An insight into the chemical exposome during pregnancy - A non-targeted analysis study

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Abstract

The extensive use of human-made chemicals in our daily lives results in chronic exposure to complex mixtures of potentially harmful substances. We investigated chemical exposures in pregnant women in New York City by applying a non-targeted analysis (NTA) workflow to 95 paired prenatal urine and serum samples (35 pairs of preterm birth) collected as part of the New York University Children's Health and Environment Study. The goal was to i) study chemical exposures in this population, ii) explore differences in the chemical profiles comparing urine vs. serum samples, and comparing preterm vs. term birth samples, and iii) investigate potential associations between exogenous chemicals and endogenous metabolites. We analyzed all samples using liquid chromatography coupled with Orbitrap high-resolution mass spectrometry (LC-Orbitrap HRMS) in both positive and negative electrospray ionization modes (ESI^+ and ESI^-), employing full scan and data-dependent MS/MS fragmentation ($ddMS^2$) scans. We detected a total of 1,524 chemical features for annotation, with 12 chemicals confirmed by authentic standards. Two confirmed chemicals dodecyltrimethylammonium and n,n-dimethyldecylamine n-oxide appear to not have been previously reported in human blood samples. We observed a statistically significant differential enrichment between urine and serum samples, as well as between preterm and term birth ($p < 0.0001$) in serum samples. When comparing between preterm and term births, an exogenous contaminant, 1,4-cyclohexanedicarboxylic acid (tentative), showed a statistical significance difference ($p = 0.003$) with more abundance in preterm birth in serum. An example of chemical associations (12 associations in total) observed was between surfactants (tertiary amines) and endogenous metabolites (e.g., bioactive lipid mediators and fatty acid amides).

Keywords: Non-targeted analysis, High-resolution mass spectrometry, Preterm birth, Exogenous chemicals, Exposure

Synopsis

Non-targeted analysis of urine and serum samples from pregnant women reveals a potential link between environmental contaminants and preterm birth.

1. Introduction

Human beings are already exposed to hundreds of thousands of synthetic chemicals through exposure to consumer products, packaged and processed food, contaminated drinking water, and polluted air, and the number is only increasing.[1] Many of these chemicals may be adsorbed by the human body and potentially pose a threat to human health. In addition new compounds, also known as transformation products, might form through biotic and abiotic processes when these chemicals are exposed to different environments.[2, 3] Approximately 350,000 registered chemical substances have been used for commercial production and use over the past 40 years across 19 countries and regions.[4] Moreover, the United States Environmental Protection Agency (US EPA) has listed over 1,218,248 chemicals of environmental importance on EPA's CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/). Recent estimates suggest that only 10% of chronic human diseases can be attributed to genetics, leaving 90% potentially related at least in part to environmental factors.[5]

Pregnant women are routinely exposed to human-made chemicals from the ambient environment that may result in adverse outcomes for both the mother and fetus. Previous studies have highlighted that maternal exposure to environmental contaminants can increase the risk of obesity,[6] asthma,[7] and various conditions in offspring, including pre-term birth.[8] The timing of exposure is also an important factor as the effects of an exposure likely depend on the developmental processes that it coincides with. Epidemiological evidence indicates that exposure to environmental contaminants at any time between preconception and birth can restrict fetal growth, resulting in a fetus not reaching its full growth potential (lower birth weight than expected).[9] The fetal brain is particularly susceptible to prenatal exposure to endocrine-

disrupting chemicals, as neurulation and neuronal proliferation begin within the first trimester, while other processes such as neural migration, myelination, synaptogenesis, and apoptosis start mid-gestation and continue rapidly until birth.[10, 11] Investigating chemical exposure during the critical windows can provide insight on underlying biological mechanisms.

Traditional monitoring of contaminants in human samples relies on prior hypotheses, the availability of analytical standards, and the existence of a validated chromatographic method. Approximately 450 environmental chemicals are regularly measured in human samples (e.g., whole blood, serum, and urine) by the US National Health and Nutrition Examination Survey (NHANES).[12] This only accounts for approximately 0.5% and 0.04% of chemicals listed under a US federal law of Toxic Substances Control Act (TSCA) and EPA's CompTox Chemicals Dashboard, respectively. Such conventional approaches cannot capture the totality of chemical exposures and consequently important associations with various health outcomes may be missed. Recent advancements in high-resolution mass spectrometry (HRMS) have improved our ability to analyze thousands of different chemicals in a single run due to its high resolving power (> 30,000 FWHM), mass accuracy (1-5 ppm), and high scan speed.[13] Combined with a pre-separation technique such as gas or liquid chromatography (GC/LC), HRMS shows great promise in detecting unknown chemicals across various domains.[14] In recent years, non-targeted analysis (NTA) using HRMS has successfully been used to screen human samples, resulting in the discovery of numerous exogenous compounds (e.g., pesticide metabolites, endocrine-disrupting compounds, and poly- and perfluoroalkyl substances).[15-17] Numerous studies for unknown compounds have focused on the possible compounds that were postulated with suspect lists.[18] There is currently a great need for the application of NTA to characterize

different pathways of exposures in public health studies.

Based on previous NTA methods,[17, 19, 20] we developed a workflow to comprehensively profile all detectable chemical exposures and metabolites in biospecimens from a racially and socioeconomically diverse sample of pregnant women from New York City. The aims of this study were threefold: (1) to analyze 95 paired serum and urine samples from pregnant women using NTA and study their chemical exposures, (2) characterize differences in chemical enrichment between urine and serum, within each biospecimen type, between preterm and term births, and (3) explore the associations of endogenous metabolites with exogenous chemicals.

2. Materials and methods

2.1 Study participants information

For this study we used paired urine and serum samples collected between 2020 and 2022 during the same prenatal study visit from 95 participants in the New York University Children's Health and Environment Study (NYU CHES). NYU CHES is an ongoing pregnancy and birth cohort study that has been recruiting pregnant patients ≥ 18 years of age and < 18 weeks of gestation from NYU Langone Health-affiliated hospitals since March, 2016. The samples were mostly collected in the first trimester with 2 and 5 pairs for the second and third trimesters, respectively. Participant characteristics are presented in **Table 1**. All samples, including 10 blinded quality control (QC) samples consisting of synthetic urine and serum, were stored in bisphenol- and phthalate-free polypropylene tubes at -80°C.

Table 1. Characteristics of NYU CHES participants included in this analysis (N=95).

Demographic parameters	Value
Participant's race/ethnicity n(%)	
Hispanic	31.1
Non-Hispanic White	43.4
Non-Hispanic Black	2.8
Asian	18.9
Other	1.9
Mixed race	1.9
Pre-pregnancy body mass index (BMI, kg/m^2)	
Underweight (BMI < 18.5), %	3.8
Normal weight (BMI = 18.5 – 25), %	58.1
Overweight (BMI = 25 – 30), %	25.7
Obesity (BMI > 30), %	12.4
Maternal Education* (%)	
High school or less	26.0
Some college but no degree	6.0
Associate degree	4.0
Bachelor's degree	28.0
Post-graduate degree	36.0
Missing	6.0
Income* (%)	
< $30,000	12.2
$30,000 – $49,999	8.2
$50,000 - $74,999	10.2
$75,000 - $99,999	2.0
≥$100,000	49.0
Missing value	24.5
Number of preterm births	35
Maternal age at enrollment (years), mean (std)	31.6 (5.1)
Pre-pregnancy weight (kg), mean (std)	64.5 (16.3)
Maternal height (cm)	161.2 (7.4)
Gestational Age (weeks), mean (std)	38.3 (2.3)
Smoking* (%)	1.9
Alcohol use during pregnancy* (%)	11.9
Missing value (%)	2.8

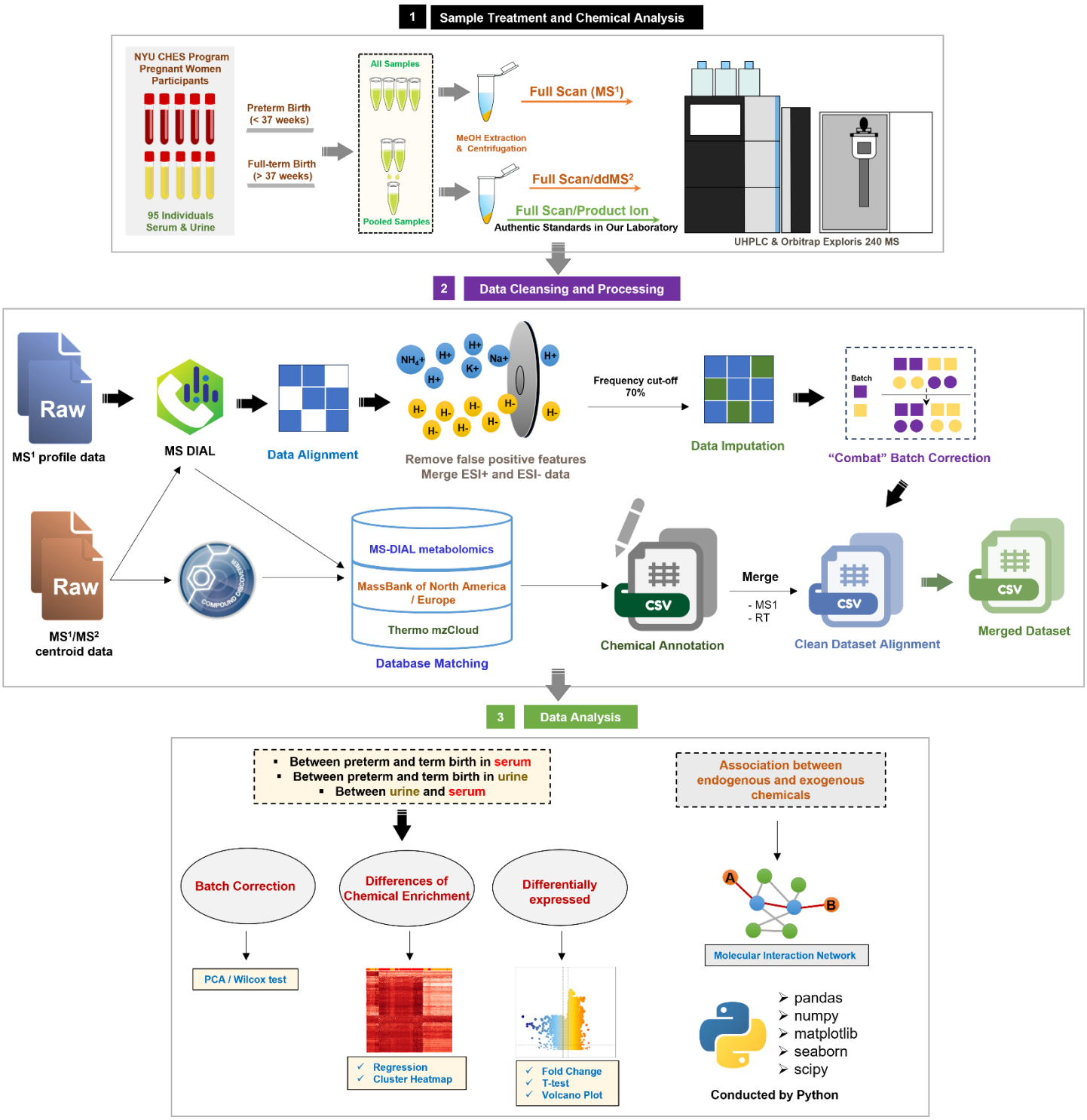
* When a parameter has missing data, it means that the participant chose the option "Prefer not to answer" / "Don't Know" from the questionnaires. The values in the parentheses correspond to the unit in the column of demographic parameters. Std indicates the standard deviations.

2.2 Workflow

The NTA workflow contained three major steps: (1) sample treatment and chemical analysis, (2) data cleansing and processing, and (3) data analysis (**Figure 1**). In this work, the individual samples and pooled samples were aimed to obtain MS^1 and MS^1/MS^2 spectra, respectively. The MS^2 spectra from pooled samples were matched to MS^1 spectra from the individual samples for database match. We used MS^1 data to examine the differences in chemical enrichment between different groups of samples, MS^2 spectra to match available databases composed of authentic standards and *in silico* predicted spectra, and to match to authentic standards in our laboratory. The chemical abundances in the diluted urine samples were adjusted using the creatinine normalization approach (**Details in Text S1, Supporting Information**). Chemical identifications and annotations were ranked based on the system proposed by Schymanski, et al. [21] The various confidence levels are as follows: Level 1, structure confirmed by a chemical standard with MS/MS and retention time (RT) matching; Level 2, probable structure deduced by spectrum database matching or other diagnostic evidence (e.g., parent ion information and MS/MS); Level 3, tentative candidate(s) supported by partial evidence for possible structure(s), but insufficient evidence for the exact structure(s); Level 4, an unequivocal molecular formula can be assigned through the spectral information but no enough information to propose possible structures; Level 5, only exact mass (m/z) with insufficient information to assign a formula.

Considering the complexity and heterogenous components in the present samples, different methods and tools were applied to explore and analyze the MS data (**Figure 1**). Following this workflow, we first used MS-DIAL to export the MS data for statistical analysis and MS/MS database matching. Python was used as the programing language for data analysis. All python

scripts are available on GitHub at the following link: https://github.com/jixiaowen4321/Jixiaowen. We also applied Thermo FreeStyle 1.8 for ion peak identification and Compound Discoverer 3.2 for matching with the Thermo mzCloud database. The details of each step in this workflow are described in the sections below.



136 **Figure 1.** Workflow diagram of sample treatment and chemical analysis, data cleansing and

137 processing, and data analysis for the urine and serum samples collected from 95 pregnant

138 participants in NYU CHES.

2.3 Sample preparation and analysis

All samples were completely thawed at room temperature (~21°C) and homogenized using a vortex mixer before extraction. For individual samples, 100 μL of sample was pipetted into a microcentrifuge tube. For pooled samples, 15 pools each of serum and urine were constructed from 10 individual 20 μL samples (200 μL total) randomly selected based on sample IDs using Python's random.choices() method. For extraction, 400 μL methanol was added to the tube, which was then shaken using a vortex mixer and centrifuged at 5000 rpm for 10 min. The upper clear layer of methanol was immediately filtered into an auto-sampler vial with an insert using a nylon membrane (pore size: 0.2 μm, Phenomenex, Torrance, CA). Triplicates of HPLC water were used as laboratory blanks and followed the same sample preparation procedure.

Analysis of the extracts was conducted using a Vanquish UHPLC and Orbitrap Exploris 240 MS (Thermo-Scientific, Waltham, MA). LC separation was achieved with an Ascentis® 3 μm C18 HPLC column (150 × 2.1 mm) (Sigma-Aldrich Supelco, St. Louis, MO) by gradient elution with 5% methanol + 95% HPLC water (A) and 100% methanol (B), both containing 0.1% formic acid at a flow rate of 0.2 mL min^{-1} and column temperature of 45 °C. The gradient method started at 5%B, ramping linearly to 100%B over 15 min, held for 5 min, and returning to starting conditions for column re-equilibration between 20.1 – 25 min.

The compounds in the samples were ionized using a heated electrospray ionization (HESI) probe in both positive (ESI^+) and negative (ESI^-) modes. The Orbitrap MS method used the following global parameters: sheath gas flow = 35; aux gas flow = 10; sweep gas flow = 1; vaporizer temperature = 400 °C; spray voltage = 3300/2000 (positive/negative); S-lens RF = 70%; ion transfer tube temperature = 352 °C. A full MS/data-dependent MS^2 spectra acquisition

(ddMS^2) method was used with the following scan settings: 90,000/12,000 resolution, normalized AGC target = standard, max injection time = auto, normalized HCD collision energy (%) = 30, 50, 70, full MS scan range of 100-1000 m/z and ddMS^2 isolation window of 0.7 m/z and scan number of 10. To confirm the selected chemicals with annotations from Levels 2 and 3, a full MS/product ion scan was conducted for authentic standards and samples.

2.4 Chemical annotations and source attributions

It is critical to discern whether the detected compounds are exogenous or endogenous, especially those expected in urine and serum samples. Many compounds enter the human body through food ingestion (e.g., nutrients and natural products) and drugs (including intermediate chemicals during pharmaceutical production) and their derivatives. The metabolic processes in the human body create a plethora of transformation products from the parent compounds.

A challenge that we encountered when trying to attribute sources to the detected compounds was that compounds often have multiple uses and can be both endogenous and exogenous.[22] Another challenge when dealing with chemical databases related to the human exposome is that, in many cases, only the monoisotopic mass of the chemical is available for matching, and the MS^2 spectra are missing. To confirm the chemicals in our samples, all data were first matched by the databases containing MS^1 and MS^2 from authentic standards, i.e., MS-DIAL metabolomics, MassBank of North America, Massbank Europe, and mzCloud. Afterwards, the sources of compounds were attributed by searching the ChemSpider database (http://www.chemspider.com/), Blood Exposome Database (https://bloodexposome.org/), Human Metabolome Database (https://hmdb.ca/), EPA CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard/).

We compiled information from multiple sources to reflect whether the compounds are intentionally ingested and whether they are industrial or natural products. The integrated data of identified compounds (Levels 1 and 2) are listed in **Supporting Information Spreadsheet S1**, where we present five categories of sources and uses:

(1) Endogenous Metabolites: Substances naturally produced from human issues during the metabolism process.

(2) Natural Products: substances derived from food or nutrients.

(3) Drugs: Substances intentionally ingested by people for different treatments, such as therapeutics/prescription drugs.

(4) Personal Care Products (PCPs): Substances used in cosmetics or other personal care products.

(5) Exogenous Contaminants: Substances present in human working/living environments, such as additives in house furnishings.

If there was no source indicated, the source of the compound was marked as "unknown". While it is generally expected that one compound will be attributed to one category, it is often the case that one compound can have multiple sources. For example, d-camphor (CAS: 464-48-2) was attributed to several sources because it is a constituent of various foods, medicines (such as treatment of colds and topical analgesics), and various cosmetics in the US. Some derivatives were annotated based on their parent compounds.

2.5 Data processing

2.5.1 Imputation and batch effects

All data processing was done using Python (version 3.11.5) as the programming language

and the following packages for data handling, data analysis and visualizations: pandas, numpy, matplotlib, seaborn, and scipy. The scripts were written using the JupyterLab and Spyder interfaces. Before data analysis, the dataset was processed for imputation (substituting missing data) and batch correction. We first calculated the frequency of each chemical feature among the samples and selected a frequency of 70% as the cutoff for imputation. The method detection limits (MDLs) were set as the minimum peak area (≥ 10,000). To fill in the data points below the MDLs, we used a previously developed imputation method.[17] Briefly, the peak areas were first log-transformed and then the missing values imputed from the left tail of the distribution that was fit to the data. The imputation algorithm inputs random values between the absolute minimum value (0) and the measured minimum value that originated from the cut-off points generated during processing of chromatographic peaks with MS-DIAL.

In total, 190 samples (95 serum and 95 urine) were analyzed in four batches (~47 samples each batch) for instrumental analysis. For each batch, randomly positioned samples consisted of both urine and serum samples. To avoid systematic differences between batches, urine samples were run with their corresponding serum samples. The remaining batch effects were corrected using a batch correction package called "ComBat" (https://github.com/brentp/combat.py). The details of the batch correction method have been described in the study of Johnson et al.[23] This package employs parametric and non-parametric Bayes methods for adjusting data for batch effects.

2.5.2 Data analysis

2.5.2.1 Unsupervised clustering

We conducted a principal component analysis (PCA) to examine the differences before and

after “Combat” batch correction among four batches. We also conducted a correlation analysis for the correlation of the PCs 1-3 with sample type and batch.

The differences for groups of similar data points of chemical composition between urine and serum samples, and between preterm birth and term birth samples were evaluated by employing hierarchically-clustered heatmap using the Seaborn Python package[24]. The differential enrichment of chemical features was quantified by comparing the relative abundance of chemical features between urine/preterm birth and the corresponding serum/term birth samples.

2.5.2.2 Relationships of chemical features in different sample types

The relative abundance and detected percentage were used to explore the relationships of chemical features between urine and serum samples. The abundance was first log-transformed and then averaged across all 95 samples for each chemical feature. The average values were used for the linear regression model to examine the correlation between urine and serum samples.

We used a volcano plot of average areas of chemical features to assess statistical significance ($p < 0.05$) through a t-test and the magnitude of change (fold change > 1.2) between preterm birth and term birth samples (in serum and urine, respectively), as well as between serum and urine samples. This approach helps identify chemicals that differ significantly between preterm and term birth samples and between serum and urine samples.

2.5.2.3 Molecular network analysis for different annotated chemicals

After annotating chemical features as described in Section 2.4, Pearson correlations between chemicals annotated as endogenous metabolites and all other annotated chemicals were used for molecular network analysis. In this study, the network indicates the association between chemical features. The purpose of the network is to visualize the inter-and intra-molecular

associations between endogenous metabolites and other chemical features, including exogenous contaminants. After calculating *p* values and t-scores between endogenous metabolites and other chemicals (Schymanski Levels 1-3), the *p* values were adjusted using the Benjamini–Hochberg false discovery rate of 5% method for multiple comparisons. For the visualization d3.js (https://d3-graph-gallery.com/graph/network_basic.html) was used to show the networks for relationships between endogenous metabolites and other chemicals. Because of the large number of associations in the complex network, we only focused on the Level 1 and 2 compounds with an absolute correlation coefficient (R) > 0.5 and revisualized the networks based on these chemicals.

2.5.2.4 Statistical analyses

For conducting correlations, we used Pearson's R, and for statistical differences between two groups (e.g., preterm and term birth), we used a *t*-test. The *p*-values were adjusted using the Benjamini–Hochberg test with a null hypothesis of 5% false positives. Statistical significance for two data groups derived from the same dataset (e.g., differential analysis of PC1 for preterm and term in urine and serum samples) was determined using the Wilcoxon Mann-Whitney Rank Sum test combined with Bonferroni correction.

2.6 Quality Assurance/Quality Control (QA/QC)

Batch analyses of samples were conducted by running three blanks, i.e., solvent blank, laboratory blank, and field blank. Two solvent blanks were run for each five samples. The QC samples were run at each batch to monitor the stability of the instrument, including RT shifts, mass accuracy, and peak intensity (**Spreadsheet S2**). EPA Phthalate Esters Mix (Sigma-Aldrich, St. Louis, MO) was used for QC with a five-point calibration curve ranging from 50 – 1000 ng

272 mL^{-1}. After running each batch, a Python package was run to filter the targeted m/z from
273 monoisotopic masses (mass tolerance: 5 ppm) and to check the aligned other values, e.g., m/z,
274 RT, and R values (> 0.5 for all expected compounds) obtained from the linearity (**Spreadsheet**
275 **S2**). All QC compounds were used for ESI^+ while only dibutyl phthalate was used for ESI^- due
276 to other compounds being poorly charged in negative polarity. In addition to commercial
277 standard mixes for QC, we also made a mixture solution consisting of 17 analytical standards,
278 following the same running and checking procedure as the EPA mixture (Results are shown in
279 **Spreadsheet S2**). The field and laboratory blanks used HPLC water to do the same extraction
280 for the same containers used during the collection procedure. The data collected from all blank
281 samples were used to remove the chemical features of which the abundances were 3 times lower
282 in real samples than those in the blank samples.

283 # 3. Results

284 ## 3.1 Filtering and confirmation of chemical features

285 After the alignment of 4 batches, the total amount of chemical features in both urine and
286 serum samples (*n* total = 190 samples) without clean-up processing from the full scan was
287 112,737 for ESI^+ and 82,335 for ESI^- (**Figure S1**, **Supporting Information**). After eliminating
288 the features that were adducts that were linked to other ion(s) and frequency below 70%, the
289 processed dataset was decreased to 21,952 features for ESI^+ and 10,006 features for ESI^-. By
290 merging the ESI^+ and ESI^- datasets (± monoisotopic H: 1.00782), the pair of 2219 features in
291 both ESI^+ and ESI^- was observed based on the RT time difference < 0.5 min and mass difference
292 ≤ 5 ppm.

293 These features were then matched to the dataset from the pooled samples run by full

scan/ddMS2 (ESI^+: 408,610; ESI^-: 270,533), then further reduced by filtering the ions with product ions, resulting in 1,524 features (Levels ≥ 3). The features identified from pooled samples using $ddMS^2$ scans matched those found in individual samples using full scans.

3.2 Batch correction

In the dataset without batch correction for serum samples, no clusters of PC1 and PC2 loadings were observed for preterm and term birth sample types (**Figure 2a-1**). After batch correction, two distinct clusters corresponding to preterm and term birth samples were observed in serum (**Figure 2a-2**). Post-correction, a negative correlation (R = -0.752) between PC1 and preterm-term birth sample types was observed (**Figure S2a**). Additionally, significant differences were found between PC1 and preterm-term birth sample types ($p < 0.01$), as well as between PC2 loadings and preterm-term birth samples ($p < 0.01$) (**Figure S2b**). No batch effect was observed after correction (**Figure 2a-4**), compared to the four distinct clusters of PC1 and PC2 loadings before correction (**Figure 2a-3**).

In urine samples, clusters of PC1 and PC2 loadings for preterm and term birth were not separated before batch correction (**Figure 2b-1**), and were only partially separated after batch correction (**Figure 2b-2**). Post-correction, no correlation was observed between PC loadings and sample type or batch (**Figure S2a**). However, a significant difference was observed between PC1 loadings and preterm-term birth sample types ($p < 0.01$) (**Figure S2b**). The batch effect in the four batches of urine samples was not pronounced before correction (**Figure 2b-3**) and was absent after correction (**Figure 2b-4**).

In the combined serum and urine dataset before batch correction, PC1 and PC2 loadings were able to separate serum and urine samples, though some data points were not well separated

316 (**Figure 2c-1 & 2**). After batch correction, a positive correlation (R = 0.521) was observed
317 between PC1 loadings and sample type (serum and urine) (**Figure S2e**). Significant differences
318 were observed between PC1 loadings and sample type or batches ($p < 0.01$), as well as between
319 PC3 loadings and sample type or batches ($p < 0.01$) (**Figure S2f**). Similar to urine samples, the
320 batch effect was not strong in the combined dataset before correction (**Figure 2c-3**) and was
321 eliminated after correction (**Figure 2c-4**).

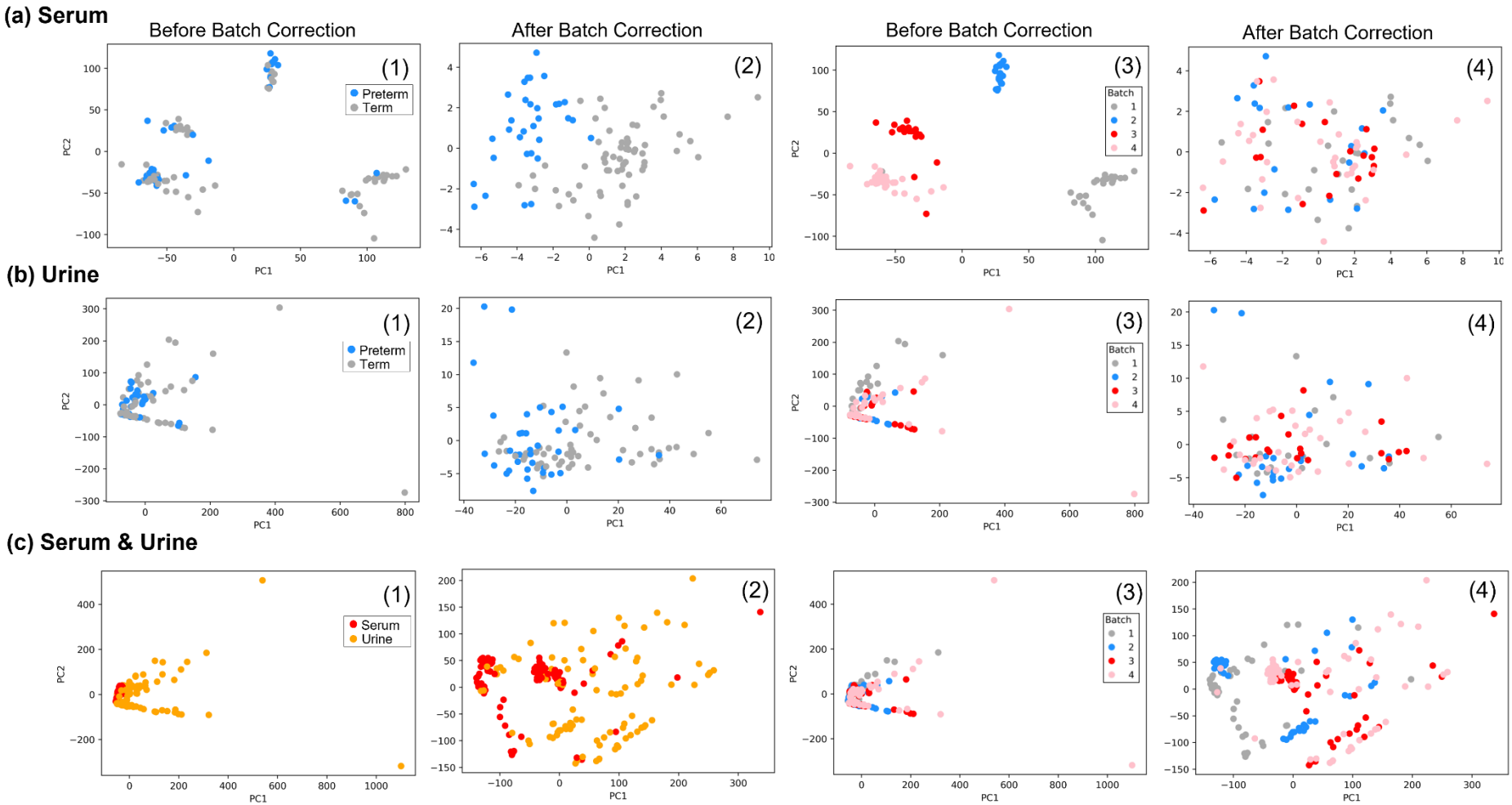


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Figure 2. Data analysis before and after "Combat" batch correction for the four individual batches in serum (a), urine (b), and combined urine & serum samples (c). The example results demonstrate the following: For preterm and term birth samples in serum or urine (a/b): (1) color-labeled principal components analysis (PCA) loadings by preterm and term birth, and (2) color-labeled PCA loadings by batch. For combined serum and urine samples (c): (1) color-labeled PCA loadings by sample type (serum and urine), and (2) color-labeled PCA loadings by batch.

3.3 Chemical Annotation

The processed chemical features merged from ESI^+ and ESI^- modes were used for database matching. Out of 1,524 chemical features with MS^2 information, we were able to annotate 344 features, with a match score of over 90% using MS-DIAL and Compound Discoverer, and 18 features were found to be common in both ESI^+ and ESI^- (**Spreadsheet S1**). The classification of the 327 chemicals was as follows: endogenous metabolites (203), exogenous contaminants (96), drugs (101), natural products (38), and PCPs (45). Additionally, many compounds were annotated in multiple categories: 37 in more than two categories, 29 in more than three, 16 in more than four, and 2 in more than five (**Figure S3**).

From the analytical standards in our laboratory, twelve chemicals were confirmed by comparing RT, and precursor ion/product ions (difference < 5 ppm) (example shown in **Figure S4**). The matches were confirmed for chemicals with an RT difference < 0.05 and a mass difference < 5 ppm. These included two organophosphorus compounds (triisobutyl phosphate and tributyl phosphate), three amines (triisopropanolamine, tributylamine, and diphenylamine), three phenol derivatives (4-nitrophenol, 3-aminophenol, and 2-aminophenol), dodecyltrimethylammonium, n,n-dimethyldecylamine n-oxide, propiconazole, and 2,2,6,6-tetramethyl-4-piperidinol (**Spreadsheet S3**). The enrichment of chemicals (Levels 1-3) differed between serum and urine samples (**Figure S5**), with most chemicals being more prevalent in serum and categorized as Level 3 (unknown, no database match observed). The annotated chemicals (Levels 1-2) identified as exogenous contaminants, which were detected more frequently in preterm birth samples (serum and urine, respectively), are shown in **Figure 3a-b**. The detection frequency of all annotated exogenous contaminants is shown in **Figure S6**. We

found that four confirmed chemicals (2-phenylindole, n,n-dimethyldecylamine, propiconazole, and triisopropanolamine) were detected more frequently in preterm birth samples, in both serum and urine (**Figure 3a & b**)

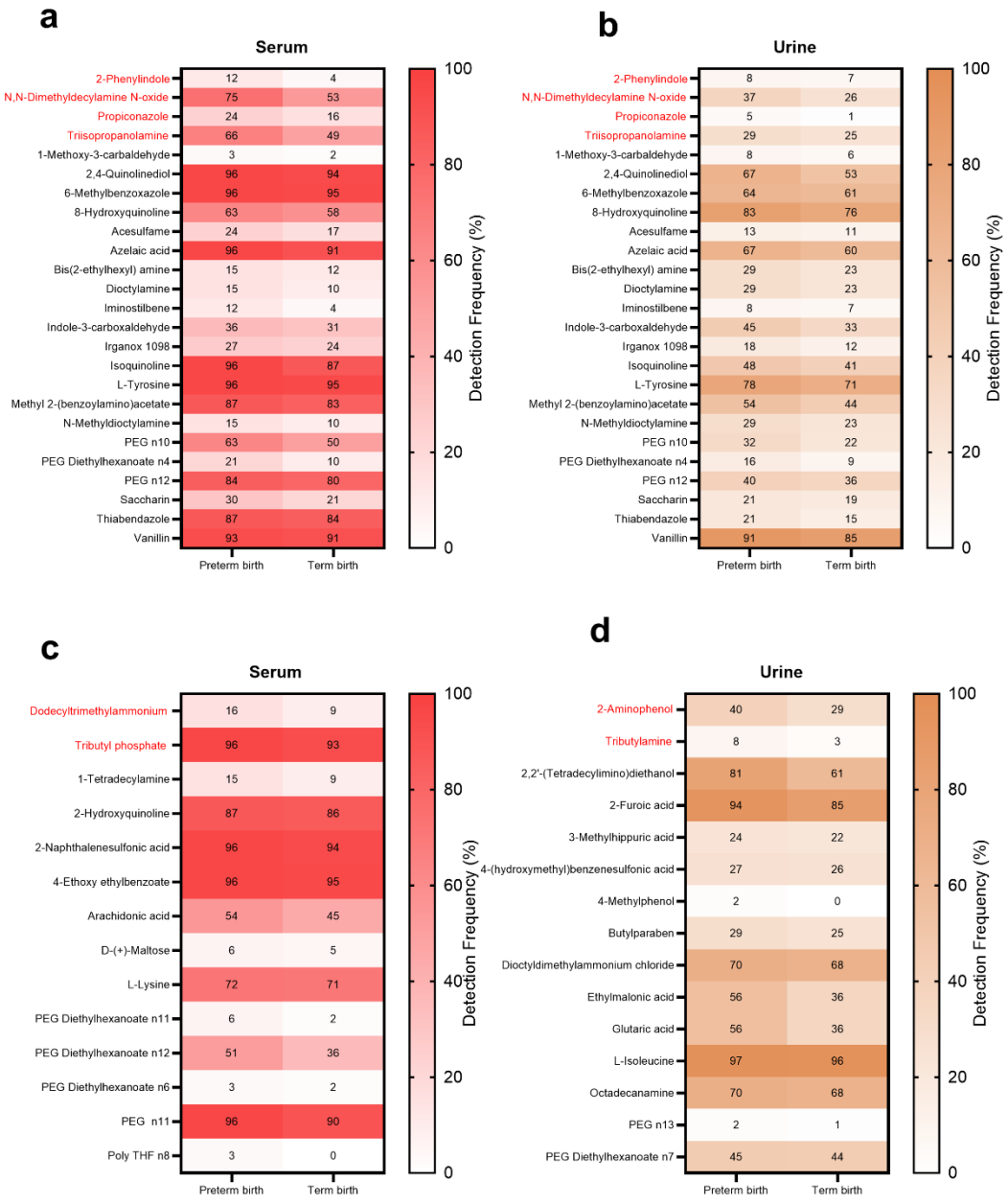

Figure 3. The detection frequency (%) of annotated chemicals (Levels 1 and 2) classified as exogenous contaminants in preterm and term birth samples: chemicals with higher detection frequency in preterm birth for both serum: (**a**) and urine (**b**); chemicals with higher detection frequency in only serum (**c**); and chemicals with higher detection frequency in only urine (**d**). The chemical names in red represent the confirmed chemicals (Level 1) by the authentic standards.

3.4 Data analysis

3.4.1 Difference between preterm and term birth

In serum, clusters of different chemicals' enrichment were observed between preterm and term birth samples (**Figure 4a**). The statistical differences in PC1 loadings between preterm and term birth samples were significant after batch correction ($p < 0.0001$) (**Figure 4a**). Among the 1,547 significantly different LC-MS features between preterm and term birth samples ($p < 0.05$), 3 out of 17 chemicals from the downregulated area ($\log_2$fold < -1.2) and 8 out of 72 chemicals from the upregulated area ($\log_2$fold > 1.2) could be tentatively annotated (**Spreadsheet S4**). For example, the annotated chemicals in the downregulated area have polyethylene glycol (PEG) n6 (m/z: 283.1755 [M+H$^+$]) and centrimonium (m/z: 284.3313 [M+H$^+$]) (**Figure 5a**). Those in the upregulated area have n-acetylhistidine (m/z: 198.0848 [M+H$^+$]), and deoxycholic acid (m/z: 391.2858 [M+H$^-$]) (**Figure 5a**). The annotated chemicals in the upregulated area include an exogenous contaminant (1,4-cyclohexanedicarboxylic acid, m/z: 173.0783 [M+H$^+$]) and other seven compounds identified as natural products, drugs, and endogenous metabolites, while those in the downregulated area were identified as exogenous contaminants, drugs and personal care products (**Figure S7a**).

In urine, we did not observe distinct chemical enrichment between preterm and term birth samples (**Figure 4b**), despite significant differences in PC1 loadings ($p < 0.0001$). Among the 9,225 significantly different LC-MS features between preterm and term birth samples ($p < 0.05$), 19 out of 427 features were tentatively annotated and they were all situated in the downregulated area (**Spreadsheet S4**). Some of these features annotated were shown as in the volcano plot, e.g., didecyldimethylammonium (m/z: 326.3782 [M+H]$^+$) and adebosine (m/z:

268.1002 $[M+H]^+$) (**Figure 5b**). The largest number of annotated chemicals belonged to endogenous metabolites and exogenous contaminants (**Figure S7b**). Only one feature (unknown, m/z: 704.5230 $[M+H]^+$) was present in the upregulated area.

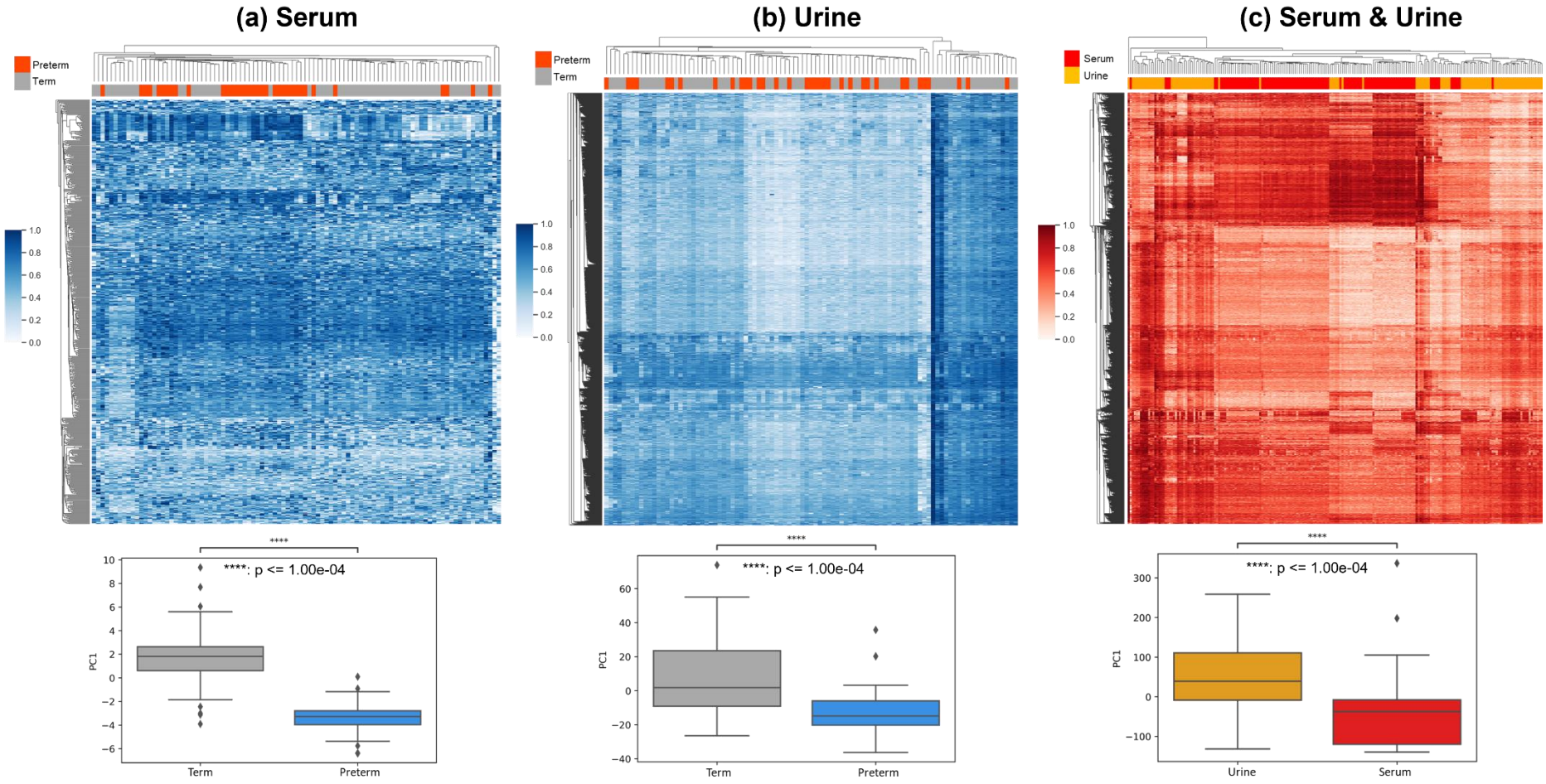

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Figure 4. Clustering heatmap after batch effect correction for serum and urine samples. The chemical features reveal the differential enrichment in preterm versus term births among serum samples (**a**) and urine samples (**b**), and between serum and urine samples (**c**) after multiple testing correction (Benjamini-Hochberg test, 5% false discovery rate). For the differential enrichment in preterm versus term birth samples, 1,524 out of 31,958 chemical features in serum and 812 out of 37,270 in urine showed significant differences ($p < 0.05$). For the differential enrichment between serum and urine samples, 26,038 out of 37,270 chemical features exhibited significant differences ($p < 0.05$). The boxplots show the statistical difference of principal component 1 (PC1) between preterm and term birth samples, and between urine and serum samples using the Mann-Whitney-Wilcoxon test (two-sided) with Bonferroni correction. The bottom and top of the boxes represent the 25th and 75th percentiles, the error bars denote the 10th to 90th percentiles, and the solid line indicates the median value.

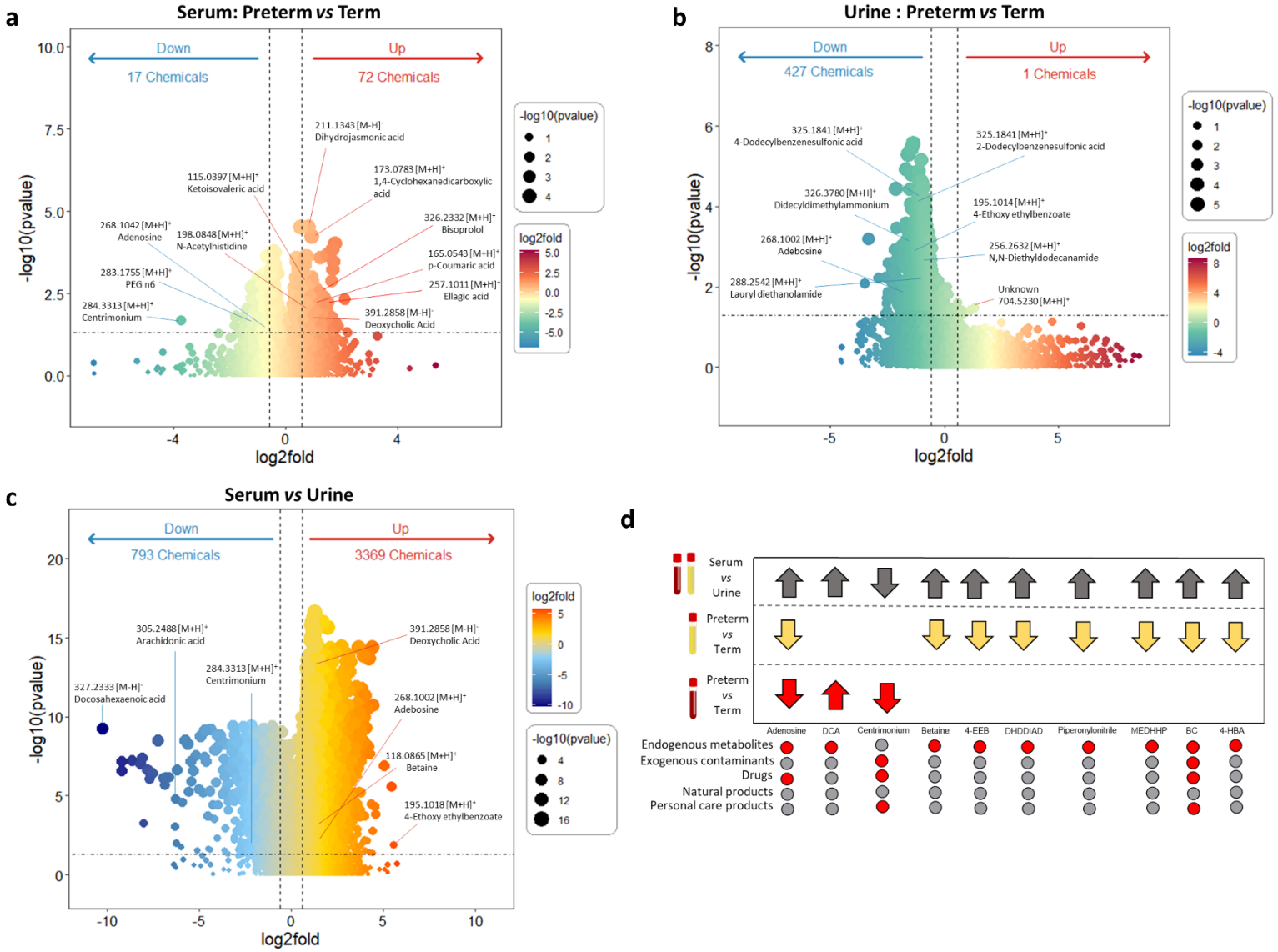


Figure 5. The volcano plot of the log-transformed ratios and corresponding p-values of chemical features with a cut-off frequency of 70% from ESI^+ and ESI^- modes illustrates the data: the statistical differences in chemical features between preterm births and term births in serum (**a**) and urine (**b**), and between serum and urine (**c**). The horizontal dashed line indicates the cutoff for the log *p*-value ($p < 0.05$), and the vertical dashed lines indicate the cutoff for fold change (Log_2 fold change = 1.2). The arrow graph (d) indicates the regulation status of the same annotated chemical across different volcano plots (a, b, and c). Up arrows represent up-regulated areas, while down arrows indicate down-regulated areas. Red balls denote annotated categories, and grey balls represent non-annotated categories. DCA: Deoxycholic Acid, 4-EEB: 4-Ethoxy ethylbenzoate, 4-HBA: 4-Hydroxybenzaldehyde, MEDHHP: Methyl 2-[4-ethenyl-2,6-dihydroxy-3-(3-hydroxyprop-1-en-2-yl)-4-methylcyclohexyl]prop-2-enoate, DHDDIAD: 1,4-dihydroxy-1,4-dimethyl-7-(propan-2-ylidene)-decahydroazulen-6-one, BC: Benzoic Acid.

3.4.2 Difference between urine and serum

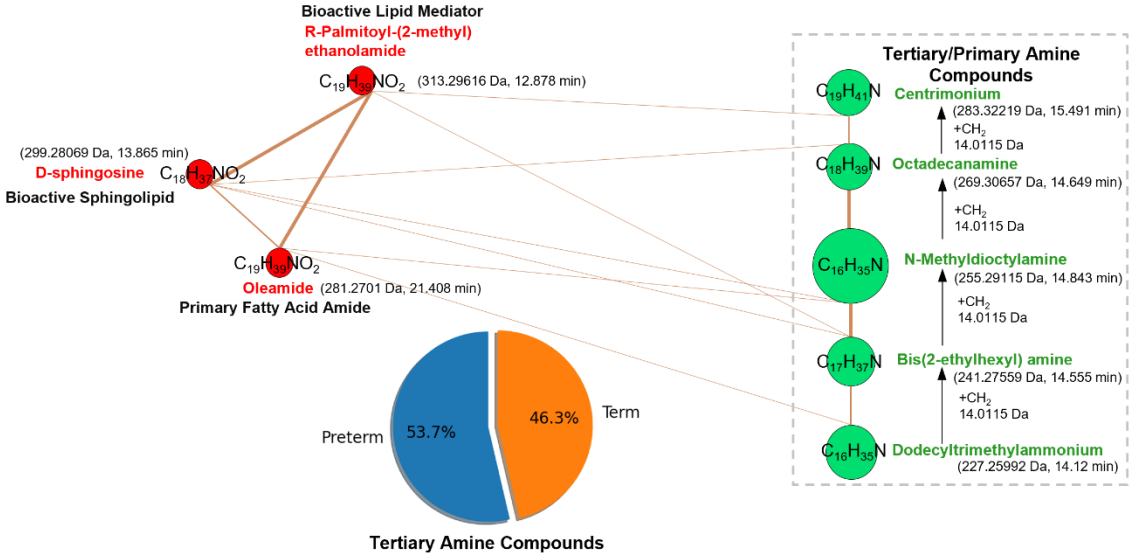
The mean log abundances of chemical features from urine and serum samples showed a positive correlation ($R^2 > 0.5$), with some chemical features diverging from the regression line before imputation and batch correction for the initial dataset (**Figure S8a**), after imputation and batch correction for the initial dataset (**Figure S8b**), and after imputation and batch correction for the chemical features that have a frequency >70% (**Figure S8c**). A significant difference in most chemicals was observed between urine and serum samples after batch correction (Figure 4c), with two distinct clusters separated with a p-value < 0.0001 for PC1 between urine and serum samples (**Figure 4c**).

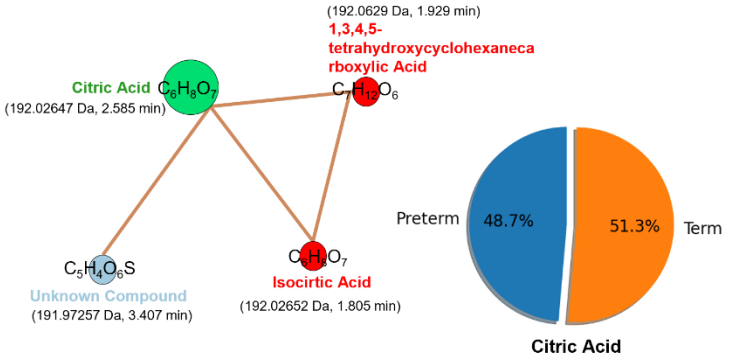
From the volcano plot of 25,885 chemical features (serum versus urine, $p < 0.05$), chemicals were more predominant in serum (3,369 chemicals in the upregulated area vs. 739 chemicals in the downregulated area) (**Figure 5c**). The chemicals with the largest fold change in the downregulated and upregulated areas were tentatively annotated as docosahexaenoic acid (log2fold = -10.25, m/z: 327.2333 $[M-H]^-$) and 4-ethoxy ethylbenzoate (log2fold = 5.54, m/z: 195.1018 $[M+H]^+$). In the upregulated and downregulated areas, 109 and 20 chemicals, respectively, were tentatively annotated (**Spreadsheet S5**), with endogenous metabolites and exogenous contaminants being the most frequently annotated (**Figure S6c**).

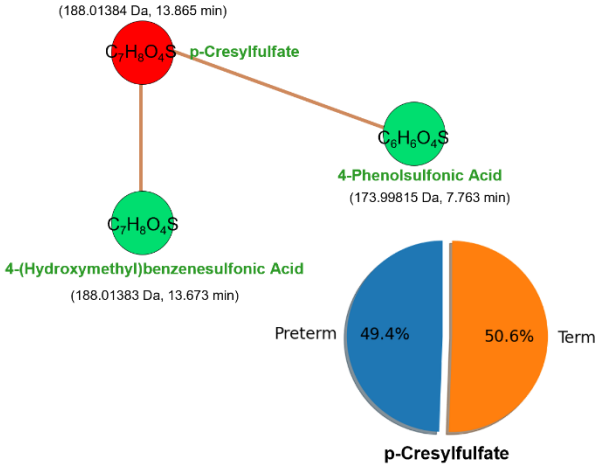
3.4.3 Association among different chemicals

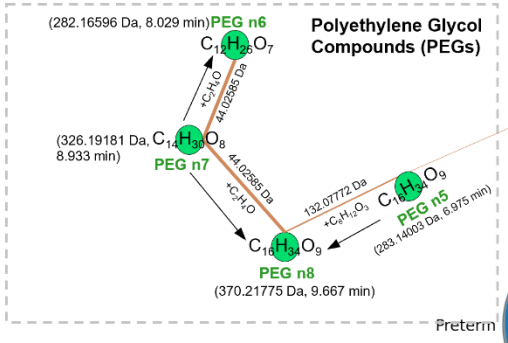
Twelve significant associations (absolute Pearson R > 0.5) in all samples were found between endogenous metabolites and exogenous contaminants (**Spreadsheet S4**), which only was observed in serum samples. For example, p-cresyl sulfate positively correlated with 4-(hydroxymethyl)benzenesulfonic acid and 4-phenol sulfonic acid (**Figure S9**).

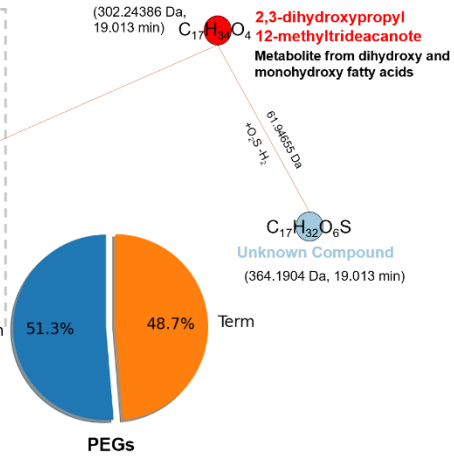
The molecular network for significant associations (R^2 > 0.5) between endogenous metabolites and exogenous chemicals were shown in **Figure 6**. Endogenous-exogenous compound correlations included d-sphingosine with n-methyldioctylamine, octadecanamine, and bis(2-ethylhexy)amine. The amine compounds like octadecanamine (primary amine) and bis(2-ethylhexyl) amine (tertiary amine) showed significant associations. Other correlations involved r-palmitoyl-(2-methyl) ethanolamide and centrimonium, and oleamide with bis(2-ethylhexyl) amine and bis(2-ethylhexyl) amine and dodecyltrimethylammonium, slightly more occurring in preterm birth (~53%). Among exogenous compounds, PEG n5 was positively correlated with an endogenous metabolite, 2,3-dihydroxypropyl 12-methyltrideacanote. Citric acid was positively correlated with two endogenous metabolites, isocirtic acid and 1,3,4,5-tetrahydroxycyclohexanecarboxylic acid.











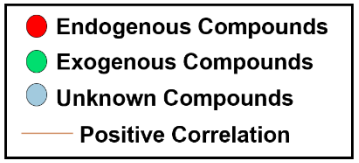


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Figure 6. Molecular interaction networks for endogenous (red) and exogenous compounds' features (green) in serum samples (N=95). The network indicates that the features of MSn had a score of 50, a coverage value of 70, and a minimum number of fragments of 3. The correlation in the networks had R values > 0.5. The correlations shown in the network are all positive (brown line). The thickness of the line indicates the strength of the correlation. The red circle and green circle represent the endogenous and exogenous compounds, respectively. The blue circle represents the unknown chemicals. The size of the circle indicates the size of the integrated area of the chemical feature. Endogenous and exogenous compounds belong to Level 2 and unknown compounds belong to Level 3 based on Schymanski, Jeon, Gulde, Fenner, Ruff, Singer and Hollender [21] for the annotation confidence (Details in Chapter 2.1). The pie charts show the average percentages of preterm and term births associated with exogenous compounds in all serum samples where these compounds were detected, such as the average percentage of polyethylene glycol compounds (PEGs).

4. Discussion

Among the confirmed compounds, two chemicals, dodecyltrimethylammonium and n,n-dimethyldecylamine n-oxide, widely used in PCPs and as surfactants for various industrial products, appear to not have been previously reported in human samples, based on our searches with the Blood Exposome Database and the Human Metabolome Database. In addition, we found that the azole fungicide propiconazole, a heavily used agricultural agent with carcinogenic[25] and endocrine-disrupting effects on humans.[26] Three tertiary amine compounds (triisopropanolamine, tributylamine, diphenylamine) are used in numerous industrial applications such as surfactants and stabilizers, with diphenylamine and its derivatives listed as propriety pollutants by the European Union.[27] Two phosphate ester flame retardants, tributyl phosphate and triisobutyl phosphate, were found to have higher detection rates and average concentrations in serum samples compared to paired urine samples (semi-quantification shown in **Figure S10**). This is similar to previous reports where tributyl phosphate was the predominant substance in blood samples from Beijing[28] and Shenzhen[29], China. However, triisobutyl phosphate has not been reported in human samples. 2,2,6,6-Tetramethyl-4-piperidinol, found in PCPs such as cosmetics, was detected in human blood.[30] 4-Nitrophenol, a metabolite of the organophosphate pesticide methyl parathion, which is illegally applied to the interiors of homes in the US,[31] it was also detected in our samples. For aminophenols, 2-aminophenol and 3-aminophenol could not be differentiated based on RTs (difference < 0.05 min) and were confirmed by product ions (**Figure S4b**). Aminophenols and their derivatives are commercially important in dyes, petroleum additives, and pharmaceutical industries. Interestingly, the commonly used 4-aminophenol was not detected in our samples, while 2- and

3-aminophenols, which we did detect, are less frequently reported in human samples. All pairs of samples found both 2-aminophenol and 3-aminophenol with good correlation between urine and serum (R^2= 0.988), suggesting that products exposing pregnant women might contain both aminophenols. We also found that 39 out of the 325 chemicals were not included in the blood exposome database (**Spreadsheet S7**).[30] Among these chemicals, except for dodecyltrimethylammonium (Level 1), citroflex (Level 2) was annotated as exogenous contaminants and PCPs but it is not included in the Human Metabolome Database and the Blood Exposure Database. According to the blood paper count from the Blood Exposure Database (**Spreadsheet S8**), several compounds showed a very limited number of studies: dodecyltrimethylammonium (0), n,n-dimethyldecylamine n-oxide, triisopropanolamine (1), and tributylamine (3). Additionally, we identified eleven tentatively annotated compounds with similarly limited study numbers (**Spreadsheet S8**). These compounds require further investigation to determine their presence in the human body.

Based on the chemical profiles of the samples, we were able to distinguish between preterm birth and term birth in only serum (**Figure 4a**). Preterm birth is a medical condition with a complex pathogenesis.[32] Previous reports have shown potential associations of environmental contaminants with preterm birth compared with the control samples, e.g., the pesticide DDT (dichlorodiphenyltrichloroethane),[33] lead,[34] and phthalates.[35-37] For phthalates, diheptyl phthalate (Level 2) was found in preterm birth samples. This is not surprising since phthalate esters are widely used in the plasticizer industry and have been detected in human samples from adults and children in Asia and North America.[38] While previous studies have reported significant associations of phthalates and their metabolites with the gestational age in other

New York City pregnancy cohorts, [39] [40] we were unable to find any associations of chemical features between preterm birth and term birth in either blood or urine samples. Due to the limited sample numbers, we do not further elucidate this observation. It should be noted that phthalates are ubiquitous and can leach from medical supplies[41] and laboratory equipment,[42] as seen in our current raw dataset where many phthalates were present in laboratory controls and even in solvent blanks, complicating source identification. Therefore, we do not further speculate on the sources of diheptyl phthalate from our samples.

In serum, among all annotated chemicals with features significantly different ($p < 0.05$) in preterm birth samples and 1.2-fold higher abundances compared to term birth samples **(Figure 5a**), only 1,4-cyclohexanedicarboxylic acid was categorized as an exogenous contaminant. This compound is used in the production of nylon and polyester resins for various purposes, such as enhancing plasticizing efficiency and hardness.[43] Exposure to this compound may occur through ingestion and inhalation of its products in the environment. Although 1,4-cyclohexanedicarboxylic acid is currently under the TSCA, it is not listed in the Blood Exposome Database. To our knowledge, no studies have reported the detection of 1,4-cyclohexanedicarboxylic acid in human samples. Other compounds, such as p-coumaric acid, ellagic acid, and bisoprolol, are commonly used in drugs or health products for dietary antioxidants, antioxidant activity, and hypertension management. These chemicals may suggest that some preterm births, which are often medically necessary, could be linked to the mother's use of medications for underlying complications. Regarding endogenous metabolites, deoxycholic acid, a bile acid, is one of the main bile acids present in the meconium of preterm infants, entering the fetus through placental transfer. More recent studies have also shown that

changes in total bile acids are directly related to preterm birth rates.[44, 45]

For the annotated chemicals that were significantly different ($p < 0.05$) in preterm birth samples, with lower abundances compared to term birth samples, we identified two exogenous contaminants in serum and six in urine. However, these contaminants were not detected with higher frequency in preterm birth samples or in either urine or serum. The negative fold change in these chemicals might be attributed to individual sample variations compared to endogenous metabolites and differences in sampling times for urine. We do not further explain this observation.

We also observed that adenosine (an endogenous metabolite), which was significantly different in preterm birth, showed decreased abundances in both serum and urine samples (**Figure 5d**). Adenosine is a common endogenous nucleoside that generally counteracts ATP-induced effects, such as inflammation.[46] It has been demonstrated that adenosine levels can increase during normal pregnancy due to platelet activation and elevated nucleosidase activity.[47] Interestingly, adenosine, a marker of oxidative stress, has been found to be significantly higher in pregnant women with preeclampsia compared to those without the condition.[48] Lower levels of adenosine in both urine and serum might be linked to preterm birth outcomes. Although endogenous metabolites were not the primary focus of this study, the levels of adenosine associated with preterm birth have not been reported. his warrants further attention from researchers, especially since adenosine is also used as a drug for treating supraventricular tachycardia during pregnancy.[49] Generally, we observed a broader range of chemicals, both endogenous and exogenous, in serum samples (**Figure 3c**). This allows for the identification of both biomarker chemicals and exogenous contaminants. Nonetheless, some exogenous

534 contaminants, such as centrimonium, were found to be more enriched in urine samples.

535 We found that paired prenatal urine and serum samples have different enrichment of chemical
536 features (**Figure 4c**), despite some endogenous chemicals showing a significantly higher
537 proportion in the serum samples (**Spreadsheet S1**). Of the tentatively identified compounds we
538 detected (Level 2, **Spreadsheet S1**), many were endogenous compounds or pharmaceuticals
539 and their transformation products as part of metabolism in the human body.

540 Some endogenous chemicals showed an association with exogenous contaminants. For
541 example, p-cresyl sulfate (p-CS) correlated with 4-phenolsulfonic acid (4-PSA) and 4-
542 (hydroxymethyl)benzenesulfonic acid (4-HMBSA) (**Figure S9**). p-CS is a prototype protein-
543 bound molecule derived from the secondary metabolism of p-cresol, where increased
544 concentrations can be associated with deteriorating kidney function.[50] 4-PSA is a common
545 intermediate/component of surfactants, detergents, pharmaceuticals, and dyes. 4-HMBSA is a
546 derivative of substituted benzenesulfonic acids, widely used as intermediates for organic
547 compound synthesis. 4-PSA has been listed in the ToxCast database,[51] while the human toxicity
548 for both 4-PSA and 4-HMBSA is not clear. In the current network, significant relationships
549 were observed among PEGs, composed of polyether compounds with repeating ethylene glycol
550 units. PEGs are used as components in drugs and PCPs. Narrowly defined molecular weight
551 ranges of PEGs are often produced as a commercial mixture,[52] similar to our data showing a
552 correlated pattern with the loss of ethylene oxide (C_2H_4O, 44.02585 Da) among PEGs n5-8.
553 PEG n5 was observed to have a positive connection to 2,3-dihydroxypropyl 12-
554 methyltrideacanote, an endogenous metabolite from the 12-methyltridecanoate fatty acid chain,
555 and a complex microbial-related metabolite in gastric cancer.[53] Only high-molecular-weight

PEGs (> 400 Da, e.g., PEG n8) have shown toxic effects in animals[54], and we were not able to find any toxicity studies on the various PEGs. Another interesting correlation was observed between a group of tertiary amine compounds, used as chemical intermediates/surfactants, with a mass defect of -CH_2- group (14.0115 Da), e.g., centrimonium and octadecanamine, and fatty acid amide (oleamide) and bioactive lipid metabolites (d-sphingosine and r-palmitoyl-(2-methyl) ethanolamide) (**Figure 6**). This suggests that these amine compounds might interfere with lipid and fatty acid metabolism. This can be referenced by a relevant report indicating that surfactants solubilize lipid membranes and transform them into lipid-surfactant micelles, while fatty acids transform lipids into cubic and hexagonal phases.[55] All these associations indicate the potential direct or indirect intervention of exogenous contaminants on the metabolism processes in human bodies.

This NTA analysis of urine and serum samples used full scan and MS/MS spectra match from pooled samples by $ddMS^2$ scan. Batch effects were significant but could be corrected by the Combat package (**Figure 2**). This is consistent with previous study that found differences in characteristics of LC/MS metabolomics data before batch correction.[56, 57] The raw dataset showed many features in blanks and field controls. Chemical features in QC samples showing peak areas that were five times higher than those of the blanks. ESI^+ and ESI^- revealed the most ions eluted from 10 to 20 min during chromatography (**Figure S1**). ESI^+ covered a higher range of charge-to-mass ratios at the beginning and end of the run. Only 344 features (~0.05% of the merged features) were matched across ESI^+ and ESI^- datasets, highlighting varied chemical properties in current samples.

In our dataset, most matched chemical features could not be fully confirmed without

analytical standards. The endogenous metabolites and exogenous contaminants groups had significantly more compounds in them than drugs, natural products and personal care products (**Figure S3**). Given the abundance of environmental contaminants and their observed associations with endogenous metabolites, many of these contaminants could substantially contribute to the exposome [58] disturb metabolic pathways such as lipid metabolism and inflammation regulation.[59]

Our study provided a comprehensive non-targeted analysis of small molecules in serum and urine samples from pregnant women, highlighting differences between sample types and between preterm and term births. Approximately ~22% of features (Level ≥ 3) were tentatively annotated by matching to spectral databases, and 12 chemicals were confirmed by authentic standards. NTA is a critical tool in the assessment of a broad spectrum of environmentally-concerned chemicals in biological samples. At present, there is a need for larger MS^n databases and analytical standards in order to increase the number of confirmed compounds.

5. Limitations and recommendations

While our study presents some evidence associating chemical exposures with preterm birth, our study is not a comprehensive epidemiological study, but a human exposure study. Our main goal was to identify new target chemicals and highlight them for further toxicity studies. We have four limitations in our study that need to be acknowledged:

(1) We were limited to only 95 participants with paired urine and serum samples (including 35 pairs from preterm births).

(2) Although we observed clustering in the serum heatmap at a chemical detection frequency cut-off of 70% (as well as at 60% and 80%, as shown in **Figure 4a** and **Figure S11**)

between preterm and term births, we did not observe a similar pattern of chemical enrichment in the paired urine samples across detection frequencies of 60-80% (**Figure 4b** and **Figure S12**). This discrepancy may be due to the different sampling times for urine and the more pronounced matrix effects in urine.

(3) This study focused on environmental contaminants. We observed that numerous annotated chemical features had a very low detection frequency. For example, among the 344 annotated chemical features, 38 and 49 chemicals identified as exogenous contaminants were detected in less than 60% of serum and urine samples, respectively. This may suggest that serum is a more comprehensive matrix for detecting small molecule contaminants.

(4) The analytical instrument presents challenges related to varying setting parameters across different mass spectrometers and manufacturers, especially for soft ionization techniques. In non-targeted analysis (NTA), the desired mass resolving power may not be achieved for specific masses. For Orbitrap HRMS in NTA, the upper limit of mass resolving power can lead to ion loss and dephasing of oscillations. The limited number of ions per unit time entering the C-trap (AGC targets) could significantly affect the sensitivity for small molecule chemicals with lower detection frequencies in our study. We recommend multiple scans for pooled samples with a dynamic MS^2 data window to mitigate the limited AGC targets per scan. Additionally, it is advisable to combine various analytical approaches to expand chemical space coverage, such as using GC separation for volatile and highly nonpolar chemicals in conjunction with Quadrupole Time-of-Flight (QTOF) MS.

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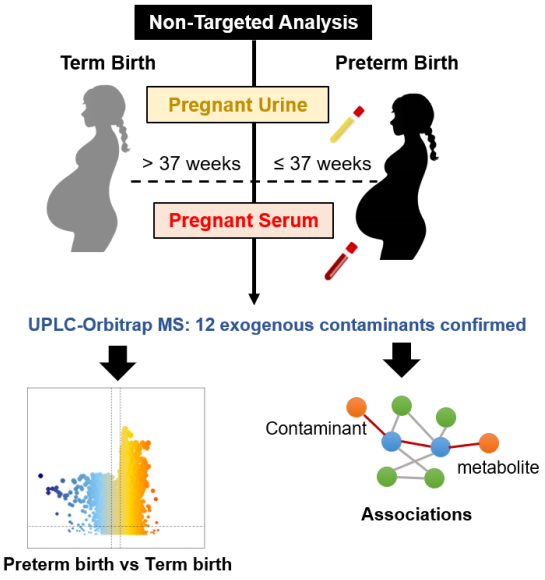
627 # TOC only



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Supporting Information

An insight into the chemical exposome during pregnancy - A non-targeted analysis study

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Contents of Text and Figures

Contents of SI Spreadsheets

Spreadsheet S1. 345 annotated chemical features, ranging from Level 1 (confirmed by authentic standards) to Level 2 (tentatively annotated by MS1/MS2 database matches), were identified in both ESI^+ and ESI^- modes. These features include those with the best match rates, and their frequency in urine/serum samples from preterm and term births.

Spreadsheet S2. Quality control standards, including EPA phthalate esters mixtures and an in-house analytical standards mixture, were used to assess the stability of the UHPLC-Orbitrap mass spectrometer for each batch. The evaluation included mass accuracy, retention time (RT) shifts, and r-values, all derived from the average results across the four batches.

Spreadsheet S3. The chemical features were confirmed in our laboratory using authentic standards by matching retention time (RT, min), MS^1 (m/z), and MS^2 (m/z).

Spreadsheet S4. The annotated chemicals that showed significant differences ($p < 0.05$) between preterm and term birth samples were identified in the upregulated region (fold change > 1.2) and the downregulated region (fold change < -1.2) of the volcano plot, in either serum or urine samples.

Spreadsheet S5. The annotated chemicals that showed significant differences ($p < 0.05$) between serum and urine samples were identified in the upregulated region (fold change > 1.2) and the downregulated region (fold change < -1.2) of the volcano plot.

Spreadsheet S6. The Pearson correlation (R) matrix was analyzed between exogenous contaminants and endogenous metabolites in serum samples (no correlation was found in urine samples).

Spreadsheet S7. The chemicals (both endogenous and exogenous) were not listed in the Blood Exposome Database (https://bloodexposome.org/).

Spreadsheet S8. The blood paper count for chemicals annotated as exogenous contaminants, which have a higher detection frequency in either serum or urine samples, was obtained from the Blood Exposome Database.

Text S1. The Creatinine normalization approach to diluted urine samples.

Urine samples can be diluted under various conditions, such as increased water intake or diuretic use. According to the Substance Abuse and Mental Health Services Administration (SAMHSA) guidelines (https://www.federalregister.gov/d/2023-21734), a urine sample is considered diluted if the creatinine concentration is below 20 mg/dL. In our study, we used the creatinine normalization method, as employed in similar research.[1, 2] Based on the creatinine concentrations from 95 pregnant women in our study, we established 25.22 mg/dL as the reference concentration for undiluted samples. For quantification, we employed a six-point calibration curve ranging from 1 to 95 mg/dL, spiked with 50 µg/L of an internal standard (creatinine-d3), using isotope dilution (with linearity > 0.99 for creatinine). Data acquisition and processing were carried out with Xcalibur v. 4.3 (including Freestyle 1.6 and Quan browser). The chemical abundances in diluted urine samples were adjusted using the following equation:

$$\text{Chemical abundance after normalized creatinin} = \text{initial abundance} \times \frac{\text{Reference Creatinine}}{\text{Sample Creatinine}}$$

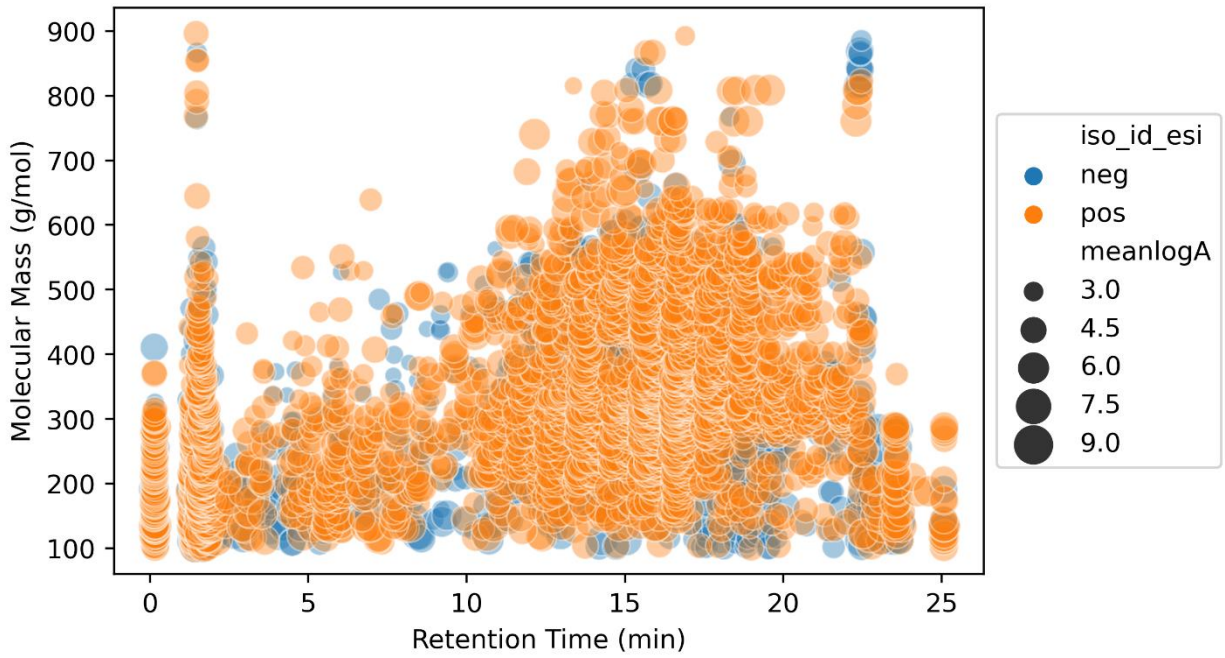

Figure S1. The highest point or peak of a chromatographic or mass spectrometric signal and abundances (log transformed) of retention times (RT) and molecular mass (g/mol). The plot shows ions from full scan from both positive and negative electrospray ionization modes.

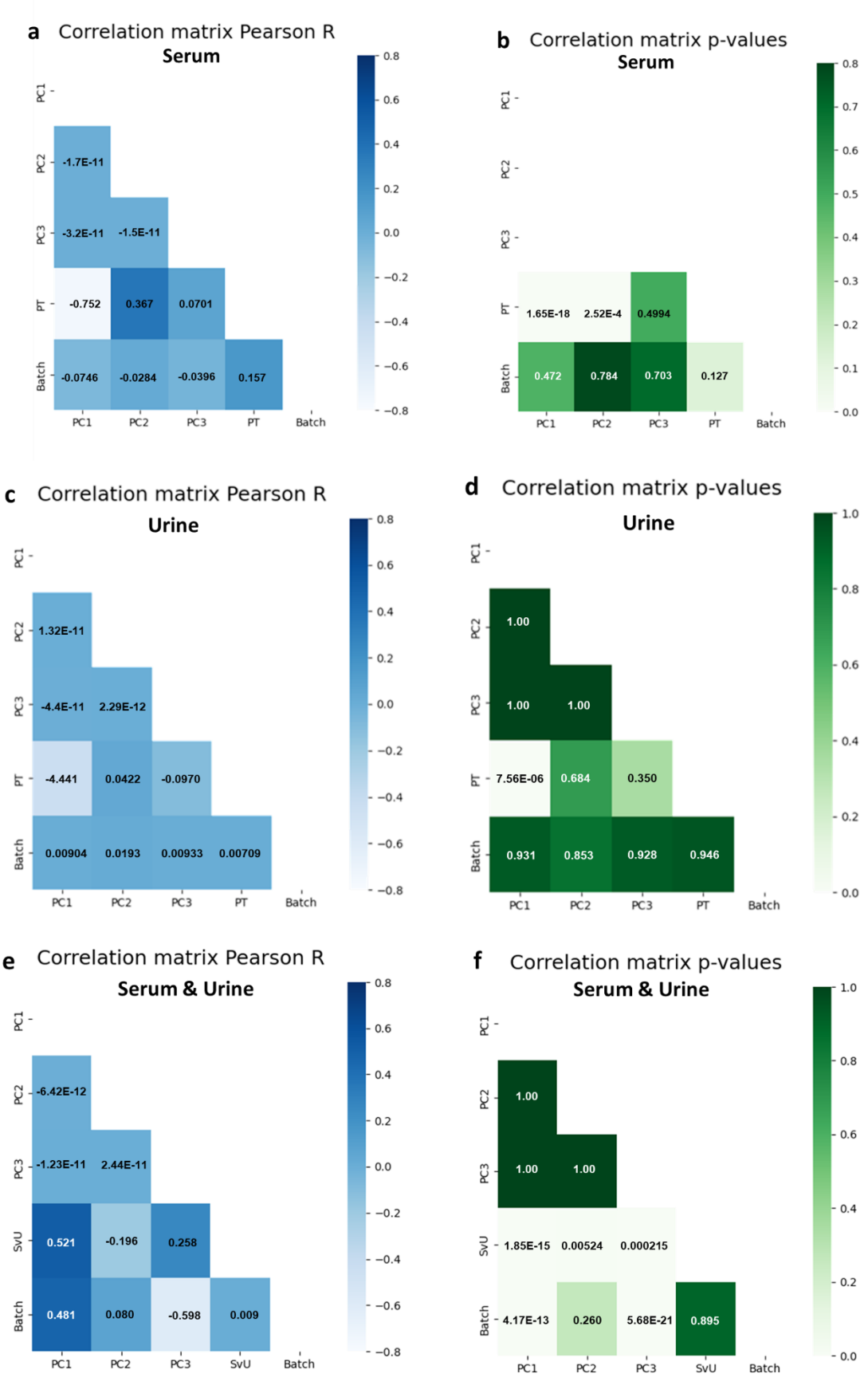

Figure S2. The dataset analysis was conducted after processing with the "Combat" batch correction to examine correlations and statistically significant differences among principal component (PC) loadings, batch, and sample type. The batch correction was applied for preterm and term birth samples across four batches in both urine and serum, as well as for combined urine and serum samples in four batches. The Pearson R values and p-values were reported for urine (**a** and **b**), serum (**c** and **d**), and combined serum & urine (**e** and **f**). PT: preterm and term birth sample types, SvU: serum and urine sample types.

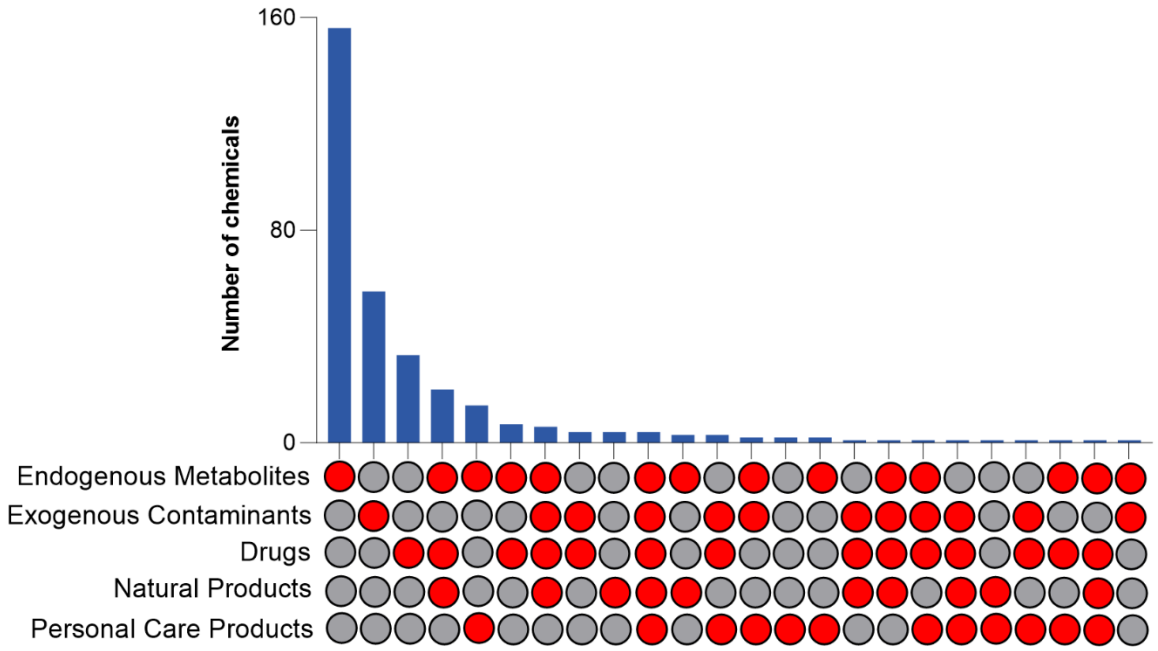

Figure S3. Level 2 chemicals annotated as endogenous metabolites, exogenous contaminants, drugs, natural products, and personal care products. The bar chart shows the full tally of annotated chemicals in each specific category, plotting with the decreasing number of chemicals. The red and grey balls represent the annotated category and non-annotated category, respectively.

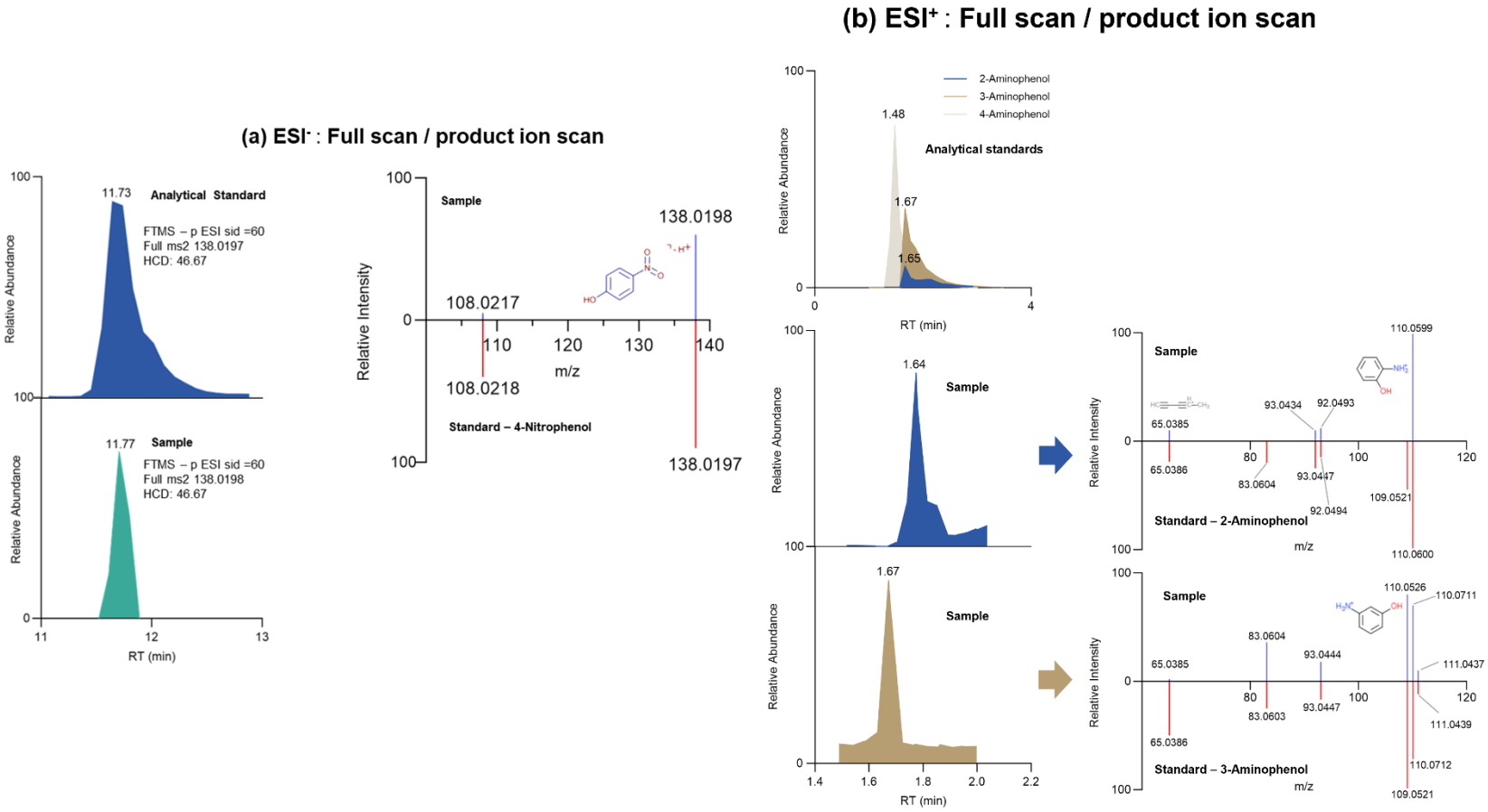

Figure S4. Examples of compounds using authentic standards detected by UHPLC-Orbitrap MS with ESI- (**a**) and ESI+ (**b**) polarities. The chromatographic plot of 4-nitrophenol and aminophenol isomers in the authentic standards and real samples. The compounds were initially selected by comparing different databases and were further confirmed by authentic standards. The differential plot of the deconvoluted spectrum between real samples and authentical standards.

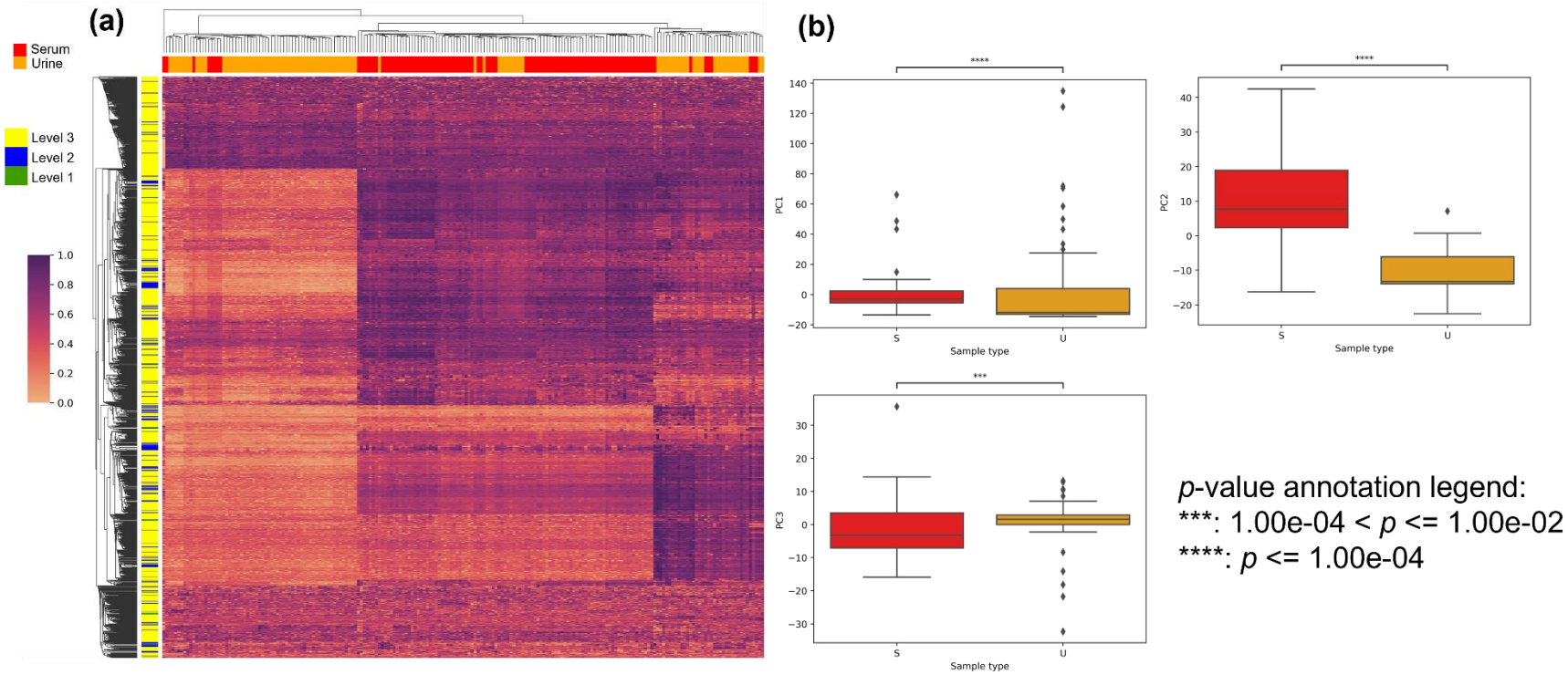

Figure S5. Clustering heatmap of chemical abundances after batch effect correction for serum (S) and urine samples (U) (**a**) and the boxplot for the significant difference (p < 0.001) of PCs 1-3 between serum and urine samples using Mann-Whitney-Wilcoxon test two-sided with Bonferroni correction (**b**). The bottom and top of boxes represent the 25th and 75th percentiles, the error bars denote 10th to 90th percentiles, the solid line means the median value. The total number of chemical features (combing ESI^+ and ESI^-) is 1524 with the annotation levels 1-3 from the classification of Schymanski, et al. [3].

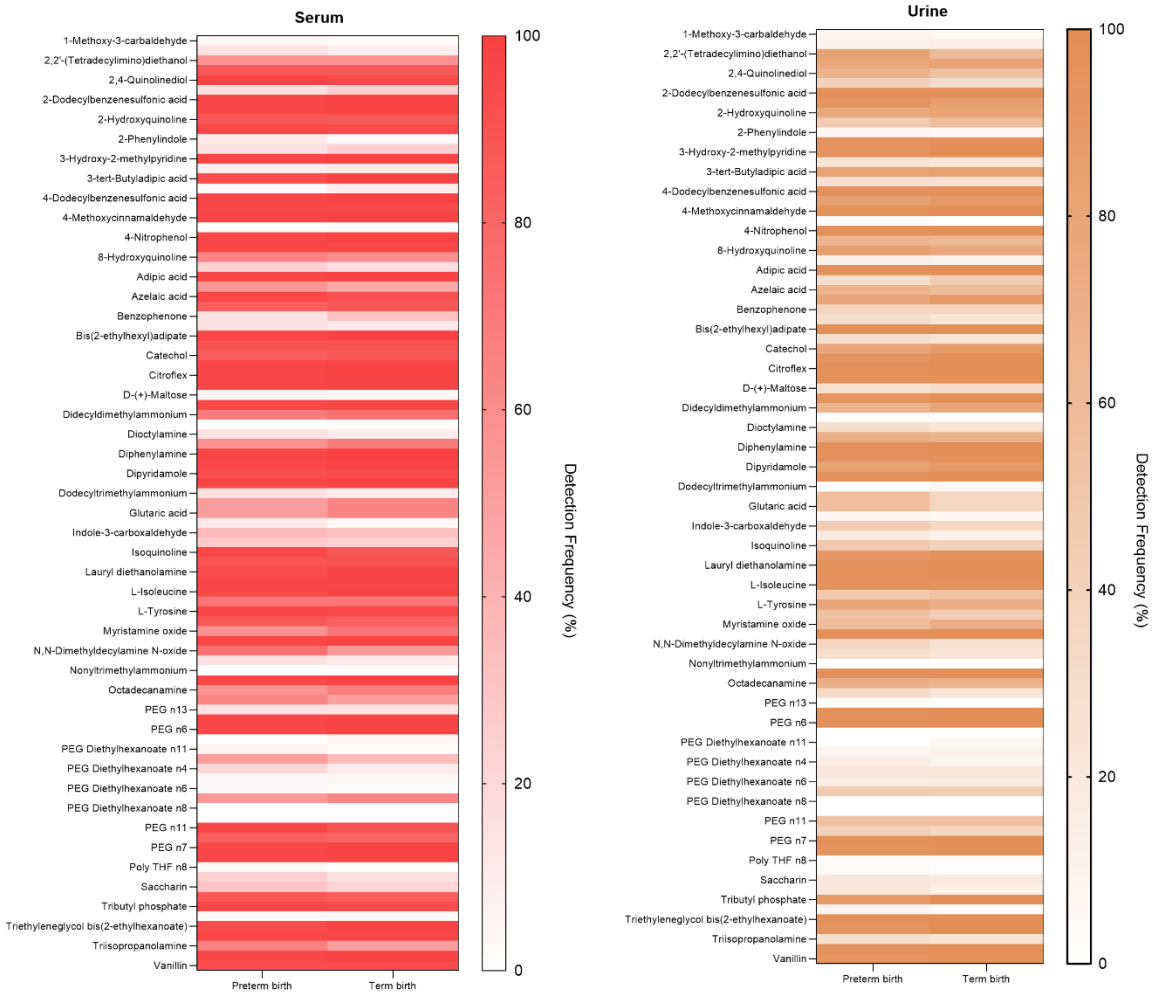

Figure S6. The heatmap of detection frequency (%) for annotated chemicals (Levels 1 and 2) classified as exogenous contaminants in preterm and term birth in serum and urine samples, respectively. The specific number of frequencies is shown in the spreadsheet S1.

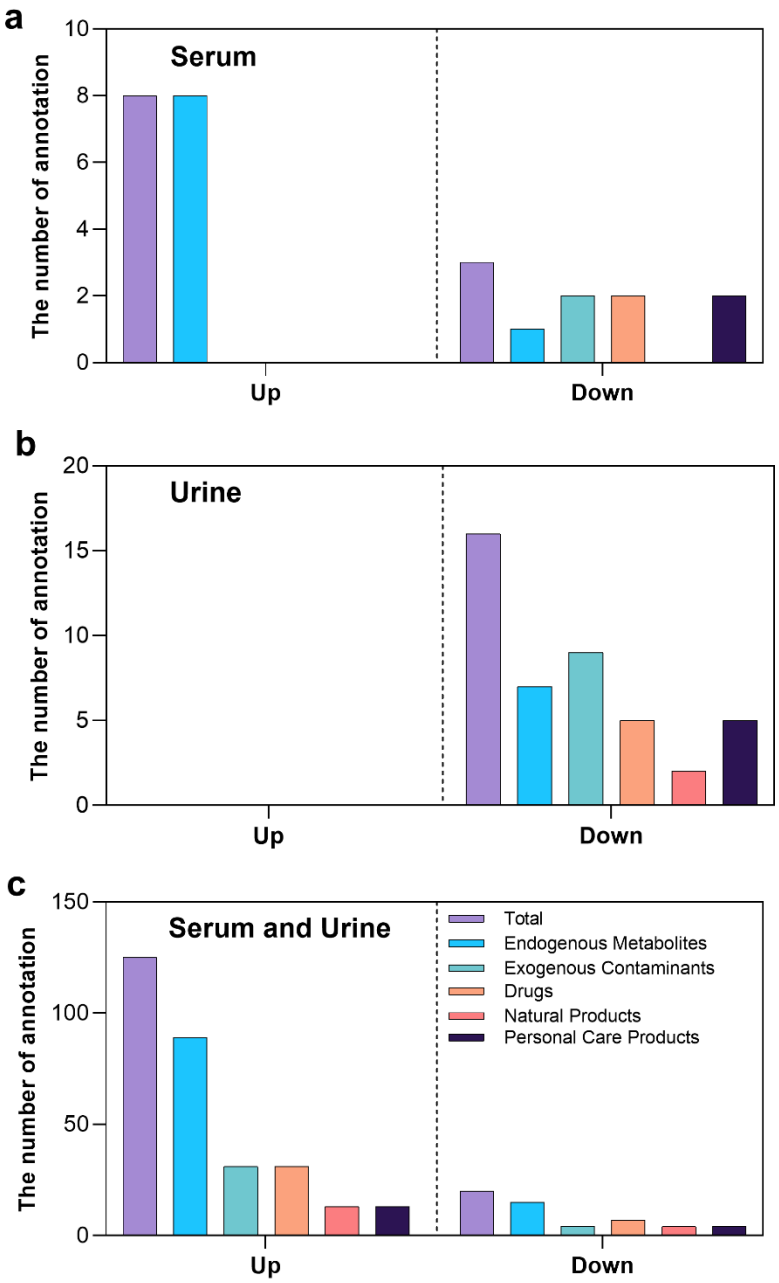

Figure S7. The bar chart of the number of chemicals that were successfully annotated, located in the down- ($p < 0.05$, $\log_2$fold < -1.2) and up- regulated areas ($p < 0.05$, $\log_2$fold >1.2) of the serum vs. urine samples / preterm and term birth samples in serum or urine from the volcano plot (Figure 5).

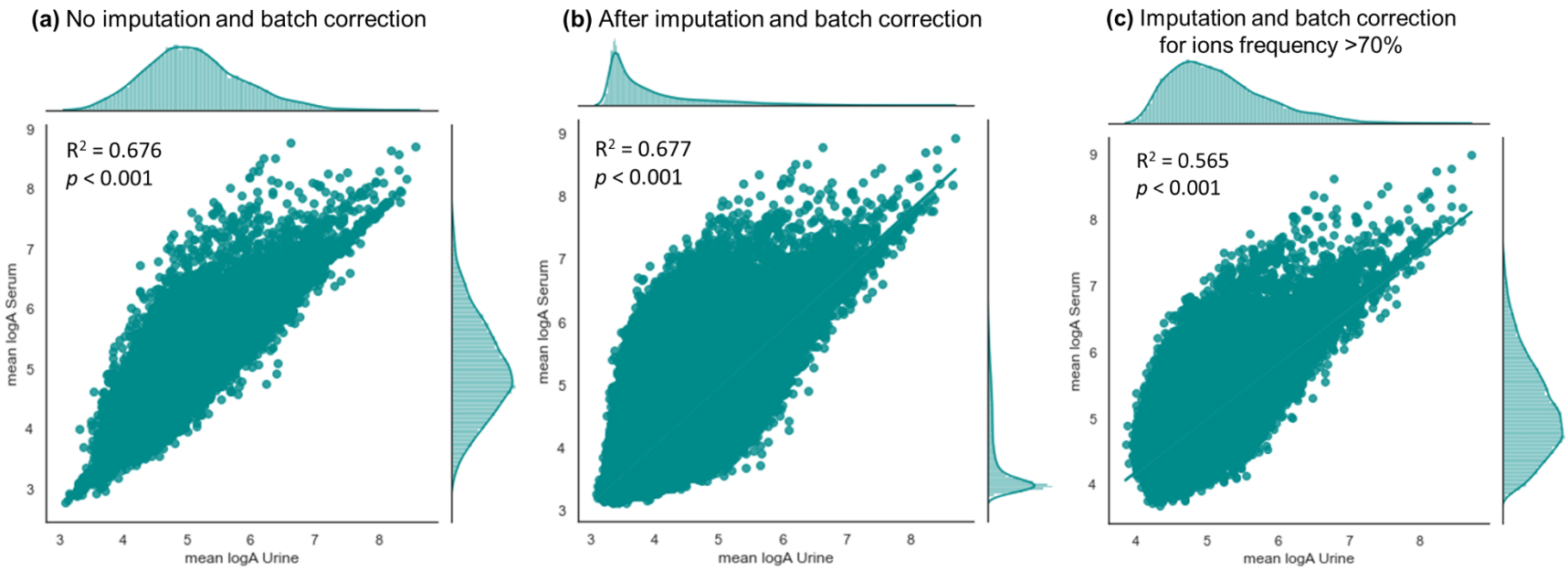

Figure S8. Linear regression correlation analysis between urine and serum abundances in logarithm scale (n = 190 urine and serum). The results show: (**a**) all original dataset before imputation and batch correction, (**b**) all dataset after imputation and batch correction, and (**c**) the ions filtered by the cut-off frequency of 70% for imputation and batch correction.

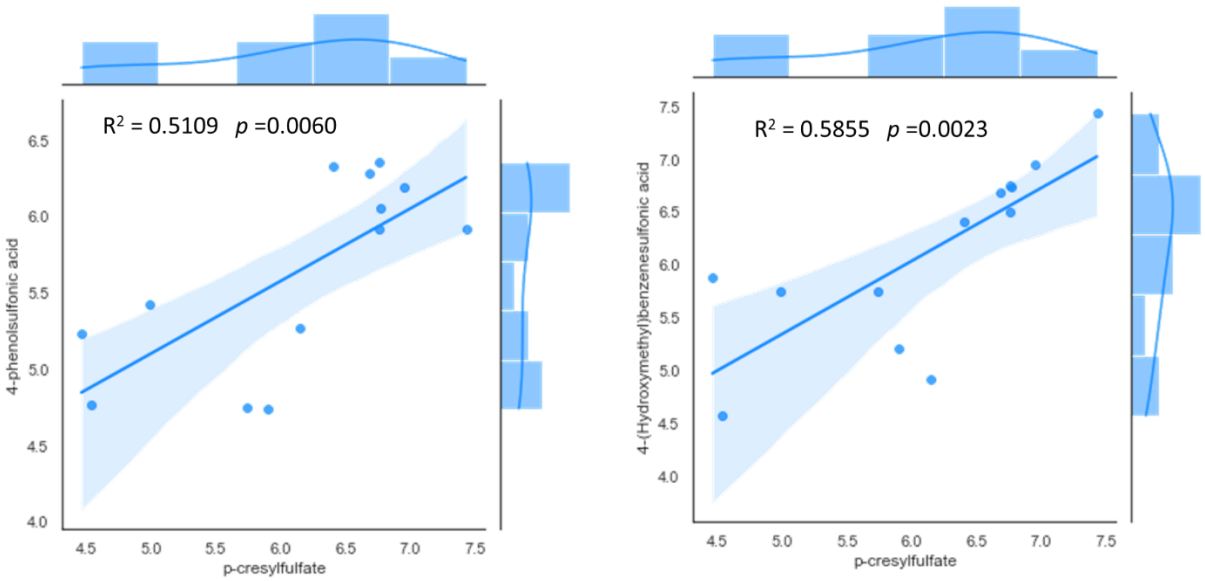

Figure S9. Correlation between p-cresylfulfate and 4-(hydroxymethyl)benzenesulfonic acid, and p-cresylfulfate and 4-phenolsulfonic acid in logarithm scale (n = 190 urine and serum).

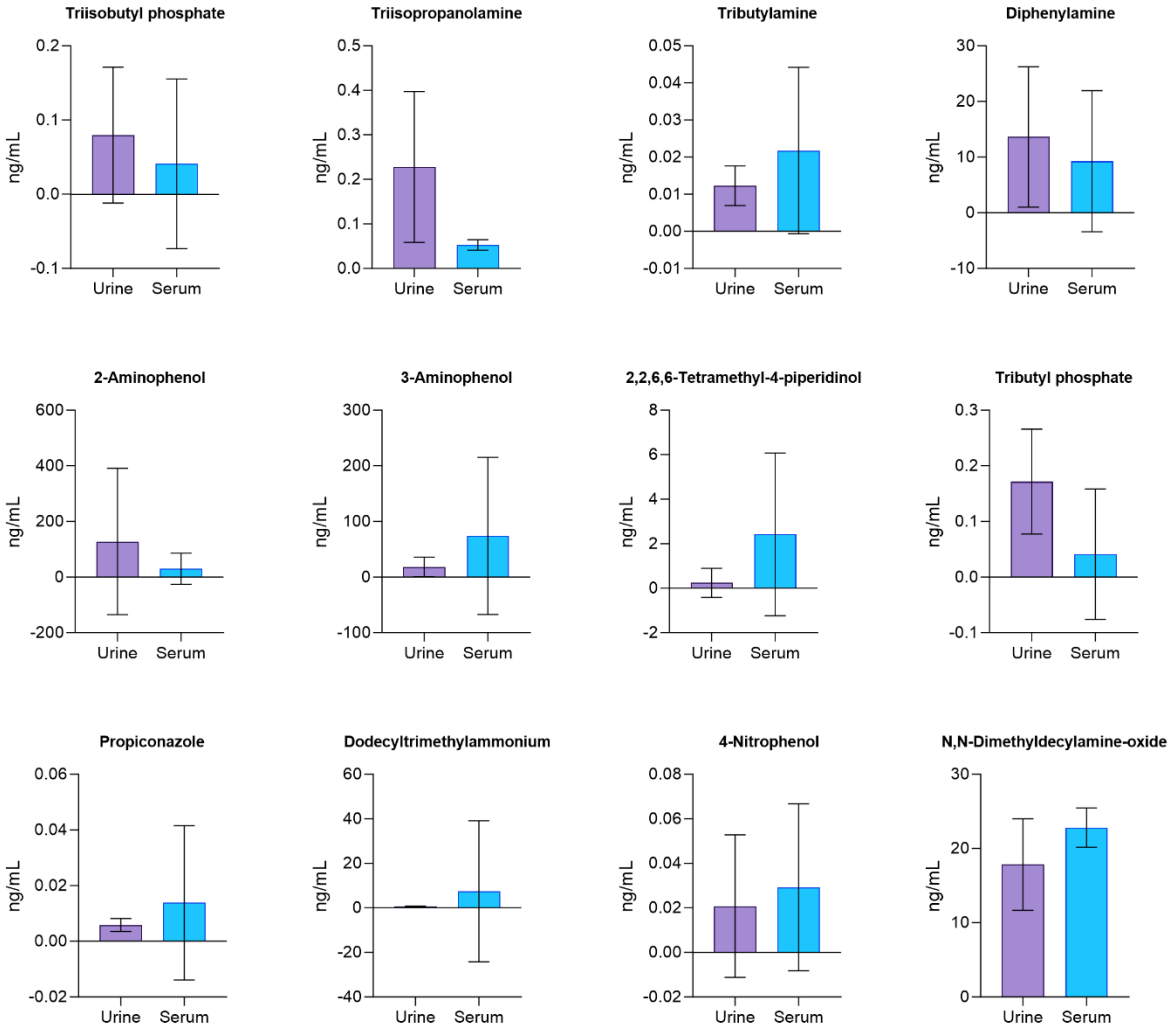

Figure S10. The semi-quantification of confirmed compounds (level 1) in urine and serum samples based on the integral peak areas of 500 ng/mL analytical standards.

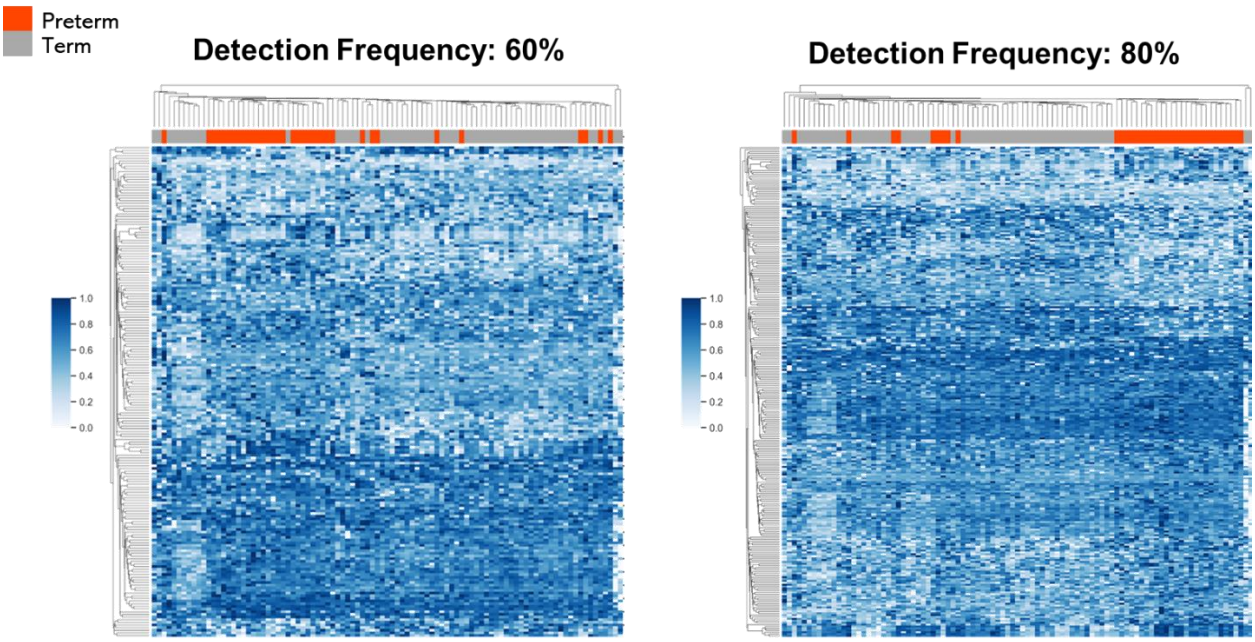

Figure S11. Clustering heatmap after batch effect correction (between preterm and term birth) for serum samples. The chemical features reveal the differential enrichment in preterm versus term births among serum with the cut-off detection frequencies of 60% and 80% after multiple testing correction (Benjamini-Hochberg test, 5% false discovery rate). For the differential enrichment in preterm versus term birth samples, 1,791 out of 43,450 chemical features in a detection frequency cut-off of 60% and 1,214 out of 25,323 in a detection frequency cut-off of 80% showed significant differences ($p < 0.05$).

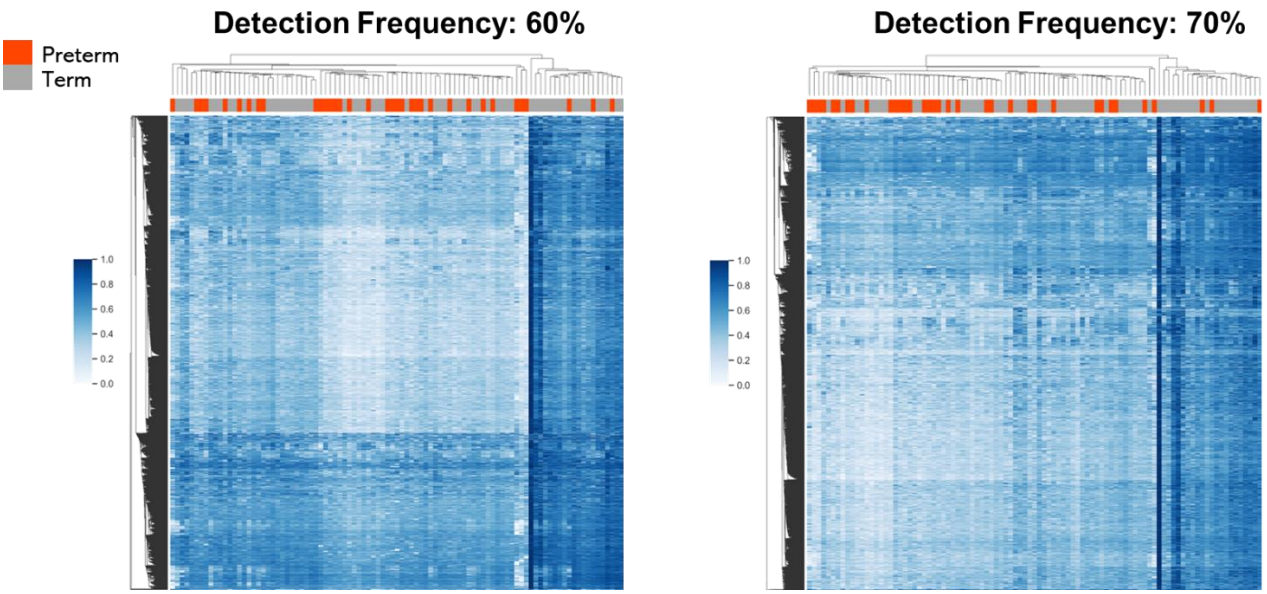

Figure S12. Clustering heatmap after batch effect correction (between preterm and term birth) for urine samples. The chemical features reveal the differential enrichment in preterm versus term births among serum with the cut-off detection frequencies of 60% and 80% after multiple testing correction (Benjamini-Hochberg test, 5% false discovery rate). For the differential enrichment in preterm versus term birth samples, 9,518 out of 49,350 chemical features in a detection frequency cut-off of 60% and 8,398 out of 29,448 in a detection frequency cut-off of 80% showed significant differences ($p < 0.05$).

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