121-71-1) and 2′-Hydroxyacetophenone are isomers that cannot be distinguished based on retention time (RT) only via the current LC-QTOF/MS analysis and was not listed in the table due to no information found on its uses from the CPCat database.

difficult, potentially resulting in increased false negatives. Still, false negatives could occur for chemicals with very low abundance, even using the current parameters optimized for detecting EOAs. For example, when running the reference standard of the compound 4-Hexyloxyphenol, we found that the peak of this compound matched to a small unintegrated peak at RT = 5.8 in our sample, but not the integrated (during the feature extraction step performed by the Agilent MassHunter Qualitative Analysis software) peak originally selected for confirmation at RT = 4.8. False negatives may also occur for EOAs that have short biological half-lives. Last, our study is moderately powered and thus may not detect demographic differences of suspect features, especially for nativity (31 observations with missing and nonimputed data).

Our suspect screening method is a semitargeted approach, where we can identify "known, unknown" environmental chemicals present in pregnant women. This method enables us to search for novel chemicals more effectively under the negative ionization mode in the LC-QTOF/MS analysis by searching in a predefined chemical space (EOAs), i.e., restricting feature extraction to compounds with the same mass as those from chemicals in the EOA database. However, the number of suspect features that we could find depended on the number of chemical formulas included in the current EOA database. In addition, some of the pesticide metabolites in our EOA database were based on predictions and might not exist or be the actual metabolite in human species.

We note that our EOA database consists of a fraction of the environmental chemicals that can be detected in a LC-QTOF/MS platform with a negative ionization mode and is an incomplete list of all environmental contaminants with anticipated exposures in pregnant women. For example, there are about 8,000 chemicals in commerce whose production and use are in large quantities (U.S. EPA 2016a), which could result in human exposures to these various chemicals. In addition, we limited the application of our suspect screening method to sera only. Nevertheless, our analysis provides a demonstration of the novel methods that can be used to more holistically scan and prioritize a large number of chemicals to which different populations may be potentially exposed. Further, our analysis reinforces the need to optimize suspect and nontargeted screening methods and to apply this approach to different matrices (e.g., sera, urine, and other tissues) to more fully characterize human exposure to a wide array of environmental chemicals.

Although similar suspect screening approaches have been used for environmental monitoring studies (Rager et al. 2016; Sjerps et al. 2016; Zhou et al. 2012), only a limited number of studies have focused on human samples (Hernández et al. 2009; Liotta et al. 2010; Plassmann et al. 2015). To our knowledge, our findings are the first from a suspect screening of serum from pregnant women. We combined chemical and statistical analysis to create a proof-of-concept tool for identifying novel chemicals for further study in four steps: (1) perform chemical analysis using LC-QTOF/MS, (2) identify suspect features by matching suspect peaks to a curated chemical database and performing peak review and isomer grouping, (3) prioritize suspect candidates for further confirmation based on a set of criteria, and (4) confirm selected suspect candidates by running their reference standards. Our method used visual inspection to reduce false positives, and we performed isomer grouping based on RT to allow for separation of suspect features. Our method also included information from our cohort to help prioritize chemical confirmation, in this case demographic data. This general workflow can be tailored to meet the needs for screening and identifying novel chemicals that are of interest to researchers in a larger population of interest. This study also provides a more comprehensive picture of the potential presence of environmental organic acids in a racially and economically diverse population of pregnant women.

Our study serves as an important starting point to apply a suspect screening approach to identify prevalent novel environmental chemical exposures in biospecimens from pregnant women. We anticipate that as the analytic methods mature, we will have a complementary approach that can be integrated with targeted methods to identify and prioritize chemical analyses. Suspect screening may also serve as a method for biomonitoring certain known chemicals upon further confirmation. For example, we identified previously that $\geq 99\%$ of U.S. pregnant women sampled in the NHANES were exposed to at least 43 different chemicals, with a portion of these women exposed to at least 139 chemicals out of the 163 evaluated (Woodruff et al. 2011). One of the suspect candidates (not confirmed) that was detected among all 75 women in our current study —— perfluorooctane sulfonic acid (PFOS) —— was also highly detected among pregnant women in NHANES (2003–2004 cycle) (Woodruff et al. 2011) and among a similar study population of pregnant women from University of California, San Francisco (UCSF) whose samples were collected between 2010 and 2011 (Morello-Frosch et al. 2016). Also, suspect candidates (not confirmed) 4-tert-Octylphenol and mono-2-ethylhexyl phthalate (MEHP) were prevalent among the U.S. pregnant women (detected in 69% and 89% of the samples based on the NHANES 2003–2004 data) (Woodruff et al. 2011) and had relatively high DFs in our sample (Table 2), albeit the matrix is different (urine in the NHANES versus serum in our study). Yet, the suspect candidate (not confirmed) perfluorooctanoic acid (PFOA) had a relatively low detection frequency in our sample (16%) compared to the detection frequencies based on targeted methods in the NHANES study (99%) and our previous UCSF study (66%). This may be due to both a lower sensitivity of the QTOF/MS method than the targeted analysis used in NHANES and differences in the timing of biospecimen collection (samples collected were collected in 2014–2016 for this study versus 2003–2004 for NHANES). We will further assess the value of the suspect screening approach as we develop targeted analytical methods for the six novel EOAs we confirmed in this study. Follow-up studies will quantify their serum levels in a cohort of 200 women and examine the association between these EOAs and pregnancy outcomes, including birth outcomes, and as well as developmental effects in offspring.

To further enhance the utility of this suspect screening method, we are pursuing several areas of improvement. First, we are developing more systematic approaches, including scoring methods similar to the ToxPi approach (Rager et al. 2016; Reif et al. 2010), which will integrate additional metadata (e.g., demographic or outcome information derived from the study cohort) into our chemical prioritization process. Second, we will expand suspect screening to both positive and negative ionization modes that will allow for evaluation of a broader set of "known, unknown" chemicals. Concurrently, we are using chemical information and resources from the U.S. EPA's CompTox Chemical Dashboard (McEachran et al. 2017) to expand our chemical database. Third, we are incorporating the use of open-source packages into our workflow to improve feature detection (Smith et al. 2006; Tautenhahn et al. 2008; Uppal et al. 2013) and better annotation (matching suspects to specific chemicals) (Edmands et al. 2017; Uppal et al. 2017) for analyzing a larger number of samples.

Conclusions

We reported the novel findings from a proof-of-concept suspect screening biomonitoring approach that identifies an average of 56 suspect EOAs in maternal serum, with six confirmed EOAs that may be of high priority for future biomonitoring among pregnant women. Based on this study, we find that suspect screening is a valuable supplement to existing targeted biomonitoring methods as it offers efficient high-throughput capacity to identify and prioritize

novel chemicals for future biomonitoring studies and will be important to assess exposure to the thousands of chemicals registered for commercial use in the United States. Characterizing and further quantifying previously unidentified environmental chemical exposures can provide critical guidance to the selection of chemicals for *in vitro* and *in vivo* studies to assess health risks as well as to future epidemiologic studies.

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