Environmental Science & Technology

pubs.acs.org/est

Article

Open Access

This article is licensed under CC-BY-NC-ND 4.0

Application of a Non-targeted Biomonitoring Method to Characterize Occupational Chemical Exposures of Women Nurses Relative to Office Workers

Published as part of Environmental Science & Technology special issue "Non-Targeted Analysis of the Environment".

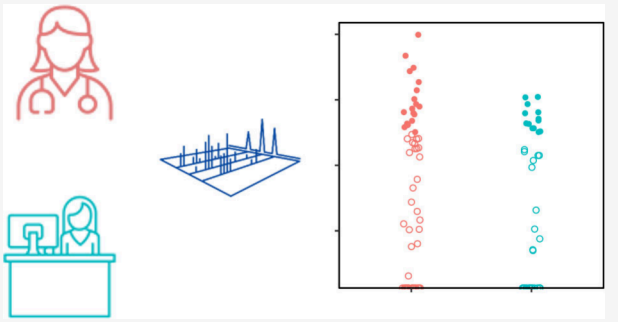
Kristin E. Knox,* Dimitri Abrahamsson, Jessica Trowbridge, June-Soo Park, Miaomiao Wang, Erin Carrera, Lisa Hartmayer, Rachel Morello-Frosch,* and R. A. Rudel

Cite This: *Environ. Sci. Technol.* 2025, 59, 9437−9448

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: We analyzed blood serum samples from two unique female occupational cohorts − 60 nurses and 40 office workers in San Francisco, CA − using liquid chromatography and high-resolution mass spectrometry (quadrupole time-of-flight). Applying a non-targeted analysis (NTA) approach, we sought to isolate occupationally related chemical exposures that were unique to nurses by flagging features that were different from office workers in abundance (mean; 95th percentile) or detection frequency. Of 9828 negative electrospray ionization (ESI-) and 6898 positive electrospray ionization (ESI+) detected chemical features, 1094 and 938, respectively, were higher in nurses, possibly due to workplace exposures. We deciphered the molecular structures of these chemical features by applying data-dependent acquisition (DDA) and targeted MS/MS approaches to pooled samples from each occupational group, and we annotated them using spectral MS/MS databases in MS-DIAL. Nurses had higher concentrations of 14 chemicals that we identified at Schymanski Level 1 (N = 6) or 2 (N = 8), as well as 20 tentatively identified chemicals without spectra. Several chemicals may be occupationally relevant for nurses, including a PFAS (6:2 fluorotelomer sulfonic acid), tridecanedioic acid, salicylic acid, and the medications acetaminophen and theophylline. To our knowledge, this study is the first to apply NTA to elucidate novel chemical exposures in nurses.



KEYWORDS: *Non-targeted analysis, occupational exposures, nurses, environmental contaminants, mass spectrometry*

INTRODUCTION

Nurses work on the front lines to protect community well-being, yet their exposures to chemicals, particularly those potentially relevant for breast cancer, are understudied. Nurses face numerous hazardous exposures in the hospitals where they work, including sterilizing agents that are associated with outcomes such as time to pregnancy, miscarriage, and respiratory problems.[1−4] In addition, nurses are exposed to complex mixtures of potentially carcinogenic and endocrine-disrupting chemicals, including disinfectants, chemotherapeutic agents, and medications,[5] but few studies have used workplace biomonitoring to characterize exposures. Nurses face a higher incidence of breast cancer compared to the general population,[6−8] which has been attributed in part to shift work and exposure to ionizing radiation, among other exposures.[9−11] However, to our knowledge, no studies have applied a discovery driven approach to characterize the extent to which nurses are occupationally exposed to endocrine disrupting chemicals, particularly those of potential relevance to breast cancer. We sought to address this data gap by applying a non-targeted analysis (NTA) approach in this study comparing occupational chemical exposures between women nurses and office workers in San Francisco, California (CA).

Non-targeted analysis (NTA) can capture a wide range of chemical exposures and is especially well-suited to occupational cohorts where exposure levels are likely to be higher. Our goal was to apply a non-targeted analytical approach to elucidate occupationally related exposures unique to nurses, by comparing them to a control group of office workers. To our knowledge this

Received: December 31, 2024
Revised: April 11, 2025
Accepted: April 14, 2025
Published: May 5, 2025

ACS Publications

© 2025 The Authors. Published by American Chemical Society

https://doi.org/10.1021/acs.est.4c14790
Environ. Sci. Technol. 2025, 59, 9437−9448

study is the first to apply NTA methods to elucidate novel chemical exposures in uniquely exposed occupational groups among exclusively women workers.

METHODS

Study Population and Recruitment. This study is part of the Women Workers Biomonitoring Collaborative (WWBC),[12] a community-based, participatory biomonitoring project that is developing a biospecimen archive to characterize occupationally related chemical exposures among female workers in diverse occupations (including nurses and other hospital workers, firefighters, office workers and domestic workers). Our study population for this analysis included 60 female registered nurses from the University of California, San Francisco (UCSF) Parnassus Medical Center and 40 female office workers employed by the City and County of San Francisco, California.

Participant recruitment and sample collection took place between 2018 and 2019. Nurse collaborators assisted with the recruitment of eligible nurse participants, by holding informational meetings, and following up with potentially interested participants by direct email and one-on-one informational meetings. Consideration was given to ensure the inclusion of nurse participants from various hospital units known to have a high likelihood of chemical or medication exposures (e.g., due to frequent disinfecting and/or administration of medications), including emergency, intensive care, acute care, peri-operative, transplant, and oncology units. Similarly, office workers, who were employees of the City and County of San Francisco, were recruited at offices via public meetings led by research staff, tabling at health fairs, and through employee listserv emails. Study inclusion criteria for both nurses and office workers included being female, over 18 years old, a full-time employee (90% effort for nurses and at least 32 h/week for office workers), not pregnant at the time of recruitment and sample collection, and a nonsmoker. All participants were consented into the study following protocols approved by the Institutional Review Board of the University of California, Berkeley (# 2017−07−10124). Demographic characteristics of the study population are shown in Table 1.

Table 1. Demographics of Study Population

	Nurses (N = 60) N (%)	Office Workers (N = 40) N (%)
Age in Years		
18−39	31 (52%)	12 (30%)
40−49	16 (27%)	9 (23%)
50−59	10 (17%)	11 (28%)
60−79	3 (5%)	8 (20%)
Educational Attainment		
High school diploma/ GED	0 (0%)	1 (3%)
Some college	1 (2%)	1 (3%)
Bachelor's degree	48 (80%)	19 (48%)
Graduate degree	11 (18%)	19 (48%)
Race/Ethnicity		
Non-Hispanic White	37 (62%)	19 (48%)
Asian and Pacific Islander	14 (23%)	13 (33%)
Latina	3 (5%)	5 (13%)
Black/African American	3 (5%)	1 (3%)
Multiracial/Native/ Other	3 (5%)	2 (5%)
Nativity		
Born in the U.S.	45 (75%)	35 (88%)
Not born in the U.S.	15 (25%)	5 (13%)

Sample Collection and Preparation. Serum samples were collected using Covidien Monoject Noncoated Red Top Tubes and within a 2-h window were centrifuged for 10 min at 3000 rpm to separate the serum from the platelets and then aliquoted and frozen at −80 °C until analysis. Aliquots were transported on dry ice to the Environmental Chemistry Laboratory (ECL) of the California Department of Toxic Substances Control (DTSC) in Berkeley. The samples were then thawed at room temperature and aliquots of 250 μL were transferred to clean tubes. The samples underwent protein precipitation with the addition of 2 mL of cold methanol (with 0.1% formic acid) and were shaken for 10 min. They were then centrifuged for 10 min at 3500 rpm to separate the supernatant from the proteins. The supernatant was then transferred to clean glass tubes and the samples were evaporated at room temperature with a gentle stream of nitrogen to near dryness. Finally, the samples were reconstituted to 200 μL with methanol, then were transferred to LC vials and stored at 4 °C until analysis.

In addition to the 100 individual samples, we created two pooled serum samples, one for nurses and another for office workers. The two pooled samples were created by combining 20 randomly selected nurse samples and 20 office worker samples.

Instrumental Analysis. Each sample was analyzed using an ultrahigh pressure liquid chromatography (UPLC) system coupled with a 6550 quadrupole time-of-flight mass spectrometer (QTOF-MS) from Agilent Technologies (Santa Clara, CA). The instrument operated in both positive and negative electrospray ionization modes (ESI+ and ESI-). Since there are advantages and disadvantages to each mode in terms of the compounds they can detect, combining them allows us to detect a larger set of compounds.[13] Full-scan accurate mass spectra (MS) were collected within a 100−1000 Da range, with a resolving power of 40,000 and a mass accuracy below 5 ppm. For MS/MS fragmentation, ion spectra were recorded at collision energies of 10, 20, and 40 eV, maintaining a mass accuracy of 10 ppm. The QTOF was calibrated before each batch, and mass accuracy was continuously monitored using reference masses 112.985587 and 1033.988109. Detailed instrumental parameters are summarized in Table S1. For chromatographic separation, an Agilent Eclipse Plus C18 column (2.1 × 100 mm, 1.8 μm) was used. The mobile phase included two solutions: (A) 5 mM ammonium acetate (Sigma-Aldrich, ≥98%) in HPLC water (Sigma-Aldrich, ≥99.5%) with 0.1% methanol (MeOH) and (B) 5 mM ammonium acetate in methanol (≥99.9%) with 10% HPLC water, following a gradient program as follows: 0 min with 10% B and 90% A, gradual increase to 100% B from 0−15 min, and equilibration at 100% B from 16−20 min. The gradient flow was set to be 0.3 mL/min. All samples were analyzed in duplicate injections ("run 1" and "run 2"), and water blanks were run at the start of each batch. In all, there were 5 batches of samples.

Quality Assurance/Quality Control. For quality control, internal standards were spiked into all samples to monitor the general method performance (Perfluoro-n-[1,2−13C2] octanoic acid (M2PFOA) was used in negative ionization mode; triphenyl phosphate D15 and DL-cotinine (methyl D3) for positive). In-house quality control samples were prepared using commercially available human AB serum (Corning Human AB Serum) and spiked with 10 PFAS compounds and 6 OPFR compounds (SI Table S2), with a final concentration of 10 ng/mL. The blank samples and the QC samples were treated the

https://doi.org/10.1021/acs.est.4c14790
Environ. Sci. Technol. 2025, 59, 9437−9448

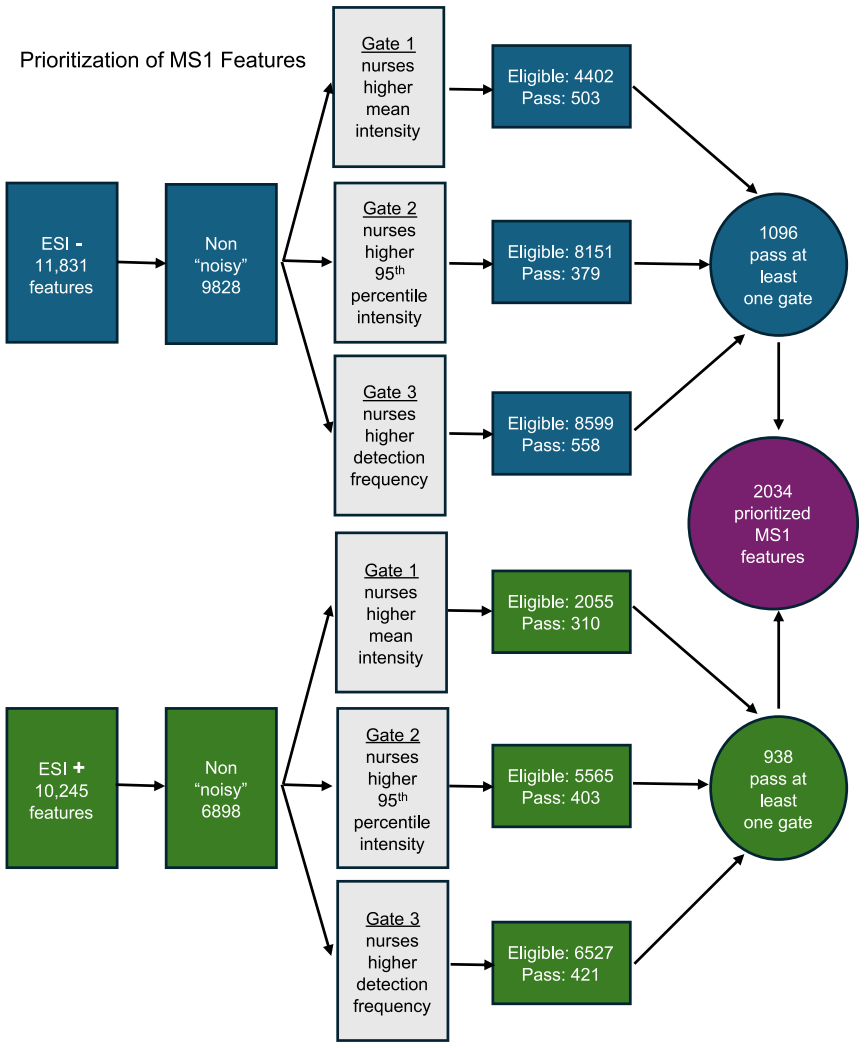


Figure 1. Prioritization of MS1 features. This flowchart shows how the initial set of 22,076 features from MS1 data on individual participants is reduced to a prioritized set of 2034 features.

same way as the samples and analyzed with every batch of the samples.

Blanks. Field blanks were collected from each occupational group every 15 samples or once per month, whichever came first. We collected a total of 6 field blanks, 3 from each nurse and office worker sample collection site. For the field blanks, the deionized water was pulled through the needle into the tube using the same sampling equipment as was used for blood collection. The lab was blinded to these blanks, so each field blank sample was run twice, like the regular participant samples. In addition, the lab ran two deionized water blanks with each batch, resulting in a total of 10 deionized water blanks. We computed the max blank value as the maximum value from among the following 22 values: run 1 and run 2 of each of the 6 field blanks and the 10 deionized water blanks. We use this max blank value in the data cleaning described below.

Data File Processing. The NTA data files, containing total ion chromatograms, were converted from .d to.abf files using Reifycs Analysis Base File and then analyzed using MS-DIAL, an open-source software developed by UC Davis and RIKEN (Japan).[14] Features were aligned across samples, with a tolerance of 10 ppm in mass difference. Chemical features with peak areas at least 3 times higher in participant samples compared to blanks were classified as true positives. All MS-DIAL processing parameters are detailed in Table S3.

Data Cleaning. We undertook some initial data cleaning with the following steps, also shown in Figure 1. We first computed the average intensity for each person-chemical feature as the mean of run 1 and run 2. We also computed the maximum intensity of the blanks for each feature. Next, we removed "noisy" features, defined as (a) those features with a maximum average intensity (across all participants) that was less than 3000,[15] or (b) those features with a maximum average intensity that was less than 3 times the maximum for the blanks. We then estimated the average relative standard deviation between run 1 and run 2 for each feature (across all participants) and flagged those features with a mean relative standard deviation between runs above 50% as having potentially low reproducibility.

We computed the following summary statistics for each feature overall, and separately for nurses and for office workers: mean intensity, 95th percentile intensity, standard deviation of intensities, and detection frequency (where a detection was defined as an intensity greater than or equal to 3000.)

Selecting Features of Potential Relevance for Nurses. Our goal was to identify features that may be work-related by comparing abundances and detection frequencies between nurses and office workers. Using the MS1 data on our individual

https://doi.org/10.1021/acs.est.4c14790
Environ. Sci. Technol. 2025, 59, 9437−9448

samples, we took three different approaches to broadly identify and prioritize key features of potential interest. We refer to these as "gates," and those features which are prioritized in each approach as "passing" the gate (Figure 1).

Our first gate prioritized those features where nurses had a higher mean intensity than office workers. For this gate, we subset our sample to only include those features that had a detection frequency (≥3000) of at least 65% (over all participants) and used imputation of nondetects and batch correction prior to comparing mean intensities between nurses and office workers. For all measures above 0, we used the machine-read value. For intensities equal to 0, we replaced the 0 with an imputed value by taking the average of three random draws from a truncated normal distribution, with a lower bound of 0, an upper bound equal to the minimum nonzero value, with mu equal to the minimum nonzero value, and sigma equal to the standard deviation of the nonzero intensities. We then used the ComBat() function from the sva R package to perform batch correction and eliminate potential batch effects in our analysis.[15,16] Using the batch-corrected data, we estimated generalized linear models (GLM) models of ln(intensity) on an indicator variable for worker (=0 for office workers and = 1 for nurses) and adjusted p-values for multiple testing using Benjamini and Hochberg (1995).[17] We assumed a 5% significance level and prioritized those features where nurses have a significantly higher mean intensity than office workers. We plotted the log 2-fold change of nurses relative to office workers using a volcano plot.

Our second gate prioritized those features where nurses had a higher 95th percentile intensity compared to office workers. We subset our sample to those features with a detected (≥3000 intensity) 95th percentile for nurses and for office workers. We were not able to perform batch correction on this data because it contained features with detection frequencies that were too low (below 65%) to replace the zeros with imputed values. We computed the fold-difference of the 95th percentile for nurses/95th percentile for office workers and then prioritized those features with a fold-difference of at least 3.

Our third gate prioritized those features that were more frequently detected in nurses than in office workers. We subset our sample to features that had an overall detection frequency below 100%. We used the one-sided Fisher Exact Test to assess whether detection frequencies were significantly higher ($p < 0.05$) for nurses than office workers. We adjusted p-values for multiple testing using Benjamini and Hochberg (1995).[17]

All statistical analyses were performed in R (version 4.4.0). Code can be found here: https://github.com/SilentSpringInstitute/Knox_et_al_2025_nurses.

Identification of Prioritized Features. We collected MS/MS spectra from pooled nurse and office worker samples and annotated these in MS-DIAL using the MS/MS databases on the MS-DIAL Web site[18] and MassBank of North America (MoNA).[19] (MS-DIAL settings are found in Table S3.) We matched these annotated features to the MS1 features prioritized as higher in nurses using the three gates described above. This matching of MS1 prioritized features with annotated MS/MS features from pooled samples used mass and retention time as described below. MS/MS spectra on pooled samples were collected using the same instrumentation and conditions as the MS1 data from the individual nurse and office worker samples, in both positive and negative ionization mode at 10, 20, and 40 eV collision energies and a mass accuracy of 10 ppm.

The MS/MS data on pooled samples were acquired using two approaches: (a) a data-dependent acquisition (DDA; "autofrag" in the Agilent software) where the instrument continuously scans MS1 data and selects the most abundant ions for fragmentation; and (b) a targeted peak-picking approach where features that matched a list of chemicals of interest by mass were prioritized for fragmentation. We annotated the MS/MS data on our two pooled samples in MS-DIAL. As a first step, we selected all features that post alignment had an assigned MS/MS library match (MS/MS-matched = true). We then matched these tentatively annotated features from the pooled samples with our prioritized features from the MS1 data on the individual samples based on mass and retention time. Specifically, for a feature to be considered a match, we required the mass to be within 10 ppm (denoting a mass difference of less than or equal to 5 ppm as "M' and a mass difference greater than 5 ppm but less than or equal to 10 ppm as "m"), and the retention time to be within 0.5 min (denoting a retention time difference of less than or equal to 0.1 min as "R" and a retention time difference greater than 0.1 min but less than or equal to 0.5 min as "r"). This resulted in many-to-many matches. These matched features were the subset on which we performed manual curation (Figure 2, SI Tables S6−S7).

To perform the manual curation, we compared the spectrum of the feature from the pooled sample in MS-DIAL to the reference spectrum in MS-DIAL and required that there be at least two ions in common, with one of them being the parent ion. We also required the dot-product and reverse dot-products, measures of how well the experimental spectrum matches the library spectrum, to be at least 750. Based on these criteria and considering the framework of Schymanski, Jeon, Gulde, Fenner, Ruff, Singer, and Hollender (2014),[20] we classified features that met these criteria as Level 2 matches, meaning the experimental spectrum matched to a library spectrum.

We purchased analytical standards for the Level 2 matches that were potential environmental chemicals and collected MS/MS data on these standards. We compared our Level 2 tentative identifications to the data from the analytical standards where available to see if any of them were eligible to be upgraded to a Level 1, again following the Schymanski framework,[20] where a Level 1 match has an experimental spectrum matched to a spectrum from a standard. In cases where the experimental spectrum matched to the spectrum from the standard, we classified the feature as a confirmed match (Level 1). In cases where the MS1 data on the standard did not match our MS1 experimental data on our tentatively identified participant feature, we considered these annotations as not confirmed.

Since we also had MS1 data on mixtures of standards, and this data was run on the same instrument, we compared this data to the MS1 data from our participant features that passed at least one of our gates. We again matched on m/z and RT, as described above. We classified these matches as Level 2 without spectra to indicate that these are tentative confirmations given that their MS1 spectra came from mixtures of standards rather than individual standards. In the cases of standard mixtures, it is often difficult to distinguish between compounds with very similar mass to charge ratios (m/z), even when MS2 spectra are available. This slight deviation from the Schymanski framework captures the nuances in our data and addresses limitations on the availability of standards.

https://doi.org/10.1021/acs.est.4c14790
Environ. Sci. Technol. 2025, 59, 9437−9448

Figure 2
Annotation of MS1 Features

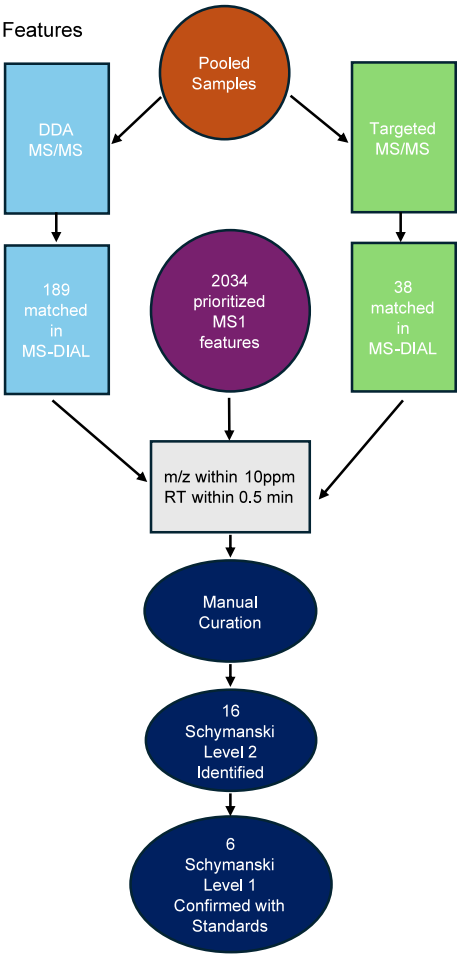


Figure 2. Annotation of prioritized MS1 features. This flowchart shows how features from the two sets of MS/MS data on pooled samples (DDA and targeted) that are annotated in MS-DIAL are then matched to the MS1 features from the participant data based on mass and retention time to produce Schymanski Level 2 identifications, then compared with standards to produce Schymanski Level 1 identifications.

RESULTS AND DISCUSSION

We detected 22,076 unique features (11,831 in ESI- and 10,245 in EIS+). After removing "noisy" features (as defined in methods), 9828 features from the ESI- run (with 784 flagged for low reproducibility) and 6898 features from the ESI+ run (with 455 flagged for low reproducibility) remained (Figure 1).

There were 4402 ESI- features and 2055 ESI+ features that had a detection frequency greater than or equal to 65% and were therefore eligible for Gate 1, which compared mean intensity between nurses and office workers. After batch-correction (Figures SI.1−2), we prioritized features with higher mean intensities in nurses. Of these, we prioritized 503 ESI- and 310 ESI+ features as ones where nurses had significantly higher (adjusted p-value <0.05) mean intensity (Figures 1 and 3).

There were 8151 ESI- features and 5565 ESI+ features that had a detected (≥3000) 95th percentile intensity for nurses, and a detected (≥3000) 95th percentile intensity for office workers. These features were eligible for Gate 2, in which we prioritized those features with higher 95th percentile intensities in nurses, that resulted in 379 ESI- and 403 ESI+ features as ones where nurses had higher 95th percentile intensities (Figure 1).

There were 8599 ESI- features and 6527 ESI+ features that had a detection frequency below 100% and were therefore eligible for Gate 3, in which we prioritized features that had higher detection frequencies in nurses relative to office workers. Of these, we prioritized 558 ESI- features and 421 ESI+ features as ones where nurses had significantly higher detection frequencies than office workers.

Annotation of Features That Were Enriched in Nurses. In total, we prioritized 1096 ESI- and 938 ESI+ MS1 features that were enriched in nurses (Figure 1). We used this subset of features to tentatively identify compounds by matching to MS/MS data from pooled samples. From the DDA MS/MS experiments on the pooled samples, we matched these to library spectra for 121 ESI- and 68 ESI+ features. From the targeted MS/MS experiments on the pooled samples, we matched these to library spectra for 25 ESI- and 13 ESI+ features (Figure 2). After manual inspection and curation, we were able to tentatively confirm 16 of these compounds with MS/MS library spectra (Level 2) (Figure 2, Figures S5−S6). We increased our identification of six of these compounds to Level 1 after confirming with analytical standards. There were two compounds for which we could not confirm the annotations. We did not have standards for the remaining eight compounds, which stayed at Level 2. We tentatively identified an additional 20 compounds as Level 2 without spectra.

Potential Occupational Exposures of Nurses. Of the 16 tentatively identified features that were enriched in nurses, we classified seven as potential occupational exposures (Figure 4), seven as dietary (Figure S3), and two as endogenous (Figure S4). Using available analytical standards data, we were able to upgrade four of the seven environmental chemical exposures and two of the seven dietary chemical exposures to Level 1. The occupational exposures that we confirmed as Level 1 included 6:2 fluorotelomer sulfonic acid, salicylic acid, theophylline, and acetaminophen. Tridecanedioic acid was tentatively identified at Level 2, however a standard for this chemical was not available. Dicyclohexyl phthalate and 4-hydroxyquinoline were included in the original set of 16, but we were not able to confirm these annotations with standards. Figure S5 shows spectra and match statistics from MS-DIAL for each of these 16 chemicals. Each is briefly discussed below.

We confirmed one PFAS chemical − 6:2 fluorotelomer sulfonic acid. Nurses had a significantly higher 95% percentile abundance of this compound than office workers. (Figure 4). This chemical is a likely substitute for PFOS and is reported to be used as a replacement for PFOS in firefighting foam, in the chromium plating industry to reduce mist formation during plating, as a polymer processing aid in synthesis of fluoropolymers (like Teflon) and in floor wax.[21−23] Since this chemical is not regulated as a hazardous substance, information about its uses is sparse. Glüge, Scheringer, Cousins, DeWitt, Goldenman, Herzke, Lohmann, Ng, Trier, and Wang (2021) report 6:2 fluorotelomer sulfonate may be used for greenhouse films, circuit boards, lithium-ion batteries, fuel and oil filtration, production of molded plastics such as artificial turf.[24] Exposure may also result from other sources that are not well documented. Risk assessments by US EPA and the Michigan Department of Environment, Great Lakes, and Energy concluded that this compound would be rapidly absorbed by inhalation or dermal contact and was similar in toxicokinetic properties to PFOS (persistent, not metabolized, slowly excreted, bound to protein).[25,26] Predicted toxicity was high despite data gaps, with Michigan setting an air level concentration of concern of 1

https://doi.org/10.1021/acs.est.4c14790
Environ. Sci. Technol. 2025, 59, 9437−9448

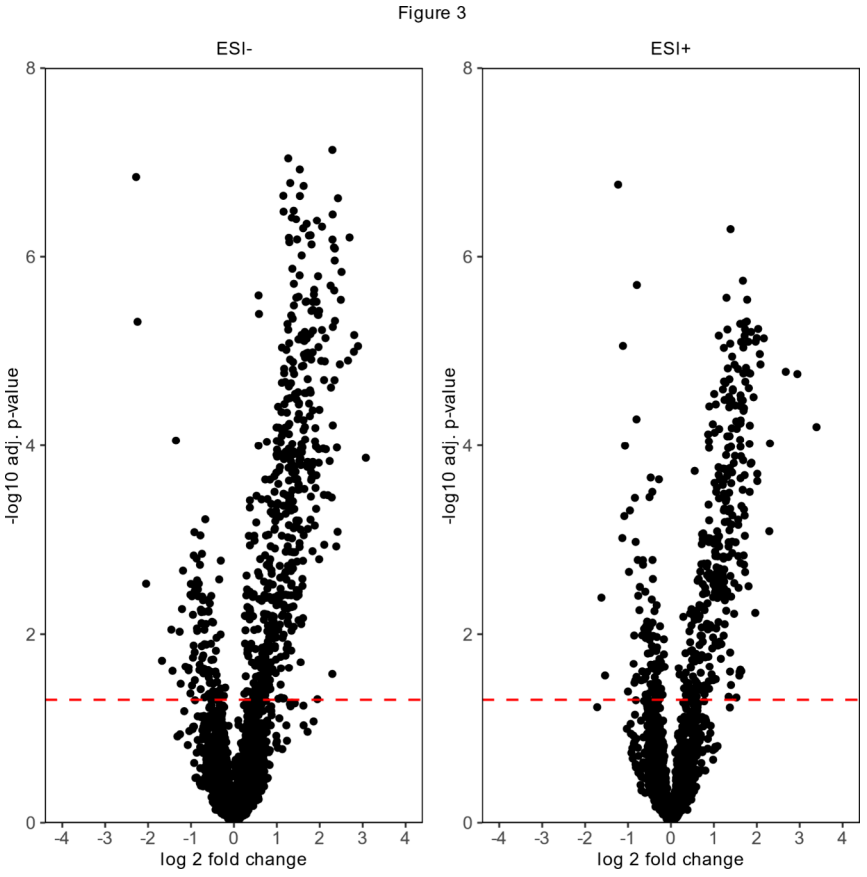


Figure 3. Volcano plots produced by estimating GLM models of ln(intensity) on a 0−1 variable equal to 1 for nurses and 0 for office workers, with Benjamini-Hochberg adjusted *p*-values. The *x*-axis shows the log 2-fold change (of nurses relative to office workers). The *y*-axis shows the negative log 10 of the adjusted *p*-value. Each point represents a feature. Points above the red dashed line have adjusted *p*-values that are below 0.05. $N = 60$ nurses and 40 office workers.

μg/m3 based on a screening level oral RfD of 0.00039 mg/kg-day, and EPA setting a slightly lower oral RfD.[25] Toxicological effects discussed in this assessment include immune system, liver, cardiac, kidney, thyroid, and body weight, as well as developmental toxicity.

We tentatively identified a chemical that has diverse sources − tridecanedioic acid also known as brassylic acid. This chemical was detected more frequently in nurses (Figure 4). It is used in the synthesis of polymers, especially nylon 1313, food packaging, biological solvents, lubricants, glues, as well as flavors and fragrances.[27] Maternal serum levels have been reported to be associated with increased risk of gestational diabetes and pregnancy hypertension.[28] Toxicity testing data are limited, though no effects were reported in a repeated dose study or in a screening study of effects on reproduction and development.[29]

We confirmed one chemical that may be used in disinfectants or antibacterial or antifungal medications − salicylic acid − but were not able to confirm the annotation for a second chemical in this category − 4-hydroxyquinoline. Nurses had higher mean intensities of both chemicals, as well as a higher 95th percentile intensity of salicylic acid compared to office workers (Figure 4). Salicylic acid is a precursor to and metabolite of aspirin (acetylsalicylic acid). It has medical uses to chemically exfoliate skin and is often found as an ingredient in antiseptic skin and acne washes.[30] It was not included in one source that lists chemical disinfectants used in health care settings,[31] but is included in a paper discussing the disinfecting of SARS-CoV-2.[32]

Quinolines as a class appear to be used or considered for use as antibacterial and antifungal agents,[33] however it was difficult to find EPA registration for this use, or any detailed use or toxicity information about 4-hydroxyquinoline. It is possible that 4-hydroxyquinoline is an impurity or metabolite of another chemical used in the hospital. Quinoline is considered likely to be carcinogenic by EPA.[34]

We also found that nurses had higher levels of two confirmed chemicals that have been used as medications. Nurses had higher mean intensities of theophylline (Figure 4), which has been used historically as a bronchodilator used to prevent and treat wheezing, shortness of breath, and chest tightness caused by asthma, chronic bronchitis, emphysema, and other lung diseases.[35] However, given the low frequency of use of theophylline as a medication for treatment in the hospital setting, other sources of this compound must be considered. Theophylline is also a metabolite of caffeine, and so the higher levels in nurses may reflect greater caffeine intake.[36] Nurses also had higher 95th percentile abundances and detection frequencies of acetaminophen (aka Tylenol) (Figure 4), which is commonly used to control pain.

We were not able to confirm the annotation for dicyclohexyl phthalate. Nurses have higher mean intensities of this chemical compared to office workers (Figure 4). This compound is used as a plasticizer in adhesives, plastics, rubber products, and resins and is included among EPA's high priority risk evaluations to be done under the Toxics Substances Control Act.[37] EPA reports that production volume is between 500 thousand and 1 million

https://doi.org/10.1021/acs.est.4c14790
Environ. Sci. Technol. 2025, 59, 9437−9448

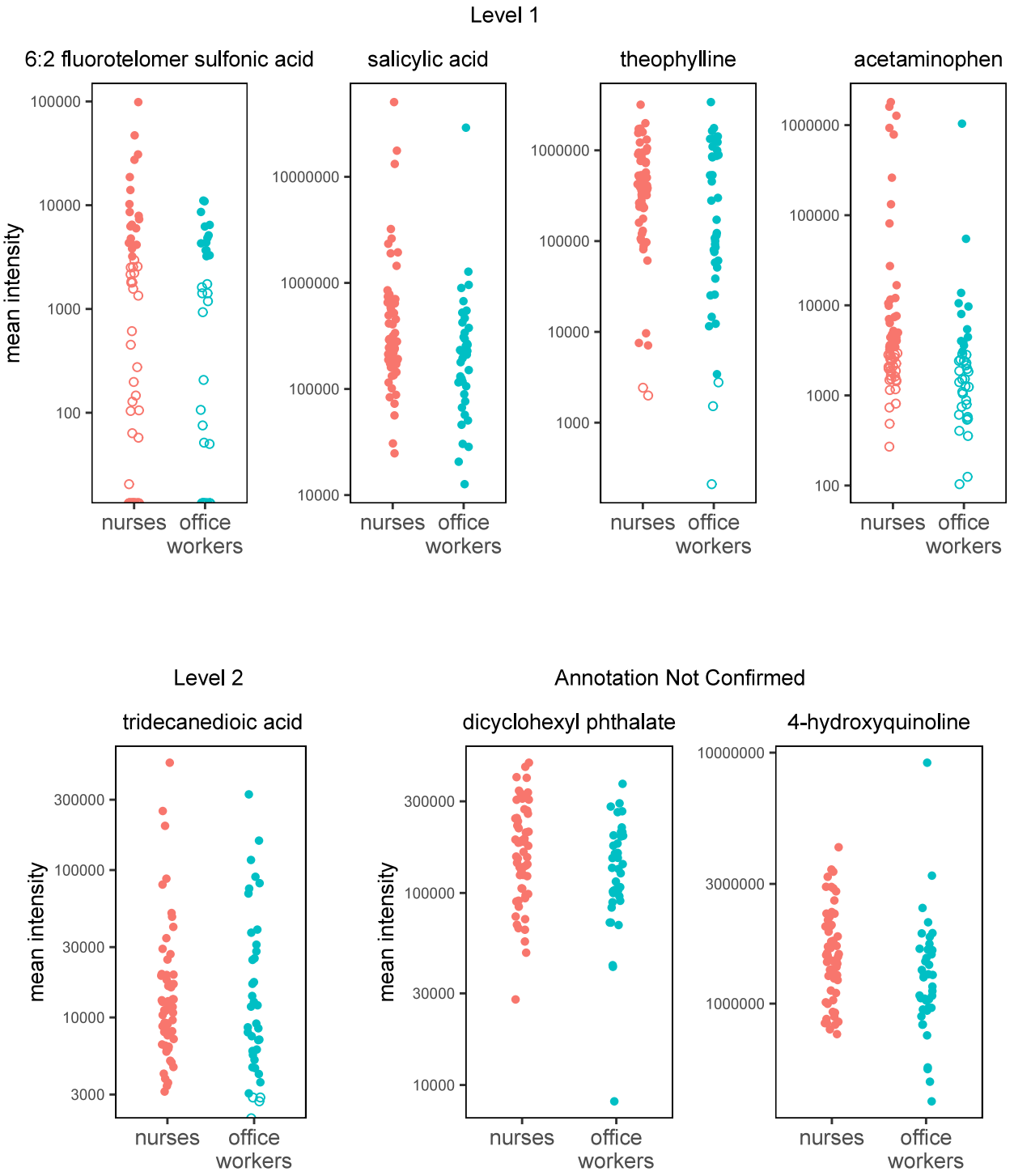


Figure 4. Panels above show nurse and office worker abundances for MS1 features that were prioritized for having higher detection frequencies or higher mean or 95th percentile abundances in nurses relative to office workers and were either confirmed or tentatively identified as environmental chemicals of occupational relevance to nurses.

pounds/year. A diverse set of end points will be considered in EPA's evaluation including thyroid and other endocrine-related effects, since studies have shown reproduction and development effects that are consistent with other phthalates that have been more restricted.[38,39] The US Consumer Products Safety Commission restricts amounts of this and other phthalates in children's toys.[40]

We confirmed two Level 1 chemicals − caffeine and piperine - and tentatively identified several other chemicals that have dietary sources (Figure S3). Nurses had higher 95th percentile intensity of alpha-hydroxyhippuric acid, which is a hippuric acid, an organic compound found in urine and produced by body's metabolism of foods with high polyphenolic content, primarily fruit and vegetables.[41] Nurses also had a higher mean intensity and a higher detection frequency for eicosapentaenoic acid. This is an omega-3 fatty acid with dietary sources that include cold-water fatty fish and fish oil supplements.[42] Nurses also had higher mean and 95th percentile intensity as well as a higher

https://doi.org/10.1021/acs.est.4c14790
Environ. Sci. Technol. 2025, 59, 9437−9448

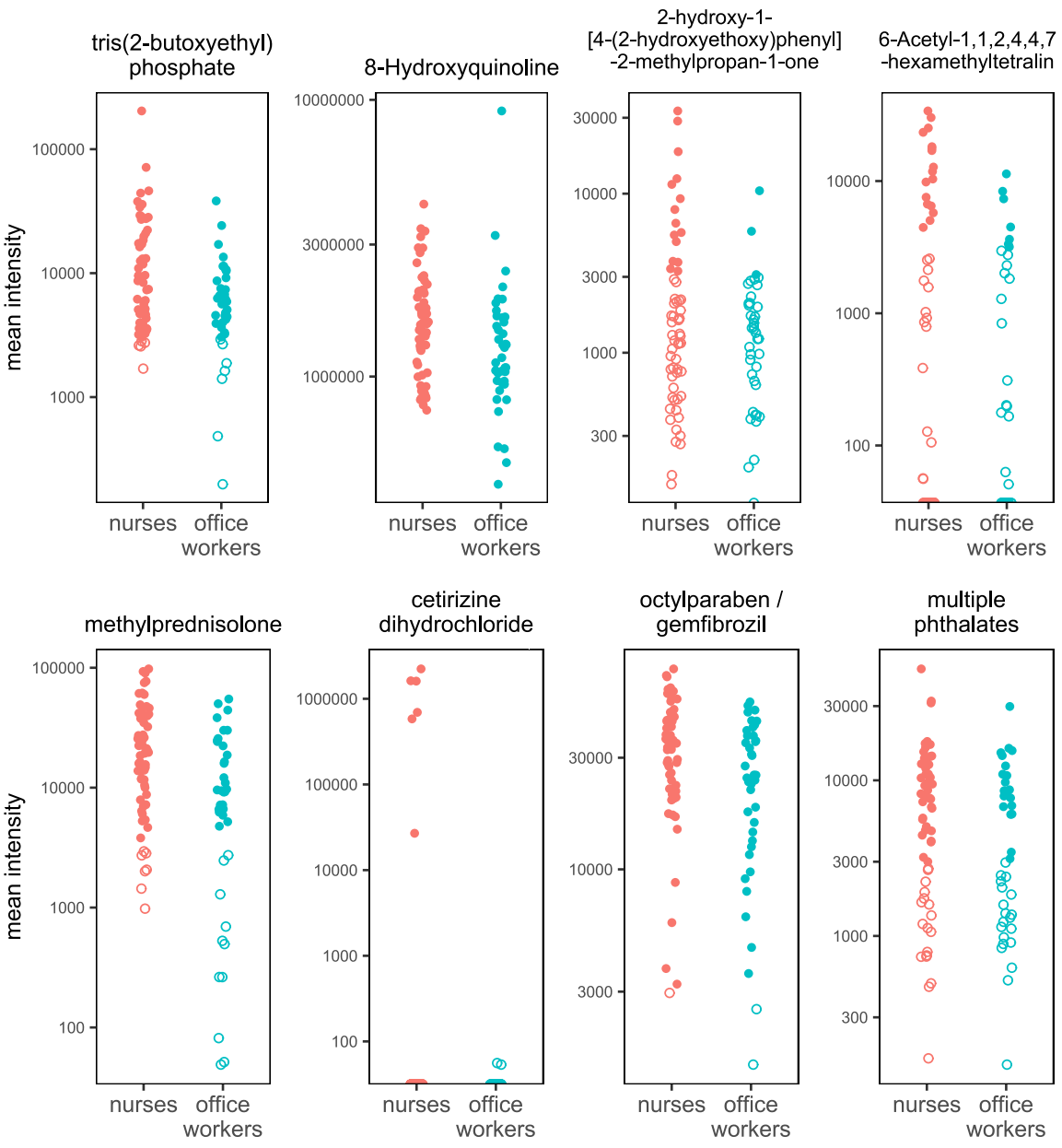


Figure 5. Panels above show nurse and office worker abundances for some of the MS1 features that were prioritized for having higher detection frequencies or higher mean or 95th percentile abundances in nurses relative to office workers and were tentatively identified as Level 2 without spectra environmental chemicals of occupational relevance to nurses. (See also Tables S8−S9 for the full set of Level 2 without spectra tentative identifications.)

detection frequency of 5-HEPE, which is a metabolite of eicosapentaenoic acid.[43] Nurses had higher mean intensity of caffeine, as well as paraxanthine, also known as 1,7-dimethylxanthine, which is a metabolite of theophylline and theobromine, two well-known stimulants found in coffee, tea, and chocolate mainly in the form of caffeine.[36] Nurses have a higher 95th percentile intensity of tryptophan, which is an essential amino acid that comes from poultry consumption, but also has a microbial metabolite in the intestine that has been associated with adverse health effects.[44] Nurses had higher intensities of piperine, an alkaloid present in black pepper.[45]

We tentatively identified two potentially endogenous chemicals (Figure S4). Nurses had a higher mean intensity of 9-HODE, a fatty acid/potential endogenous ligand that is associated with oxidative stress and inflammation.[46,47] Nurses had higher mean intensity of 5,12-DiHETE, a lipid.

From the MS1 data on mixtures of standards, we were able to tentatively confirm at Level 2 (without MS2 spectra) 20 additional chemicals, including a flame retardant and plasticizer (tris(2-butoxyethyl) phosphate),[48] an antibacterial and antifungal agent (8-hydroxyquinoline),[49] fragrance ingredients (6-Acetyl-1,1,2,4,4,7-hexamethyltetralin, also known as tonalide,[50] and 4-(4-Hydroxyphenyl)butan-2-one),[51] the aromatic ketone 2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methylpropan-1-one,[52] an insect repellant (icaridin), and a number of medications, including the steroid medications methylprednisolone and prednisolone and the allergy medication cetirizine dihydrochloride (active ingredient in Zyrtec) (Tables S8−S9, Figure 5). In a few cases, a MS1 feature in our nurse and office worker data matched to more than one MS1 feature from the standards mixtures. For example, one feature matched to diethyl phthalate, mono-n-butyl phthalate, and monoisobutyl phthalate,

https://doi.org/10.1021/acs.est.4c14790
Environ. Sci. Technol. 2025, 59, 9437−9448

while another matched to both octylparaben and gemfibrozil (a cholesterol medication). These cases of one to multiple matching are due to multiple chemicals in the standards mixtures having identical chemical formula and m/z.

Strengths of This Approach. To our knowledge, this is the first occupational study to apply a discovery-driven approach using NTA to characterize novel workplace-related chemical exposures in female nurses and office workers. The study design had three key strengths compared with other applications of non-targeted analysis in biomonitoring. First, the ability to compare features in serum between nurses and office workers who were otherwise similar in demographics, age, sex, geographic location (Table 1), and time of collection enhanced the viability of the NTA approach for discovering novel workplace-specific exposures. Second, using NTA to detect workplace exposures, which tend to be higher than those in the general population, partially mitigates the lower sensitivity of full scan mass spec methods. Using pooled samples from the same cohort run in MS/MS mode facilitated our ability to annotate features of interest in the nurses. Third, spectral databases have been primarily developed to focus on annotating endogenous metabolites and drugs. Since pharmaceuticals are an important potential exposure for nurses, these exposures are more likely to be confirmed compared with other common environmental chemicals.

An important element of our approach to identifying features enriched in nurses compared with office workers was that we used three different comparisons: for frequently detected features we compared means, for infrequently detected features we compared detection frequencies, and for most features we also compared the 95th percentile of the distribution to identify exposures that were high for a fraction of the cohort. This approach paid off, in fact of the 7 occupationally related exposures, 5 passed gate 1, 3 passed gate 2, and 1 passed gate 3, and only 2 passed two gates and none passed all three gates. We would have missed important exposures if we had not used all three of these approaches.

This NTA approach tentatively identified several novel compounds potentially related to workplace exposures among nurses, including an unregulated but toxic PFAS chemical. These findings support future work to identify sources of exposure, seek less toxic substitutes, and implement other exposure reduction strategies.

Limitations and Future Work. Several challenges limit what we were able to learn from this occupational NTA study. The sensitivity of instruments used in NTA (such as QTOFs) is lower for any given analyte compared to instruments used in targeted methods (triple quadrupole mass spectrometers). However, QTOFs are necessary for NTA due to their high mass accuracy across a wide range of masses. Consequently, we are likely missing potential chemical exposures with lower abundances that may not be detectable using NTA but still be potentially harmful to health. Figure 3 shows that there are more features elevated in nurses compared with office workers, but most of them were not able to be identified. Lack of commercially available analytical standards from industrial producers of these compounds,[53,54] and spectral databases for environmental chemicals is another important barrier to expanding the chemicals we can annotate, since Level 2 annotation/identification requires matching spectra from test samples to a spectral standard. DDA MS/MS approaches may not be ideally suited for NTA of environmental chemicals, since the DDA approach will tend to fragment larger abundance peaks, but these are more likely to be of endogenous or dietary origins, while environmental compounds may have lower abundances. Our modest sample size may have limited our ability to observe some occupational chemical exposures that are uniquely experienced by nurses. Furthermore, although the occupational exposures of male nurses would presumably be the same as those of female nurses, it is possible that levels of some chemicals in female nurses may potentially be lower or higher than in male nurses, in part due to differences in metabolism between men and women, including excretion pathway differences due to the binding affinity of certain compounds, including PFAS, with fatty acid-biding proteins of the blood which can increase excretion of chemicals during menstruation.[55,56] Because we did not include men in our study, we are unable to speculate further on how the pharmacokinetics of chemical exposures and their metabolites might differ between male and female nurses. Finally, we were only able to do batch correction for the more highly detected features, due to missing data in less detected features, which prevented us from imputing values, a necessary prerequisite to batch correction. Consequently, we only used batch-corrected data for comparing nurse and office worker mean intensities, and not for the comparisons of 95th percentile intensities or detection frequencies. Despite these limitations, NTA provides an important complement to targeted analyses, for identifying novel chemical exposures, while targeted methods have greater sensitivity.

Using NTA, we showed many features with higher intensities in nurses versus office workers and we tentatively identified nurses' workplace exposures to a novel PFAS, a common and hazardous phthalate, a ubiquitous commercial chemical, as well as several disinfectants and drugs.

If more sensitive analytical approaches for NTA were developed, we might detect more medication-related or other workplace exposures in nurses. However, NTA can viably complement and augment the limitations of targeted analytic methods that require *a priori* selection of chemicals to study, which can be limited by a lack of information about where, how and the extent to which chemicals are used in occupational settings and other sources of exposure.

The NTA of these biomonitoring samples allowed us to detect thousands of features − including many that are higher in nurses − but only confirm a small number. Annotation and identification would be possible for more features if spectral databases were available for a larger fraction of environmental chemicals. For example, approximately 2% of chemicals on the EPA dashboard have analytical standards available, limiting the chemical space for which features can be matched on spectra and confirmed with a standard.[57] One way to strengthen NTA for environmental samples would be for environmental regulations to systematically require analytical standards for all commercially used chemicals. Right now, making standards commercially available is only required for pesticides and pharmaceuticals.

Our findings can be leveraged to inform policy changes, including eliminating the use of PFAS- and phthalate-containing products to reduce occupational exposures among nurses. Our finding of exposure to disinfectants and drugs in nurses may support use of least toxic cleaning products and enhanced use of personal protective equipment to reduce exposure to medications.

https://doi.org/10.1021/acs.est.4c14790
Environ. Sci. Technol. 2025, 59, 9437−9448

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.4c14790.

PCA figures showing impact of batch correction, confirmed and tentative identifications from dietary sources, tentative identification of endogenous molecules, evaluation of level 2 features against standards (PDF)

Tables of instrument parameters, list of spiked compounds for QC, data processing parameters specified in MS-DIAL, summary statistics for all non-noisy features, manual curation files, MS-DIAL output (XLSX)

■ AUTHOR INFORMATION

Corresponding Authors

Kristin E. Knox − *Silent Spring Institute, Newton, Massachusetts 02460, United States;* orcid.org/0000-0001-6425-8914; Email: knox@silentspring.org

Rachel Morello-Frosch − *Department of Environmental Science, Policy and Management, and School of Public Health, University of California, Berkeley, California 94720, United States;* orcid.org/0000-0003-1153-7287; Email: rmf@berkeley.edu

Authors

Dimitri Abrahamsson − *Program on Reproductive Health and the Environment, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, California 94143, United States;* orcid.org/0000-0002-3402-7565

Jessica Trowbridge − *Program on Reproductive Health and the Environment, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, California 94143, United States;* orcid.org/0000-0001-9414-087X

June-Soo Park − *Environmental Chemistry Laboratory, Department of Toxic Substances Control, California Environmental Protection Agency, Berkeley, California 94710, United States;* orcid.org/0009-0005-2177-4405

Miaomiao Wang − *Environmental Chemistry Laboratory, Department of Toxic Substances Control, California Environmental Protection Agency, Berkeley, California 94710, United States;* orcid.org/0000-0002-8627-0073

Erin Carrera − *Department of Nursing, University of San Francisco, San Francisco, California 94143, United States; California Nurses for Environmental Health & Justice, Bolinas, California 94924, United States*

Lisa Hartmayer − *Department of Nursing, University of San Francisco, San Francisco, California 94143, United States; California Nurses for Environmental Health & Justice, Bolinas, California 94924, United States*

R. A. Rudel − *Silent Spring Institute, Newton, Massachusetts 02460, United States;* orcid.org/0000-0002-1809-4127

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.est.4c14790

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was funded by the California Breast Cancer Research Program (grant numbers 23BB-1700 and B29BI7737).

■ REFERENCES

(1) Lawson, C. C.; Whelan, E. A.; Hibert, E. N.; Grajewski, B.; Spiegelman, D.; Rich-Edwards, J. W. Occupational Factors and Risk of Preterm Birth in Nurses. *Am. J. Obstet Gynecol* **2009**, *200* (1), 51.e1.

(2) Gaskins, A. J.; Chavarro, J. E.; Rich-Edwards, J. W.; Missmer, S. A.; Laden, F.; Henn, S. A.; Lawson, C. C. Occupational Use of High-Level Disinfectants and Fecundity among Nurses. *Scand J. Work Environ. Health* **2017**, *43* (2), 171−180.

(3) Dumas, O.; Varraso, R.; Zock, J. P.; Henneberger, P. K.; Speizer, F. E.; Wiley, A. S.; Le Moual, N.; Camargo, C. A., Jr Asthma History, Job Type and Job Changes among US Nurses. *Occup Environ. Med.* **2015**, *72* (7), 482−8.

(4) Le Moual, N.; Varraso, R.; Zock, J. P.; Henneberger, P.; Speizer, F. E.; Kauffmann, F.; Camargo, C. A., Jr Are Operating Room Nurses at Higher Risk of Severe Persistent Asthma? The Nurses' Health Study. *J. Occup Environ. Med.* **2013**, *55* (8), 973−7.

(5) Institute of Medicine Committee on Enhancing Environmental Health Content in Nursing Practice, Environmental Hazards for the Nurse as a Worker. In *Nursing Health, & Environment: Strengthening the Relationship to Improve the Public's Health*, Pope, A. M., Snyder, M. A., Mood, L. H., Eds. National Academies Press (US): Washington (DC), 1995.

(6) Shen, C. C.; Hu, Y. W.; Hu, L. Y.; Perng, C. L.; Su, T. P.; Teng, C. J.; Yen, S. H.; Tzeng, C. H.; Chiou, T. J.; Yeh, C. M.; Chen, T. J.; Wang, W. S.; Chen, P. M.; Liu, C. J. The Risk of Cancer among Taiwanese Female Registered Nurses: A Nationwide Retrospective Study. *PLoS One* **2013**, *8* (7), No. e68420.

(7) Kjaer, T. K.; Hansen, J. Cancer Incidence among Large Cohort of Female Danish Registered Nurses. *Scand J. Work Environ. Health* **2009**, *35* (6), 446−53.

(8) Lie, J. A.; Andersen, A.; Kjaerheim, K. Cancer Risk among 43000 Norwegian Nurses. *Scand J. Work Environ. Health* **2007**, *33* (1), 66−73.

(9) Rosa, D.; Terzoni, S.; Dellafiore, F.; Destrebecq, A. Systematic Review of Shift Work and Nurses' Health. *Occup Med. (Lond)* **2019**, *69* (4), 237−243.

(10) Li, X.; Hu, Y.; Aslanbeigi, F. Genetic and Epigenetic Alterations in Night Shift Nurses with Breast Cancer: A Narrative Review. *Cancer Cell Int.* **2025**, *25* (1), 20.

(11) Lie, J. A. S.; Kjærheim, K. Cancer Risk among Female Nurses: A Literature Review. *European Journal of Cancer Prevention* **2003**, *12* (6), 517−526.

(12) Ohayon, J. L.; Rasanayagam, S.; Rudel, R. A.; Patton, S.; Buren, H.; Stefani, T.; Trowbridge, J.; Clarity, C.; Brody, J. G.; Morello-Frosch, R. Translating Community-Based Participatory Research into Broad-scale Sociopolitical Change: Insights from a Coalition of Women Firefighters, Scientists, and Environmental Health Advocates. *Environ. Health* **2023**, *22* (1), 60.

(13) Malm, L.; Palm, E.; Souihi, A.; Plassmann, M.; Liigand, J.; Kruve, A. Guide to Semi-Quantitative Non-Targeted Screening Using LC/ESI/HRMS. *Molecules* **2021**, *26* (12), 3524.

(14) Tsugawa, H.; Cajka, T.; Kind, T.; Ma, Y.; Higgins, B.; Ikeda, K.; Kanazawa, M.; VanderGheynst, J.; Fiehn, O.; Arita, M. MS-DIAL: Data-Independent MS/MS Deconvolution for Comprehensive Metabolome Analysis. *Nat. Methods* **2015**, *12* (6), 523−6.

(15) Hattaway, M. E.; Black, G. P.; Young, T. M. Batch Correction Methods for Nontarget Chemical Analysis Data: Application to a Municipal Wastewater Collection System. *Anal Bioanal Chem.* **2023**, *415* (7), 1321−1331.

(16) Leek, J. T.; Johnson, W. E.; Parker, H. S.; Jaffe, A. E.; Storey, J. D. The Sva Package for Removing Batch Effects and Other Unwanted Variation in High-Throughput Experiments. *Bioinformatics* **2012**, *28* (6), 882−3.

(17) Benjamini, Y.; Hochberg, Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society: Series B (Methodological)* **1995**, *57* (1), 289−300.

(18) MS-DIAL, *MS-DIAL Metabolomics MSP Spectral Kit Containing EI-MS, MS/MS, and CCS Values*. 2024.

(19) *MoNA - MassBank of North America, Mass Spectral Repository*. 2024.
(20) Schymanski, E. L.; Jeon, J.; Gulde, R.; Fenner, K.; Ruff, M.; Singer, H. P.; Hollender, J. Identifying Small Molecules Via High Resolution Mass Spectrometry: Communicating Confidence. *Environ. Sci. Technol.* **2014**, *48* (4), 2097−8.
(21) Lu, M.; Cagnetta, G.; Zhang, K.; Huang, J.; Yu, G. Mechanochemical Mineralization of ″Very Persistent″ Fluorocarbon Surfactants - 6:2 Fluorotelomer Sulfonate (6:2FTS) as an Example. *Sci. Rep* **2017**, *7* (1), 17180.
(22) Méndez, V.; Holland, S.; Bhardwaj, S.; McDonald, J.; Khan, S.; O'Carroll, D.; Pickford, R.; Richards, S.; O'Farrell, C.; Coleman, N.; Lee, M.; Manefield, M. J. Aerobic Biotransformation of 6:2 Fluorotelomer Sulfonate by Dietzia Aurantiaca J3 under Sulfur-Limiting Conditions. *Sci. Total Environ.* **2022**, *829*, 154587.
(23) Chemours; *Capstone Fluorosurfactants Product Guide*. https://www.chemours.com/en/-/media/files/capstone/capstone-surfactants-brochure.pdf?rev=983b02234dd54159bd807f40b97aa762&hash=E0929BCDCF885450B70EC05679CC0B97 (accessed 2024−12−30).
(24) Glüge, J.; Scheringer, M.; Cousins, I. T.; DeWitt, J. C.; Goldenman, G.; Herzke, D.; Lohmann, R.; Ng, C. A.; Trier, X.; Wang, Z. An Overview of the Uses of Per- and Polyfluoroalkyl Substances (PFAS). *Environ. Sci. Process Impacts* **2020**, *22* (12), 2345−2373.
(25) U.S. Environmental Protection Agency; *ORD Human Health Toxicity Value Assessment for 6:2 Fluorotelomer Sulfonic Acid (CASRN 27619−97−2 | DTXSID6067331)*. https://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=363720&Lab=CPHEA (12/30/24).
(26) Michigan Department of Environment Great Lakes and Energy; *File for 6:2 Fluorotelomer Sulfonic Acid (CAS No. 27619−97−2)* https://www.egle.state.mi.us/aps/downloads/ATSL/27619-97-2/27619-97-2_annual_ITSL.pdf (accessed 2024−12−30).
(27) ChemBK; *Tridecanedioic Acid*. https://www.chembk.com/en/chem/Tridecanedioic%20acid (accessed 2024−12−30).
(28) Trowbridge, J.; Abrahamsson, D.; Bland, G. D.; Jiang, T.; Wang, M.; Park, J. S.; Morello-Frosch, R.; Sirota, M.; Lee, H.; Goin, D. E.; Zlatnik, M. G.; Woodruff, T. J. Extending Nontargeted Discovery of Environmental Chemical Exposures during Pregnancy and Their Association with Pregnancy Complications-a Cross-Sectional Study. *Environ. Health Perspect* **2023**, *131* (7), 077003.
(29) ECHA Chemicals Database; *Tridecanedioic Acid*. https://chem.echa.europa.eu/100.007.284/overview?searchText=brassylic%20acid (accessed 2024−12−30).
(30) Mayo Clinic; *Salicylic Acid (Topical Route)*. https://www.mayoclinic.org/drugs-supplements/salicylic-acid-topical-route/description/drg-20066030 (accessed 2024−12−30).
(31) Exner, M.; Bhattacharya, S.; Gebel, J.; Goroncy-Bermes, P.; Hartemann, P.; Heeg, P.; Ilschner, C.; Kramer, A.; Ling, M. L.; Merkens, W.; Oltmanns, P.; Pitten, F.; Rotter, M.; Schmithausen, R. M.; Sonntag, H. G.; Steinhauer, K.; Trautmann, M. Chemical Disinfection in Healthcare Settings: Critical Aspects for the Development of Global Strategies. *GMS Hyg Infect Control* **2020**, *15*, Doc36.
(32) Xiao, S.; Yuan, Z.; Huang, Y. Disinfectants against SARS-CoV-2: A Review. *Viruses* **2022**, *14* (8), 1721.
(33) New Jersey Department of Health; *Hazardous Substance Fact Sheet - Quinoline*. https://nj.gov/health/eoh/rtkweb/documents/fs/1628.pdf (accessed 2024−12−30).
(34) U.S. Environmental Protection Agency; *Quinoline; CASRN 91−22−5 Integrated Risk Information System (IRIS) Chemical Assessment Summary*.
(35) Mayo Clinic; *Theophylline (Oral Route)*. https://www.mayoclinic.org/drugs-supplements/theophylline-oral-route/description/drg-20073599 (accessed 2024−12−30).
(36) Purkiewicz, A.; Pietrzak-Fiećko, R.; Sörgel, F.; Kinzig, M. Caffeine, Paraxanthine, Theophylline, and Theobromine Content in Human Milk. *Nutrients* **2022**, *14* (11), 2196.
(37) U.S. Environmental Protection Agency; *Risk Evaluation for Dicyclohexyl Phthalate*. https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-evaluation-dicyclohexyl-phthalate#background (accessed 2024−12−30).
(38) U.S. Environmental Protection Agency - Office of Chemical Safety and Pollution Prevention; *Final Scope of the Risk Evaluation for Dicyclohexyl Phthalate (1,2- Benzenedicarboxylic Acid, 1,2-Dicyclohexyl Ester) CASRN 84−61−7* 2020.
(39) United States Consumer Product Safety Commission; *Toxicity Review of Dicyclohexyl Phthalate (DCHP)*; 2010.
(40) United States Consumer Product Safety Commission; *CPSC Prohibits Certain Phthalates in Children's Toys and Child Care Products*. https://www.cpsc.gov/Newsroom/News-Releases/2018/CPSC-Prohibits-Certain-Phthalates-in-Childrens-Toys-and-Child-Care-Products (accessed 2024−12−30).
(41) Ticinesi, A.; Guerra, A.; Nouvenne, A.; Meschi, T.; Maggi, S. Disentangling the Complexity of Nutrition, Frailty and Gut Microbial Pathways during Aging: A Focus on Hippuric Acid. *Nutrients* **2023**, *15* (5), 1138.
(42) Mount Sinai; *Eicosapentaenoic Acid (EPA)*. https://www.mountsinai.org/health-library/supplement/eicosapentaenoic-acid-epa#:~:text=Dietary%20Sources,sardines%2C%20shellfish%2C%20and%20herring. (accessed 2024−12−30).
(43) Onodera, T.; Fukuhara, A.; Shin, J.; Hayakawa, T.; Otsuki, M.; Shimomura, I. Eicosapentaenoic Acid and 5-HEPE Enhance Macrophage-Mediated Treg Induction in Mice. *Sci. Rep* **2017**, *7* (1), 4560.
(44) Roager, H. M.; Licht, T. R. Microbial Tryptophan Catabolites in Health and Disease. *Nat. Commun.* **2018**, *9* (1), 3294.
(45) Derosa, G.; Maffioli, P.; Sahebkar, A. Piperine and Its Role in Chronic Diseases. *Adv. Exp. Med. Biol.* **2016**, *928*, 173−184.
(46) Wang, L.; Gill, R.; Pedersen, T. L.; Higgins, L. J.; Newman, J. W.; Rutledge, J. C. Triglyceride-Rich Lipoprotein Lipolysis Releases Neutral and Oxidized Ffas That Induce Endothelial Cell Inflammation. *J. Lipid Res.* **2009**, *50* (2), 204−13.
(47) Vangaveti, V.; Baune, B. T.; Kennedy, R. L. Hydroxyoctadecadienoic Acids: Novel Regulators of Macrophage Differentiation and Atherogenesis. *Ther Adv. Endocrinol Metab* **2010**, *1* (2), 51−60.
(48) National Center for Biotechnology Information; *Pubchem Compound Summary for CID 6540, Tris(2-Butoxyethyl) Phosphate*. https://pubchem.ncbi.nlm.nih.gov/compound/Tris_2-butoxyethyl_-phosphate (accessed 2025−04−02).
(49) Saadeh, H. A.; Sweidan, K. A.; Mubarak, M. S. Recent Advances in the Synthesis and Biological Activity of 8-Hydroxyquinolines. *Molecules* **2020**, *25* (18), 4321.
(50) National Center for Biotechnology Information; *Pubchem Compound Summary for CID 89440, Tonalide*. https://pubchem.ncbi.nlm.nih.gov/compound/Tonalide (accessed 2025−04−02).
(51) National Center for Biotechnology Information; *Pubchem Compound Summary for CID 21648, Raspberry Ketone*. https://pubchem.ncbi.nlm.nih.gov/compound/Raspberry-Ketone (accessed 2025−04−02).
(52) National Center for Biotechnology Information; *Pubchem Compound Summary for CID 86266, 1-Propanone, 2-Hydroxy-1-[4-(2-Hydroxyethoxy)Phenyl]-2-Methyl-*. https://pubchem.ncbi.nlm.nih.gov/compound/86266 (accessed 2025−04−02).
(53) Trier, X.; van-Leeuwen, S. P. J.; Brambilla, G.; Weber, R.; Webster, T. F. The Critical Role of Commercial Analytical Reference Standards in the Control of Chemical Risks: The Case of PFAS and Ways Forward. *Environ. Health Perspect* **2025**, *133* (1), 015001.
(54) Kannan, K. Invited Perspective: Reference Standards Are Key to Environmental and Human Health Research-the Case of PFAS. *Environ. Health Perspect* **2025**, *133* (1), 011301.
(55) Zhang, Y.; Beesoon, S.; Zhu, L.; Martin, J. W. Biomonitoring of Perfluoroalkyl Acids in Human Urine and Estimates of Biological Half-Life. *Environ. Sci. Technol.* **2013**, *47* (18), 10619−27.
(56) Harada, K.; Inoue, K.; Morikawa, A.; Yoshinaga, T.; Saito, N.; Koizumi, A. Renal Clearance of Perfluorooctane Sulfonate and

Perfluorooctanoate in Humans and Their Species-Specific Excretion. *Environ. Res.* **2005**, *99* (2), 253−61.
(57) Nuñez, J. R.; Colby, S. M.; Thomas, D. G.; Tfaily, M. M.; Tolic, N.; Ulrich, E. M.; Sobus, J. R.; Metz, T. O.; Teeguarden, J. G.; Renslow, R. S. Evaluation of in Silico Multifeature Libraries for Providing Evidence for the Presence of Small Molecules in Synthetic Blinded Samples. *J. Chem. Inf Model* **2019**, *59* (9), 4052−4060.

https://doi.org/10.1021/acs.est.4c14790
Environ. Sci. Technol. 2025, 59, 9437−9448