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Article

Integrating Exposure Knowledge and Serum Suspect Screening as a New Approach to Biomonitoring: An Application in Firefighters and Office Workers

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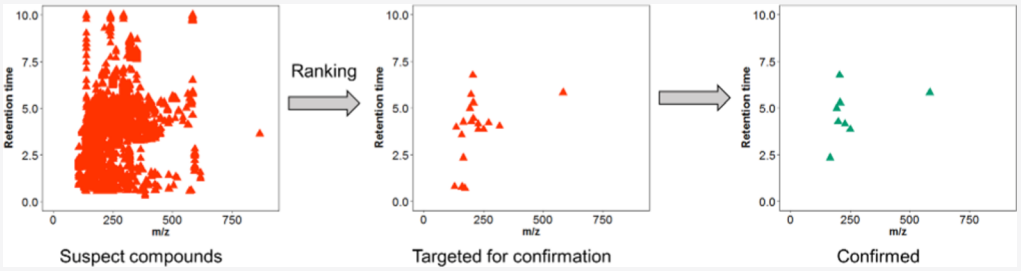

ABSTRACT: Firefighters (FF) are exposed to recognized and probable carcinogens, yet there are few studies of chemical exposures and associated health concerns in women FFs, such as breast cancer. Biomonitoring often requires a priori selection of compounds to be measured, and so, it may not detect relevant, lesser known, exposures. The Women FFs Biomonitoring Collaborative (WFBC) created a biological sample archive and conducted a general suspect screen (GSS) to address this data gap. Using liquid chromatography−quadrupole time-of-flight tandem mass spectrometry, we sought to identify candidate chemicals of interest in serum samples from 83 women FFs and 79 women office workers (OW) in San Francisco. We identified chemical peaks by matching accurate mass from serum samples against a custom chemical database of 722 slightly polar phenolic and acidic compounds, including many of relevance to firefighting or breast cancer etiology. We then selected tentatively identified chemicals for confirmation based on the following criteria: (1) detection frequency or peak area differences between OW and FF; (2) evidence of mammary carcinogenicity, estrogenicity, or genotoxicity; and (3) not currently measured in large biomonitoring studies. We detected 620 chemicals that matched 300 molecular formulas in the WFBC database, including phthalate metabolites, phosphate flame-retardant metabolites, phenols, pesticides, nitro and nitroso compounds, and per- and polyfluoroalkyl substances. Of the 20 suspect chemicals selected for validation, 8 were confirmed—including two alkylphenols, ethyl paraben, BPF, PFOSAA, benzophenone-3, benzyl *p*-hydroxybenzoate, and triphenyl phosphate—by running a matrix spike of the reference standards and using m/z, retention time, and the confirmation of at least two fragment ions as criteria for matching. GSS provides a powerful high-throughput approach to identify and prioritize novel chemicals for biomonitoring and health studies.

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INTRODUCTION

Firefighters (FFs) are exposed to complex and variable chemical mixtures that include known carcinogens. In addition to exposures during fire suppression activities,[1−5] FFs pick up chemical exposures from their equipment, such as fire extinguishing foams or protective gear,[6,7] and also from automotive diesel.[8] These compounds include benzene, polycyclic aromatic hydrocarbons (PAHs), nitro-PAHs, formaldehyde, dioxins, flame retardants, polychlorinated biphenyls, and per- and polyfluorinated substances (PFAS).[9−12] These chemicals are associated with a wide range of cancers and other health effects in human and experimental animal studies, and it is noteworthy that many of these exposures have been identified as potential breast carcinogens either because they cause mammary gland tumors in laboratory animals or because they alter mammary gland development.[13,14]

Research examining the chemical exposures and health risks faced by FFs, and women FFs in particular, is limited. A 2015 study conducted by the National Institute for Occupational Safety and Health (NIOSH) on 19,309 male US FFs observed positive associations between the total time spent at fires and lung cancer incidence and mortality, and between the total number of response to fires and leukemia mortality from 1950 to 2009.[15] An earlier report from this NIOSH cohort that included 991 women showed nonsignificant increases in breast

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cancer incidence and mortality in both men and women, compared with the general US population; these increases were largest at younger ages (<65 for men, 50−55 for women).[16] Studies in multiple countries have also documented an elevated risk of certain cancers in male FFs and other first responders, including thyroid, bladder, kidney, prostate, testicular, breast, brain, and digestive cancers, multiple myeloma, and non-Hodgkin's lymphoma.[17−23] A metaanalysis of 32 studies determined an increased risk of certain cancers in the mostly male FF population.[24] Most studies do not calculate risks to female FFs; however, in a study on cancer incidence among Florida professional FFs, female FFs showed a significantly increased risk of cancer overall, as well as Hodgkin's lymphoma disease and thyroid cancer, compared with the Florida general population.[21] Although women make up 5.1% of FFs across the United States,[25] their numbers can be higher in urban jurisdictions, including San Francisco, which has one of the highest proportions of women FFs (15%).[26] In the US population, between ages 20 and 49, breast cancer is 6 times more common than any cancer in men,[27] and so, it is a priority to identify chemical exposures that may increase risk. As fire departments diversify and increase the number of women in their ranks, it is important to characterize chemical exposures and implications for health outcomes of particular relevance to women, such as breast cancer, that might not be addressed in existing studies, which have been primarily conducted among men.

Biomonitoring is an important tool in environmental and occupational health studies seeking to link health outcomes to chemical exposures. External measurements including air, dust, and water do not always reflect internal dose, and biomonitoring studies in human tissue can integrate over multiple routes of exposure including dermal, inhalation, and ingestion. One limitation of targeted biomonitoring studies is that they rely on a priori selection of chemicals for study, but it can be difficult to know which chemicals are present in occupational settings[28,29] and to predict metabolic transformations. As a result, significant time and resources may be expended to develop analytical methods to measure chemicals without knowing whether they are present in biological specimens. For example, 20% of the 250 chemicals biomonitored in NHANES since 1999 were not detected in 95% or more of the US population, indicating that the criteria for selecting chemicals for biomonitoring have not always identified chemicals with prevalent exposure.[30] Nontargeted approaches represent an important complement to these targeted methods in order to systematically identify a broader spectrum of environmental chemicals present in the human body; this strategy is now recognized as a critical component of an "exposome" approach.[31−33]

One way to characterize the human exposome is to perform nontargeted analysis of biospecimens using high-resolution mass spectrometry (MS) to detect as many molecules as possible. Then, one can match chemical mass, retention time (RT), and, in some cases, mass spectral information of molecules detected against a curated database of environmental chemicals of interest to identify chemical exposures in the study population. This approach is known as a general suspect screen (GSS). Recent applications of the GSS approach identified novel chemical exposures among pregnant women, including benzophenone-1 and bisphenol S.[34,35] Other more agnostic approaches match mass (and anything else) against much larger compound databases such as DSSTox, which currently includes over 875,000 compounds. This approach would tentatively identify a larger number of environmental chemicals, compared to matching against a more curated database, and would require additional data reduction. Both of these methods can be applied to chemicals with a range of physical−chemical properties depending on which analytical approaches are applied. For example, sample preparation methods, choice of liquid versus gas chromatography (GC), and positive or negative ionization will determine what types of chemicals will be detected.

To better understand how women FFs are exposed to potential breast carcinogens and other understudied chemicals, we undertook a community-based, participatory biomonitoring project, a partnership among FFs, environmental health scientists, and environmental health advocates, known as the Women FFs Biomonitoring Collaborative (WFBC). Our objectives were to develop a biospecimen archive of women FFs and women office workers (OWs) in San Francisco and to characterize exposures using both targeted and nontargeted methods in a cross-sectional chemical biomonitoring study. To achieve the second objective of the WFBC, we sought to identify novel chemical exposures by applying a discovery-driven GSS using high-resolution MS. Our goal is to demonstrate an agnostic approach for prioritizing candidate compounds for confirmation and targeted methods development in order to advance the discovery of novel environmental chemicals in human biomonitoring.

■ METHODS

Study Design. The WFBC was designed to measure and compare exposures to potential breast carcinogens and other endocrine-disrupting compounds in two occupational cohorts: women FFs and OWs from the City of San Francisco, California, and to create an archive of biological specimens for exposomic research. In this part of the study, GSS was performed on serum samples collected from female FFs and OWs, and accurate mass spectra were acquired using liquid chromatography−quadrupole time-of-flight MS (LC−QTOF/MS) operating in negative ionization mode. We used negative ionization mode to capture acidic or phenolic organic compounds of interest and looked for exact masses that matched a chemical in our database of 722 chemicals of interest based on their relevance to firefighting and breast cancer etiology. Accurate mass of each unique molecule (i.e., mass-to-charge ratio, m/z) generated by LC−QTOF/MS was matched to chemical formulas from our database. We then compared detection frequencies and peak areas of candidate compounds between FFs and OWs to identify those that might be work-related. We then systematically combined expert knowledge on the sources, uses, and toxicity of candidate compounds to prioritize and select a subset of tentatively matched chemicals for confirmation. Ultimately, we sought to demonstrate how GSS methods—in conjunction with expert knowledge in exposure science and toxicology—can be used to improve human biomonitoring by broadening the spectrum of potential environmental chemical exposures and prioritizing chemicals for confirmation by targeted analysis.

Recruitment and Consent. Women were eligible to participate in the WFBC study if they were over 18 years old, nonsmokers, and employees of the City and County of San Francisco (OWs) or the San Francisco Fire Department (FFs). In addition, FFs had to have been working active duty for at least 5 years with the Department. FFs were recruited through letters, emails, and phone calls that targeted FF organizations, including United Fire Service Women, Local 798 of the International Association of FFs, the Black FFs Association,

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Asian FFs Association, and Los Bomberos (Latino FF Association). Informational meetings were held at the San Francisco Fire Department. Female office employees with the City and County of San Francisco were recruited through informational meetings, direct email, letters, and telephone calls and by networking efforts through SEIU Local 1021. The study was publicized through regular newsletters and other online communication outlets regularly sent to FFs and other San Francisco City and County employees through the Health Services System. WFBC study protocols were approved by the Institutional Review Board of the University of California, Berkeley (Protocol # 2013-07-5512). Informed consent was obtained prior to all interviews and sample collections. Subjects were not paid for participation but did receive a $20.00 gift card and reimbursement to offset the cost of parking and transportation. Blood samples were collected between June 2014 and March 2015.

Interviews and Sample Collection. Once consented and enrolled, participants were scheduled for an in-person interview and blood collection. Subjects met with a member of the research team to answer questions about their diet, home, job, other activities, and education. After completing the exposure interview, a trained phlebotomist drew four blood sample replicates, which were collected in 10 mL red-top tubes without additives. Samples were collected at sites near participants' workplaces and transported in a cooler with ice for processing within 3 h of collection. Serum was separated by allowing it to clot at room temperature and then centrifuging at 3000 rpm for 10 min at −4 °C. The serum was aliquoted into 1.2 mL cryo-vial tubes and stored at −80 °C until analysis. All samples were processed and analyzed at the University of California, San Francisco. We collected and processed samples from 86 FFs and 84 OWs. We analyzed serum samples from those who had sufficient serum for the chemical analysis, that is, from 83 FFs and 79 OW participants.

WFBC Suspect Chemical Database. To build a chemical database for our GSS, we began with a database of 682 chemicals developed previously to identify environmental organic acids (EOAs) among pregnant women, including chemicals from the following classes: phenols, such as parabens; phenolic and acidic pesticides and their predicted acidic and phenolic metabolites; per- and polyfluoroalkyl substances (PFAS); phthalate metabolites; and phenolic metabolites of polybrominated diphenyl ethers (OH-BDEs) and polychlorinated biphenyls (OH-PCBs).[35] These EOAs include many common consumer product chemicals and environmental pollutants as well as 353 predicted metabolites of common pesticides.[35] We extended this EOA database for our WFBC analysis by adding environmental chemicals that are relevant to occupational exposures faced by FFs and OWs and also chemicals implicated in breast carcinogenesis based on toxicological evidence. Specifically, we assessed the viability of adding over 100 chemicals based on the following criteria: (1) chemicals shown to be rodent mammary gland carcinogens or that affect mammary gland development and thus may increase breast cancer risk[13,14] or (2) chemicals related to firefighting that could lead to occupational exposures, including perfluorinated compounds found in firefighting foams, and other flame retardants and their metabolites.[36−38] Chemicals that fit these two criteria were added to the WFBC database if their structures were expected to be compatible with LC−QTOF/MS operating in negative electrospray ionization mode. For example, carcinogenic PAHs were not added to the database because they are unlikely to be detected using this method. We added 40 chemicals for a total of 722 in the WFBC database (Table S1). A more comprehensive approach that covers a larger chemical landscape would analyze serum samples using both positive and negative electrospray ionization and GC/MS methods. The work reported here focuses on a subset of the chemical space (weak organic acids detected with negative ionization) and demonstrates a work flow for chemical prioritization and confirmation that can be applied in other studies.

General Suspect Screening Analysis Using Liquid Chromatography and Quadrupole Time-of-Flight MS. GSS of serum was performed as previously described.[34] Briefly, 250 μL of serum was spiked with 2.5 μL of 1 μg/mL of internal standard (2.5 ng of BPA-d16, final concentration: 10 ng/mL) and centrifuged at 3000 rpm for 10 min. Analytes were extracted using solid-phase extraction (Waters Oasis HLB 10 mg, 1 cm^3) for cleanup. Extracts were dried under a stream of nitrogen gas and reconstituted in 250 μL of 10% methanol.

Extracts were analyzed on a LC−QTOF/MS system consisting of an LC 1260 and a QTOF/MS 6550 (Agilent, Santa Cruz, CA, USA). The analytes were separated by reversed-phase chromatography using a C18 column (Agilent Poroshell 120, 2.1 mm × 100 mm, 2.7 mm particle size) maintained at 55 °C. Mobile phase A consisted of water with 0.05% ammonium acetate (pH = 7.8) and mobile phase B consisted of methanol with 0.05% ammonium acetate (pH = 7.8). The elution gradient employed was as follows: 0−0.5 min, 5% B; 1.5 min, 30% B; 4.5 min, 70% B; 7.5−10 min, 100% B; and 10.01−14 min, 5% B for re-equilibration of the column. The injection volume was 50 μL.

Analyses were performed with a QTOF/MS operating in negative electrospray ionization (ESI^-) mode. Ions were collected in the m/z 80−600 range at high resolution for eluates coming out of the liquid chromatograph from 1 to 12 min. Using the Auto MS/MS mode (information-dependent acquisition), a product ion scan (MS/MS) of the three most abundant peaks at high resolution was triggered each time a precursor ion with an intensity of ≥500 counts/second was generated in the QTOF/MS scan using a collision voltage ranging from 0 to 40 V depending on ions m/z. The LC−QTOF/MS analysis produces a total ion chromatogram (TIC) for each sample. TIC is evaluated against our comprehensive database to generate suspects characterized by the following information derived from their extracted ion chromatograms: the accurate mass of each unique compound (expressed as m/z of their respective anion), peak area, RT, and spectral data on the parent and fragment ions, including isotopic pattern.

For each batch, we included seven solvent blanks (i.e., mobile phase solution) and six matrix blanks—synthetic human serum that has undergone the same analytical process as the samples—to identify potential contamination in the analytical method. After visual inspections of chromatogram peaks, we excluded mass features that appeared in either blank.

We used the Agilent MassHunter Qualitative Analysis software Find-by-Formula algorithm to analyze QTOF/MS data for novel chemical exposures among FFs and OWs using a set of optimized parameters previously reported.[34] First, all detected m/z were matched to potential compound hits in the WFBC chemical database. The algorithm imports molecular formulas from the database, automatically calculates their m/z values, and then matches them to m/z measured by the QTOF/MS with a mass tolerance value of 10 ppm. A list of possible chemical matches was generated for all serum samples,

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which included the accurate mass (m/z), mass error (i.e., the difference between the experimental and the theoretical m/z), RT, peak area, and match scores.[39] We performed visual reviews of TIC peaks to remove peaks that (1) had poor peak shape (e.g., very broad peaks, peaks with multiple shoulders, peaks with signal-to-noise (S/N) < 3) or (2) had peak areas ≤1.10 times the maximum observed peak area in the solvent or the double blanks. The initial LC−QTOF full scan identification resulted in 12,051 features (i.e., unique pair of m/z and RT), which matched 300 chemical formulas in our WFBC database with multiple RTs/formulas or 620 unique chemical formula/RT combinations.

RT Correction and Isomer Distinction. Isomers (compounds with the same chemical formula but with different chemical structures) are recognized by the LC−QTOF method as the presence of multiple RTs, (measured in minutes) per chemical formula or mass. We distinguished isomers by clustering compounds based on RT. Briefly, we first ranked all suspect detections by RT for each chemical formula. We considered a suspect peak to be from a different isomer if its RT differed from the RT of the same chemical formula in the previous row by more than 0.16 min. Cutoff points ranging from 0.15 to 0.20 with a 0.01 increment were tested, and 0.16 allowed the best distinction based on graphical examination.[35] Then, we aligned peaks originating from the same isomer to an identical RT. The final analytical sample consisted of 4791 suspect detections (level 5 annotations according to Schymanski et al.[39]) that matched 620 suspect chemicals (i.e., unique combinations of chemical formula and RT).

Chemical Selection for Validation and Confirmation. We used a multistep procedure and criteria to reduce the initial set of candidate chemical matches from LC−QTOF/MS to a smaller set of compounds for validation by prioritizing matches that showed differences in exposure between FFs and OWs or had toxicity characteristics relevant to breast cancer.[13,40] We focused our GSS on compounds in our database that were not pharmaceutical chemicals or chemicals that we had already identified for targeted analysis. We then used the following initial criteria to prioritize matches for validation: (1) at least 10% detection frequency difference between FFs and OWs; (2) a higher peak area (indicator of higher relative concentration) in FFs compared to OWs (paired t-test, $p \leq 0.1$); (3) ubiquitous chemicals detected in more than 90% of both FF and OW groups; and (4) whether a chemical had been flagged as a mammary carcinogen or mammary gland developmental disruptor.[13,40] As shown in Figure 2, this process yielded an initial list of 71 chemicals that we then narrowed down to 54 for potential confirmation based on the availability of an analytical standard.

In the second step for prioritizing tentative chemical matches for validation, we scored the remaining 54 chemicals based on the first set of selection criteria as well as the following additional characteristics: flame-retardant chemicals, chemicals identified as estrogenic or genotoxic, chemicals not detected in OWs, and chemicals not currently biomonitored in NHANES[41] or the California Biomonitoring Program.[42] The specific criteria were chemicals (1) listed as flame retardants;[36,37] (2) not detected in the OWs; (3) currently not biomonitored in NHANES or Biomonitoring California; (4) listed as "active" for at least one genotoxicity bioassay tested in PubChem;[43] and (5) listed as "active" for at least one estrogen receptor bioassay in PubChem.[44] For bioassay data, results were downloaded from the PubChem website for each chemical. Then, assay descriptions were queried for terms including "genotox", "estrogen", and "salmonella" (to flag all Ames assays). All assays matching those terms listed as "active" were tallied, and chemicals with active assays were prioritized.

We scored the chemicals by assigning one point for each of the nine criteria. The study team reviewed the top scoring chemicals and selected 20 for validation based on score as well as data on uses, toxicity, and sources using the Comparative Toxicogenomics Database,[45] PubChem,[43] Toxnet,[46] and the Toxin and Toxin Target Database (T3DB)[47] (Table S2). Peaks that matched predicted pesticide metabolites in our database were not considered for validation because of the additional uncertainty about their presence in biological samples and lack of available reference standards.

Confirmation of Selected Chemicals. We confirmed the presence of suspect chemicals in the serum samples by running the LC−QTOF/MS analysis using the corresponding reference standard spiked into synthetic serum. Tentative chemical matches from participant samples were confirmed if the m/z, at least two fragment peaks in the MS/MS spectra, and RT of the authentic standard matched those found in the serum samples, consistent with level 1 confidence in identification (i.e., chemical identity confirmed by reference standard) of small molecules via high-resolution MS as proposed by Schymanski et al.[39]

Statistical Analysis. For statistical comparisons across demographic and occupational groups, we used the Wilcoxon rank sum test to compare continuous variables or the Fisher test for categorical variables. All tests were two-sided, and $p < 0.05$ was the level of significance, except for the t-tests to compare differences in peak areas between FFs and OWs ($p < 0.1$). All data analysis and visualizations were completed using R, version 3.3.2.[48]

RESULTS AND DISCUSSION

The goal of this study was to apply a GSS approach to identify novel exposures to previously understudied chemicals—of particular relevance to firefighting and breast cancer etiology—among a cohort of women FFs compared to OW controls. Table 1 shows the demographic characteristics for the 83 FFs and the 79 OWs recruited for the WFBC study. At the time of recruitment, the San Francisco Fire Department (SFFD) had 224 active duty women FFs who made up nearly 15% of its workforce. Among our study population, the average age of women FFs is 47.9 (±4.6) years old and the average time of service in the Department is 17.4 (±4.2) years. The racial/ethnic makeup of this population in the department, which is reflected by the recruited FF participants, is 50% non-Hispanic White, 21% Asian/Pacific Islander, 17% Hispanic/Latino, and 13% African American, which is reflected by recruited FF participants. Among the OWs, the average age is 47 years old and most have worked an average of 14.0 years for the City and County of San Francisco. The racial and ethnic makeup of this workforce was statistically similar to that of the FFs, with a higher percentage of non-Hispanic Asian/Pacific Islanders (25%).

Overall, the FFs and OWs were similar in terms of average age, race/ethnicity, body mass index (BMI), parity, and hormone use (p-values ranged from 0.2 to 0.6). However, the household income for FFs was significantly higher when compared to that for OWs, probably because of the relatively higher compensation rate for firefighting versus office or clerical work. There were significantly more premenopausal

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Table 1. WFBC Study Population Characteristics[a]

characteristic	OWs (n = 79)	FFs (n = 83)	p-value[b]
Age			
mean ± SD	48.1 ± 10.6	47.9 ± 8.4	0.4
Race/Ethnicity n (%)			
non-Hispanic Asian	17 (22)	13 (16)	0.3
non-Hispanic blacks	5 (6)	9 (11)	
Hispanics of all races	7 (9)	8 (9)	
multiracial	10 (13)	16 (19)	
non-Hispanic whites	40 (50)	37 (45)	
Education n (%)			
high school or less	5 (6)	6 (7)	<0.001
some college	10 (13)	40 (48)	
college graduates or higher	64 (81)	37 (45)	
BMI			
mean (SD)	25.8 (5.2)	26.2 (3.5)	0.2
Household Income n (%)			
<$99,999	23 (29)	1 (1)	<0.001
$100,000−174,999	18 (23)	29 (35)	
$175,000−199,999	12 (15)	17 (20)	
>$200,000	26 (33)	36 (44)	
Menopausal Status n (%)			
premenopausal	44 (56)	62 (75)	0.007
postmenopausal	35 (44)	21 (25)	
Hormone Use[c] n (%)			
never	19 (26)	16 (20)	0.6
during the past	38 (53)	46 (60)	
currently	15 (21)	15 (20)	
Parity (# of Live Births) n (%)			
0	36 (46)	34 (41)	0.3
1	18 (23)	15 (18)	
>1	25 (31)	34 (41)	

[a]SD: Standard deviation. [b]Wilcoxon rank sum test to compare continuous variables by FF status or Fisher test for categorical variables. [c]Missing data on hormone use for 6 FFs and 7 OWs.

women in the FF group. Finally, OWs had a higher proportion of college graduates than the FFs.

Suspect Screening Analysis of Serum Samples. Our GSS analysis detected 12,051 candidate compounds across all serum samples, which were then compared to 722 chemical formulas from the WFBC database. RT correction tentatively identified 300 chemical formulas (level 5 annotations according to Schymanski et al.[39]), with multiple RTs per formula such that there were 620 putative chemicals in the FF and OW samples. These included phthalate metabolites, phosphate flame retardants (PFRs) and their metabolites, phenols, pesticides, nitro and nitroso compounds, and per- and PFASs. Figure 1 shows the number of chemical suspect hits per participant for each chemical class. Because the analytical approach was limited to negative electrospray ionization and LC/MS, many of the chemical matches detected in FF and OW were phenols and phthalate metabolites. The average cumulative number of suspect chemicals detected was 73 (minimum: 45, maximum: 109) and 70 (minimum: 45; maximum: 100) in FF and OW, respectively. Thus, the nontargeted LC−QTOF/MS data acquisition in ESI$^-$ was able to detect a wide range of suspect organic acids that include many common commercial chemicals.

Chemical Restriction and Prioritization for Validation. We tentatively identified 71 chemicals (level 5 annotations according to Schymanski et al.[39]) that were (1) more abundant in FFs, (2) ubiquitous and not already in NHANES, or (3) tagged as a potential concern for breast cancer. Sixty-three of these chemicals satisfied only one criterion, and eight satisfied more than one. We further reduced this list to chemicals that had commercially available authentic standards, leaving 54 to be considered for validation. These chemicals included phenols such as bisphenol F and some alkylphenols, phthalate metabolites, PFAS, flame-retardant metabolites, nitroso compounds, and pesticides (see Table S2). None of the chemicals had significantly different detection frequencies in FF versus OW. Seven tentatively identified chemicals had statistically significant differences in peak areas between FF and OW. Three PFASs (PFOS, PFOSAA, and PFOA), 4-butoxyphenol, and 4-phenethylphenol had higher mean peak areas in OW samples, while 2,4-bis(1,1-dimethylethyl phenol) and a metabolite of propham had higher peak areas in FF samples. Fewer than half were identified as mammary carcinogens or developmental disruptors. We scored the 54 tentatively

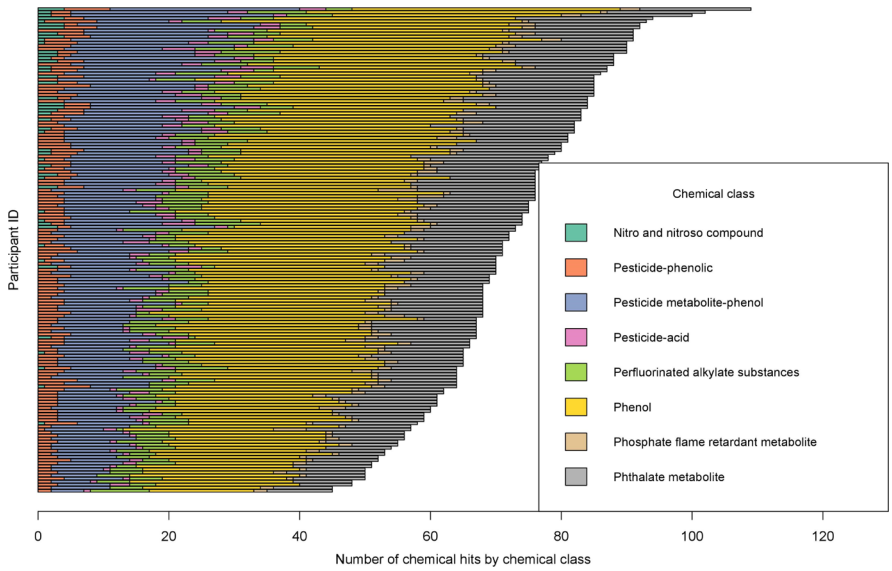

Figure 1. Cumulative number of WFBC database chemicals detected with LC−QTOF/MS ESI$^-$ in serum samples from 162 study participants (mean = 72; min = 45; max = 109).

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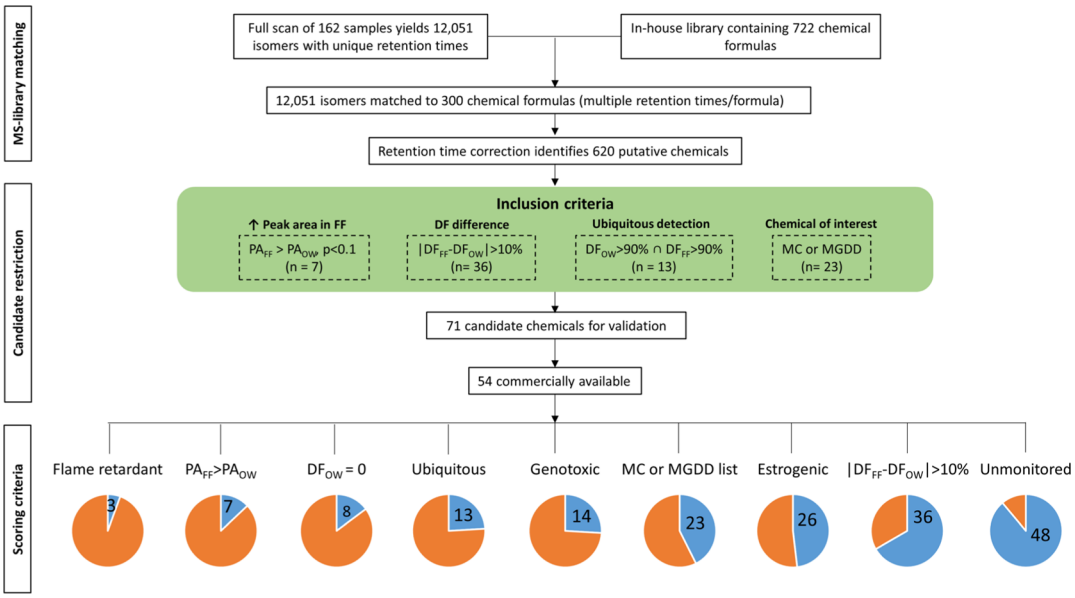

Figure 2. Scoring and ranking of chemicals detected by LC−QTOF. PA = peak area; FF = firefighter; OW = office worker; DF = detection frequency; MC = mammary carcinogen; and MGDD = mammary gland developmental disruptor.

identified chemicals based on indications of toxicity and exposure potential, as shown in Figure 2 and Table S2.

We selected chemicals for analytical validation after reviewing the priority scores across nine criteria for the 54 chemicals along with information about uses, toxicity, and sources (Table S2 provides this information for all 71 candidate chemicals).

Table 2 shows the top 20 scoring candidate chemicals and indicates the priority rank and whether the chemical was included in the confirmation testing. For example, 2,4-bis(1,1-dimethylethyl) phenol had the top ranking, meeting six of the nine criteria (Table 2), and was selected for validation. Three nitro and nitroso compounds with high scores, including 1-ethylnitroso-3-(2-oxopropyl)-urea, 1-ethylnitroso-3-(2-hydroxyethyl)-urea, and 1-amyl-1-nitrosourea, were eliminated because the cost to purchase analytical standards was prohibitive. Bis(1,3-dichloro-2-propyl) phosphate (BDCIPP)—metabolite of the common flame retardant tris(1,3-dichloro-2-propyl)phosphate—was excluded because it was already being targeted for analysis in this cohort. Estradiol was excluded because it is endogenous, and Nifurdazil, an antibacterial agent, was excluded because we were not targeting pharmaceuticals. We included the remaining 14 priority chemicals in the confirmation testing.

Validation. Authentic standards of the 14 selected chemicals were analyzed by LC−QTOF/MS to evaluate their match with RTs and mass spectra in the samples. RTs for chemical candidates and authentic standards, exact masses, and validation status are listed in Table 3. Eight chemicals were validated, including 2,4-bis(1,1-dimethylethyl)phenol, 2-hydroxy-4-methoxybenzophenone-2, bisphenol F, perfluorooctanesulfonamidoacetate (PFOSAA), diphenyl phosphate (DPP), ethyl-*p*-hydroxybenzoate (ethyl paraben), benzyl *p*-hydroxybenzoate (PHBB), and 4-hexyloxyphenol.

We found that RTs and MS/MS spectra in participants' serum did not match those of the standards for six chemicals: 1-allyl-1-nitrosourea, 4-butoxyphenol, 2,3,6-trimethylphenol, 4-phenethylphenol, and two isomers for 4-heptyloxyphenol.

Among the eight chemicals whose identity was validated by matching RT and MS/MS fragmentation of a known standard, the results suggested that exposures were different between FFs and OWs for most of them, although the magnitude of the differences was modest. Based on statistically significant differences in peak area, FFs had higher relative levels of exposure for 2,4-bis(1,1-dimethylethyl) phenol and OWs for PFOSAA and ethyl paraben (Table 2). FFs appeared to have slightly higher detection frequencies for 2-hydroxy-4-methoxybenzophenone (BP-3), bisphenol F, PFOSAA, and ethyl paraben, and OWs had a higher detection frequency for PHBB. For these confirmed analytes, concentrations in all samples will be quantified by reanalysis with the standard, and these findings will be reported separately.

The validated chemicals included two phenols (bisphenol F and PHBB), which are used as bisphenol-A substitutes,[49] and BP-3, which is a UV filter in sunscreens, textiles, and other products. The chemical 2,4-bis(1,1-dimethylethyl) phenol (aka 2,4-di-*tert* butyl phenol) is listed as a manufacturing chemical and a fuel additive; however, because it was detected in all of the participants, it may have some common consumer use or be a metabolite of a common exposure (CID 7311).[50] It is interesting to note this compound's similarity to 4-*tert* butyl phenol—a stronger estrogen mimic that is ubiquitous in residential settings.[51] Ethyl paraben is an antifungal preservative found in cosmetics, toys, sunscreen, and pesticides.[52] A PFAS chemical, PFOSAA, was also validated. Previous studies have reported higher firefighting exposures for PFASs,[53,54] and findings of targeted analysis for PFASs in this cohort are forthcoming.[55] Originally a metabolite of an active ingredient in Scotchgard stain and water repellant, PFOSAA is listed as an automotive, construction-related, and cleaning chemical, as well as an inert pesticide ingredient (CID 23691014).[50] It may also be found in firefighting foams. DPP, a common metabolite of the flame retardant and plasticizer triphenyl phosphate,[56] appeared to have similar concentrations in FFs and OWs. Quantification for targeted analysis for a suite of flame retardants in urine samples from this cohort will be reported separately.

Among the few biomonitoring studies previously conducted on FFs, one[12] observed higher exposures to environmental phenols (i.e., bisphenol A, triclosan, benzophenone-3, and methyl paraben)

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Table 2. Twenty Highest Scoring Chemicals Prioritized for Validation

Chemical name	Class	Rank	DF FF (%)	DF OW (%)	Mean peak area FF	Mean peak area OW	DF > 90% in FF and OW	|DF_FF - DF_OW| > 10%	T-test PA p<0.1	OW non-detect	Flame retardant	Unmonitored [a]	Genotoxic	Estrogenic	MC list	Total Score	Validation status
2,4-bis(1,1-dimethylethyl)phenol	Phenol	1	82 (98.8%)	76 (96.2%)	9.17E+05	7.66E+05	1	0	1	0	0	1	1	1	0	5	S
benzyl p-hydroxybenzoate (PHBB) or [b]	Phenol	2	16 (19.2%)	6 (7.6%)	2.98E+04	2.12E+04	0	1	0	0	0	1	1	1	0	4	S
2-hydroxy-4-methoxybenzophenone-2 (BP-3)											0	0	1	1	0	3	
4-hexyloxyphenol	Phenol	3	81 (97.6%)	71 (89.9%)	1.04E+05	7.51E+04	1	0	1	0	0	1	1	1	0	5	S
benzyl p-hydroxybenzoate (PHBB) or [b]	Phenol	4	30 (36.1%)	38 (48.1%)	6.04E+04	9.68E+04	0	1	0	0	0	1	1	1	0	4	S
2-hydroxy-4-methoxybenzophenone-2											0	0	1	1	0	3	
bisphenol F	Phenol	5	10 (12.0%)	0 (0%)	4.98E+05	NA	0	1	0	1	0	1	0	1	0	4	S
4-butoxyphenol	Phenol	6	77 (92.8%)	71 (89.9%)	7.21E+04	8.58E+04	1	0	1	0	0	1	0	0	0	3	S
2,3,6-trimethylphenol	Phenol	7	18 (21.7%)	7 (8.9%)	2.04E+04	1.15E+04	0	1	0	0	0	1	0	0	0	2	S
1-ethylnitroso-3-(2-oxopropyl)-urea	Nitro and Nitroso Compound	8	14 (16.9%)	10 (12.6%)	2.54E+04	2.09E+04	0	0	0	0	0	1	0	0	1	2	E-No std
perfluorooctanesulfonamidoacetate (PFOSAA)	PFAS	9	16 (19.3%)	25 (31.6%)	3.94E+04	4.56E+04	0	1	1	0	0	1	0	0	0	3	S
diphenyl phosphate (DPP)	Phosphate Flame Retardant metabolite	10	45 (54.2%)	39 (49.4%)	1.57E+04	1.68E+04	0	0	0	0	1	0	0	0	0	1	S
bis(1,3-dichloro-2-propyl) phosphate (BDCIPP)	Phosphate Flame Retardant metabolite	11	2 (2.4%)	1 (1.3%)	1.35E+04	1.13E+04	0	0	0	0	1	0	0	0	1	2	E- target analyte

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Table 2. continued

Chemical name	Class	Rank	DF FF (%)	DF OW (%)	Mean peak area FF	Mean peak area OW	DF > 90% in FF and OW	|DF_FF - DF_OW| > 10%	T-test PA p<0.1	OW non-detect	Flame retardant	Unmonitored [a]	Genotoxic	Estrogenic	MC list	Total Score	Validation status
4-phenethylphenol	Phenol	12	82 (98.8%)	76 (96.2%)	1.35E+05	1.43E+05	1	0	1	0	0	1	0	1	0	4	S
4-heptyloxyphenol [b] (isomer 1)	Phenol	13	31 (37.3%)	21 (26.6%)	6.60E+04	6.87E+04	0	1	0	0	0	1	0	1	0	3	S
Nifurdazil	Nitro and Nitroso Compound	14	4 (4.8%)	3 (3.8%)	2.37E+04	1.07E+04	0	0	0	0	0	1	1	0	1	3	E - medication
4-heptyloxyphenol [b] (isomer 2)	Phenol	15	51 (61.4%)	55 (69.6%)	2.89E+05	2.55E+05	0	1	0	0	0	1	0	1	0	3	S
1-ethylnitroso-3-(2-hydroxyethyl)-urea	Nitro and Nitroso Compound	16	3 (3.6%)	2 (2.5%)	1.57E+04	1.57E+04	0	0	0	0	0	1	0	0	1	2	E-No std
1-amyl-1- nitrosourea	Nitro and Nitroso Compound	17	7 (8.4%)	11 (13.9%)	3.56E+04	2.33E+04	0	0	0	0	0	1	0	0	1	2	E-No std
ethyl-p-hydroxybenzoate (ethyl paraben)	Phenol	18	52 (62.6%)	35 (44.3%)	1.10E+05	1.57E+05	0	1	1	0	0	0	0	1	0	3	S
1-allyl-1-nitrosourea	Nitro and Nitroso Compound	19	12 (14.4%)	5 (6.3%)	7.25E+04	3.96E+04	0	0	0	0	0	1	0	0	1	2	S
estradiol	Steroid	20	1 (1.2%)	0 (0%)	1.03E+04	NA	0	0	0	1	0	0	1	1	1	4	E-endogenous

[a]Unmonitored in NHANES or Biomonitoring California. [b]These are isomers and could not be distinguished based on molecular mass; FF = firefighter; OW = office worker; DF = detection frequency; PA = peak area; MC = mammary carcinogen; E = eliminated for validation; S = selected for validation; std = standard. Additional details and references can be found in Supplementary Table S2.

among Southern California FFs compared to the general population. Because this study also investigated FFs from California, it is difficult to decipher whether the prevalent exposures to phenols are specifically related to firefighting activities or simply more prevalent among California populations in general.

The phenols and the PFAS that were validated in this study have estrogenic activity (Table 2) or are of concern for a diverse set of toxicity endpoints, such as effects on kidney, liver, lipid metabolism, growth and development, mammary gland development, and immunotoxicity.[57] Although there were tentative matches to nitro and nitroso chemicals, which are of interest because of their genotoxicity and carcinogenicity (Table 2), we were not able to validate any of these compounds, either because the RT did not match the known standard or we could not obtain the standard.

The success of this general suspect screening technique to identify novel chemical exposures in environmental and occupational health studies could be improved further if there were chemical databases that contain mass spectral information about diverse chemicals of interest. Because most public metabolomics databases, such as HMDB, Metlin, or T3DB, contain few entries for environmental chemicals (e.g., HMDB contains 163 entries for toxins/pollutants) and there are no extensive mass spectral databases of environmental chemicals currently available, we instead made comparisons to 722 chemicals in our database based on matching exact masses. This approach allowed us to tentatively identify novel exposures by focusing the search on a set of chemicals of interest and for which the analytical method was optimized. We also demonstrated that this approach can be effective in measuring low abundant chemicals in human serum. For example, PFOS detected using GSS (Table S2) was also confirmed and quantified using targeted LC−MS/MS (median serum concentrations for the whole cohort were 4.1 ng/mL for PFOS).[55]

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Table 3. RT and Exact Mass for Chemicals Selected for Validation

chemical name	chemical class	# of isomers	m/z values	mean RT for serum samples	RT lab standard	validation status
2,4-bis(1,1-dimethylethyl) phenol	phenol	4	206.1668, 206.1666, 206.1664, 206.1673	4.33, 5.25, 5.48, 6.73	6.72	√
2-hydroxy-4-methoxybenzophenone (BP-3)	phenol	2	228.0786, 228.0787	4.33, 5.25	5.30	√
bisphenol F	phenol	2	200.0833	3.91	4.00	√
PFOSAA	PFAS	1	528.9747	5.93	5.95	√
diphenyl phosphate (DPP)	PFR metabolite	1	250.0396	3.86	3.90	√
ethyl-*p*-hydroxybenzoate (ethyl paraben)	phenol	2	166.0631, 166.0629	2.21, 3.80	2.30	√
benzyl *p*-hydroxybenzoate (PHBB)	phenol	2	228.0786, 228.0787	4.33, 5.25	4.40	√
4-hexyloxyphenol[1]	phenol	1	194.1308	5.81	5.80	√[a]
4-butoxyphenol	phenol	1	166.0994	4.19	5.10	×[b]
2,3,6-trimethylphenol	phenol	2	136.0879	3.97	4.25	×[b]
4-phenethylphenol	phenol	1	198.1047	5.71	6.02	×[b]
4-heptyloxyphenol (2 isomers)	phenol	1	208.1465	5.09	6.22	×[b]
1-allyl-1-nitrosourea	nitro and nitroso compound	1	129.0547	0.76	1.20	×[b]

[a]Validated but with high LOD. [b]Not validated because of RT mismatch.

We were also interested in identifying exposures associated with work practices that are not related to fire events, such as diesel fuel and exhaust from trucks and equipment in the station, flame retardants and PFAS chemicals from firefighting foam and protective gear, chemicals used to clean gear, and possibly others. Some of the chemicals selected for targeted analyses may be related to workplace exposures such as these, and this suspect screening approach is one way to generate hypotheses about exposures and to prioritize novel compounds for confirmation and quantification using targeted methods.

Our study has several limitations. The sample size is relatively modest, and a larger cohort would have provided more power to detect candidate chemicals that differed between FFs and OWs. In addition, because most of chemicals we detected are nonpersistent, we can expect large intraindividual variability in serum because of temporal variation in exposure. Also, only 15 FFs had their blood samples collected within 24 h of working at a fire event, so it may be that the chemicals we detected were not necessarily associated with firefighting activities. One way to better characterize chemicals originating from fighting fires would be to perform a longitudinal analysis in which biospecimens would be collected before and after a fire event (within 12−24 h).

Our WFBC general suspect chemical database (722 chemicals) contained only a small fraction of the chemicals that could be important exposures for FFs and OWs, and so, we may have missed some important compounds for this study population. The use of larger chemical databases such as the EPA Distributed Structure-Searchable Toxicity (DSSTOX; ∼875,000 chemicals)[58] or PubChem (∼96.5 million unique chemical structures)[50] would provide detection of a larger set of chemical suspects. However, increasing the number of chemicals in a general suspect database would likely also increase the number of "hits" (tentative chemical RT matches), making it more challenging to confirm matches by only looking at exact masses and RTs and increasing the rate of false positives. Even with our database of 722 chemicals, 6 (2 of which are isomers), of the top 20 tentative chemical matches that we selected for validation showed a RT mismatch such that the study serum sample RT did not match the RT generated from a reference standard. Combining LC−QTOF/MS data—collected using a data-independent acquisition approach (i.e., MS/MS fragmentation of as many metabolites as possible in a single acquisition)—with bioinformatics tools such as RT prediction, in silico MS/MS prediction, and molecular networking analysis[59,60] would help to address this issue. In addition, a careful validation of the chemical identity using an authentic standard is required to avoid reporting false-positive matches. Likewise, the number of matching fragmentation peaks required to minimize false positives can be investigated in future studies. Ultimately, the MS/MS spectra generated for any compound provide structural information specific to a compound. These data become very valuable for distinguishing isomeric compounds that may have very close RTs in chromatography but different fragmentation patterns.

Another limitation is that the use of LC−QTOF/MS in negative ionization mode limited the types of chemicals that could be detected to organic acids. The use of complementary platforms and ionization sources such as LC−QTOF/MS in positive ionization mode or GC combined with high-resolution MS would expand the investigation to more diverse classes of chemicals. For example, Wallace et al.[61] identified several VOCs and PAHs in FFs exposed to controlled structure burns using targeted and nontargeted GC−MS analysis of exhaled breath condensate. Some of these chemicals such as benzaldehyde and dimethyl sulfide have been previously associated with smoke/fire and combustion sources, while methyl *tert*-butyl ether is commonly used as an additive to gasoline. Some of the nitroso compounds with high priority scores in our analysis such as 1-amyl-1-nitrosourea and 1-allyl-1-nitrosourea could not be validated because standards were not available. Finally, follow-up studies should include targeted analyses to confirm and quantify the identified chemicals in the cohort, identification of potential sources of the exposures, extension of the approach to cover a broader and more diverse chemical space, and assessment of potential associations with health outcomes for validated chemicals.

■ ASSOCIATED CONTENT

Supporting Information

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WFBC chemical database and exposure and toxicity data on 71 tentative chemical matches used to prioritize for confirmation (XLSX)

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Notes

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