

Exploratory breath analyses for assessing toxic dermal exposures of firefighters during suppression of structural burns

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Abstract

Firefighters wear fireproof clothing and self-contained breathing apparatus (SCBA) during rescue and fire suppression activities to protect against acute effects from heat and toxic chemicals. Fire services are also concerned about long-term health outcomes from chemical exposures over a working lifetime, in particular about low-level exposures that might serve as initiating events for adverse outcome pathways (AOP) leading to cancer. As part of a larger U.S. National Institutes for Occupational Safety and Health (NIOSH) study of dermal exposure protection from safety gear used by Chicago City firefighters, we collected pre- and post-fire fighting breath samples and analyzed for single-ring and polycyclic aromatic hydrocarbons as bioindicators of occupational exposure to gas-phase toxicants. Under the assumption that SCBA protects completely against inhalation exposures, any changes in the exhaled profile of combustion products were attributed to dermal exposures from gas and particle penetration through the protective clothing. Two separate rounds of firefighting activity were performed each with 15 firefighters per round. Exhaled breath samples were collected onto adsorbent tubes and analyzed with gas-chromatography-mass spectrometry (GC-MS) with a targeted approach using selective ion monitoring. We found that single ring aromatics and some PAHs were statistically elevated in post-firefighting samples of some individuals suggesting that fire protective gear may allow for dermal exposures to airborne contaminants. However, in comparison to a previous occupational study of Air Force maintenance personnel where similar compounds were measured, these exposures are much lower suggesting that firefighters' gear is very effective. This study suggests that exhaled breath sampling and analysis for specific targeted compounds is a suitable method for assessing systemic dermal exposure in a simple and non-invasive manner.

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Introduction

Firefighters wear specialized gear designed primarily to protect against the immediate dangers of fire associated with heat, asphyxiation, and acute effects from toxic vapors, gases, and particles from combustion sources. This article addresses a secondary concern, which is the potential for lower level toxic exposures that may create longer-term adverse health outcomes. Such exposures pose a health risk by initiating adverse outcome pathways (AOPs) at the molecular or cellular level and are experienced repeatedly by the community of 330,000 career fire fighters and 770,000 volunteer fire fighters in the United States [NFPA 2011]. As such, it is important to gain detailed exposure data from representative single events to predict the overall risk to the human systems biology for long-term latency disease from repeated short-term exposures (Pleil 2009, Pleil and Sheldon, 2011). Furthermore, the availability of *in vivo* biomarker specimens is valuable in supplementing the overall exposure assessment framework and the toxicity data derived from *in vitro* (molecular and cellular level) and *in silico* (computational models) research of suspected human carcinogens (Sobus et al. 2011, Tan et al. 2012, Pleil 2012).

The U.S. National Institute for Occupational Safety and Health (NIOSH) conducted a study of dermal exposures to and biological uptake of carcinogenic compounds in firefighters during fire suppression (NIOSH 2013). Firefighters in this study wore the highest level of dermal and respiratory protection currently available to them. This study included ambient measurements, urinary biomarkers, dermal swabs, and assessments of protective fire-fighting turnout gear (Fent et al. 2014). As part of this study U.S. Environmental Protection Agency (EPA) researchers collaborated with NIOSH in assessing exhaled breath biomarkers of fire exposure represented by aromatic and polycyclic aromatic hydrocarbons (PAHs). Breath analysis has become a useful procedure for a variety of environmental and clinical health investigations (Pleil 2008, Dweik RA 2011, Miekisch et al. 2012, Davis et al. 2013) and has been featured in recent conferences and review articles as a mainstream technology (Pleil et al. 2013, Amman 2009, Corradi and Mutti 2012, Pleil and Hanzel 2012, Beauchamp et al. 2013, Braun et al. 2012, Risby and Solga 2006). Furthermore, breath has joined blood and urine as a primary human biofluid in decoding the human exposome (Sinues et al. 2013, Pleil et al. 2011).

The purpose of the breath portion of the study was to provide objective biomarker evidence of short-term exposures and was conducted as a “targeted” rather than a “discovery” assessment (Pleil and Stiegel 2013). That is, the analytes were chosen *a-priori* to optimize sensitivity and specificity. As such, we restricted ourselves to certain compounds that are presumed indicative of the totality of exposure to products of incomplete combustion; this approach has been used for environmentally derived samples for assessments of other fire related exposures including from the “9/11” New York City World Trade Center (WTC) disaster (Lorber et al. 2007, Pleil et al. 2006, Pleil et al. 2004). We note that there are previous breath based studies of firefighters for assessing carbon monoxide (CO)

exposures (Cone et al. 2005, Dunn et al. 2009, Miranda et al. 2012), but that this is the first study wherein organics exposures were directly assessed using breath biomarkers.

This article describes the application and results from breath analyses of fire fighters engaged in scripted (planned exposure) activities. Breath samples were collected before, shortly after, and 6-hrs after specific firefighting tasks in a realistic training environment. The breath results indicated that some amount of biological uptake of toxic materials occurred despite the use of dermal and respiratory protection. We compared these results to other breath studies of jet fuel exposures to gain context of occupational exposures to similar compounds. Exhaled breath analysis was found to be a valuable and non-invasive tool for assessing exposures of firefighters to toxic chemicals derived from incomplete combustion.

Methods

Study design:

The study was comprised of two rounds of controlled structure burns one year apart; each round consisted of three days of scripted firefighting activity with five firefighters studied per day. As such, there were 30 distinct subject-days of data gathered; twelve of the firefighters from round 1 repeated as subjects in round 2. Each day of firefighting activity consisted of a scripted test-burn that took place in the morning with segments of fire/entry, knockdown, and overhaul. Firefighting activity varied in time from 15 to 30 minutes depending on the heat and intensity of each of the fires. The firefighters wore protective ensembles for structural firefighting that were compliant with the National Fire Protection Association 1971/1981 standards (NFPA 2007a, b) and did not remove their ensembles until the controlled burns had been completely extinguished and they were 30 meters or more away from the burn structure. The controlled burns were conducted by the Illinois Fire Service Institute at the University of Illinois—Urbana-Champaign. The subset of samples described herein were comprised of end-tidal breath and served to confirm specific exposures. A detailed description of each round-day is available in the NIOSH study report (NIOSH 2013).

Breath sampling and analysis:

Breath samples were collected from each firefighter approximately 1-hour before the activity, about 10-min after the conclusion of the activity, and then 6-hours post activity. Subjects forcefully exhaled an entire breath through a Markes International Bio-VOC™ sampler wherein the last 127 ml of end-tidal air were retained, and subsequently transferred onto a Markes Carbograph 2TD/Carbograph 1TD thermal desorption tube (Markes International, Ltd., Llantrisant, UK). The tubes were shipped to the U.S. EPA laboratories in Research Triangle Park, NC for analysis. Tubes were sealed and stored under refrigeration from sampling, through shipment, and until immediately before analysis according to our standard internal procedures.

Adsorbent tubes were analyzed via methods developed at EPA for a variety of VOCs in all types of gas-phase matrices and sampling containers (Pleil and Lindstrom 1995, Pleil 2008, Hubbard et al. 2009). Specifically, for this project, we used an Ultra TD autosampler coupled with a Unity thermal desorber for the adsorbent tubes (Markes International, Ltd., Llantrisant, UK) coupled to a GC/MS system (6890N GC, 5973N MS, Agilent Technologies, Palo Alto, CA). The samples were analyzed in selected ion monitoring (SIM) mode with optimized ion groups and dwell times to achieve a sampling rate of approximately 2Hz and a minimum of 10 points per GC peak (Pleil et al. 1991). To insure that the peaks of interest were correctly identified, the peak area for each analyte was calculated and compared for both primary and secondary ions. Instrumentation was externally calibrated for all targeted analytes using certified standards (Accustandard, New Haven, CT, USA). Standard

instrument and tube blank analyses were performed to set the zero and span calibration slopes for quantitation. The levels of quantitation (LoQ) for the aromatic hydrocarbons are < 0.09 ppbv, and the LoQs for the 2 to 4-ring PAHs are in the range of 0.004 to 0.01 ppbv.

Human subjects:

Firefighter subjects were recruited as volunteers from the Chicago Fire Department. Inclusion criteria were non-smoking males, 45 years of age or younger, with instructor level firefighter qualifications. Subjects were instructed to refrain from eating charbroiled food and avoid second hand tobacco smoke for at least 2-days prior to their participation. Their respective Fire Chiefs ensured that volunteers had at least one day off before reporting to the study. Subjects participated with informed consent under Institutional Review Protocol HSRB 10-DSHEFS-03 administered by NIOSH.

Mathematical analysis:

This set of experiments has a complex nested data structure: 2 rounds, 3 days per round, 5 subjects per day, 3 samples per subject. We chose to composite the days within rounds into individual data sets (15 subjects each) to boost statistical power. Because twelve of the subjects appeared in both rounds, we had the opportunity to treat their results as repeat measures to gain insight into within-subject and between-subject variance components (Pleil 2009, Sobus et al. 2010, Pleil and Sobus, 2013). In addition to summary statistics comparing the temporal profiles of exhaled organics (i.e. pre, post and 6-hr post), we also assessed the individual subjects profiles by normalizing the post-work samples by the pre-work and 6-hr post-work samples as a baseline control. Data were compiled and analyzed using MS Excel (Microsoft Corp., Redmond, CA); graphics were created using GraphPad Prism (GraphPad Software Inc., La Jolla, CA).

Results

General observations:

Figure 1 presents all of the firefighter breath data in a heatmap. The data are arranged horizontally along the x-axis as three blocks (by subject number) representing pre-, post and 6-hr post-measurements. The vertical axis is arranged as two blocks representing rounds 1 and 2 with internal structure of compounds arranged in order from top to bottom by elution on the GC column. The corresponding fields in the x-y plane are color coded to represent the concentration of analytes in the breath according to the color scale (red = high to blue = low) found at the far right column of the graphic. From this observational tool, we see that there are large variances among subjects, there are no obvious major trends

between time points, and most of the apparent outliers occurred during round 1. Furthermore, the less volatile compounds (3-ring PAHs) have much lower levels in the breath than the more volatile single ring aromatics. The outlier group in the round 1, 6-hr post-work benzene levels (upper right side corner) comprised of subjects 6,7,8,10,11,12, and 15 are most likely due to some external exposures that were unnoticed by the field operatives; this was not observed in the second round. We believe that these exposures were unrelated to firefighting as the pattern of the other compounds seems to fit in well with the remaining data. We also note the occasional high outlier series of the volatiles, for example: round 1, pre-work, subject 15, round 2, pre-work, subject 4, and round 1, 6-hr post-work, subject 5. We note that the data appear log-normally distributed within compound, as expected, and as observed in the log nature of the color code. We confirmed this with subsequent calculations described below.

For context, we extracted results from previously published studies of jet fuel exposures in the U.S. Air Force (USAF) (Pleil et al. 2000, Pleil et al. 2011). The USAF studies measured the volatile JP-8 jet fuel constituents that included n-alkanes and single-ring aromatic compounds; for the comparison, we chose only those compounds that overlapped with the present study: benzene (Ben), toluene (Tol), ethylbenzene (Etb), m,p-xylene (mp-xyl), o-xylene (o-xyl), ethyl toluene (Etol) and 1,3,5-trimethylbenzene (Tmb). Figure 2 shows the direct comparisons in a two-part heatmap: the upper portion shows the pre- and post-exposure comparison for the firefighters, and the lower portion shows the pre- and post-exposure comparison for the USAF personnel. The heat map comparison shows very distinctly that the occupational exposures in the USAF studies were much more pronounced. In both the firefighter and Air Force studies the subjects wore supplied air respiratory protection, however, due to the confined spaces within the aircraft and concerns for explosion risk, the Air Force workers wore cotton overalls, and so, were much more likely to have dermal exposures from vapors and accidental contact with fuel. Figures 3a,b show typical exposure scenarios for both studies and documents the personal protective differences.

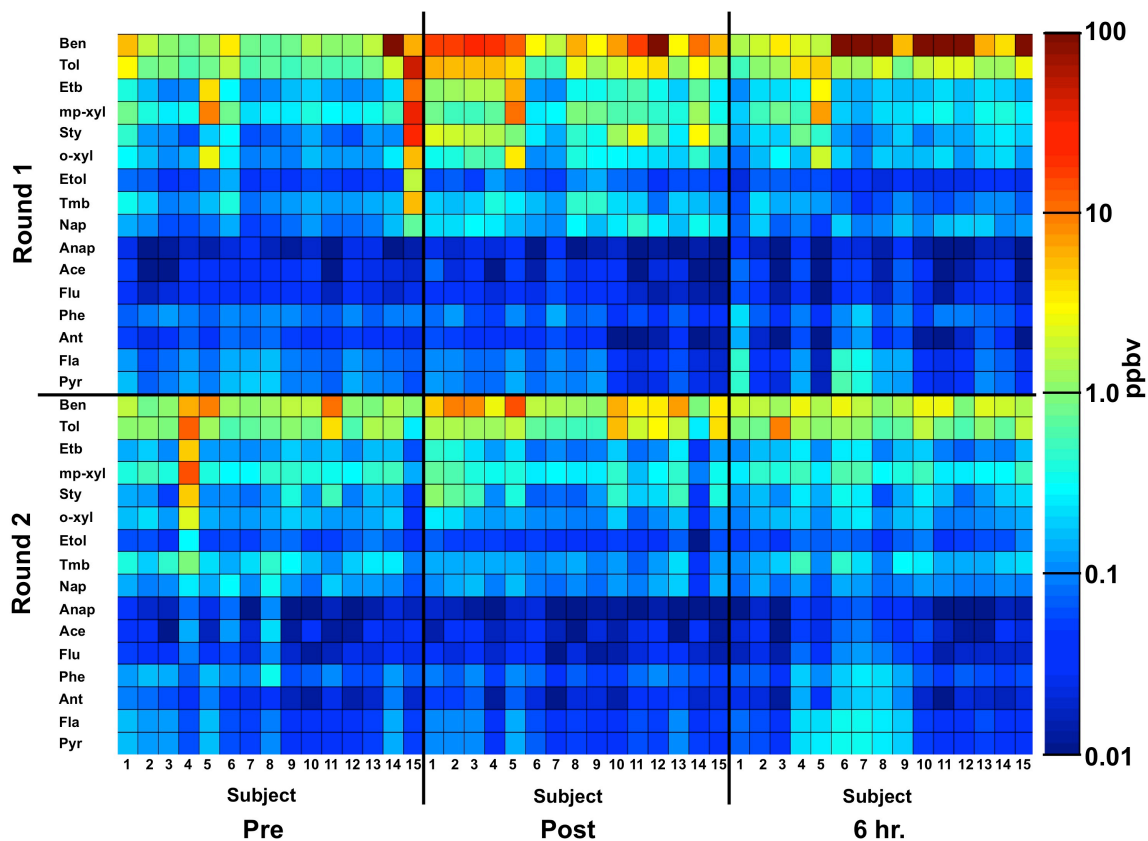


Figure 1. Heatmap of exhaled breath concentrations for NIOSH firefighters dermal exposure study grouped by exposure period (x-axis) and by experimental round and compounds (y-axis). Patterns indicate no major changes.

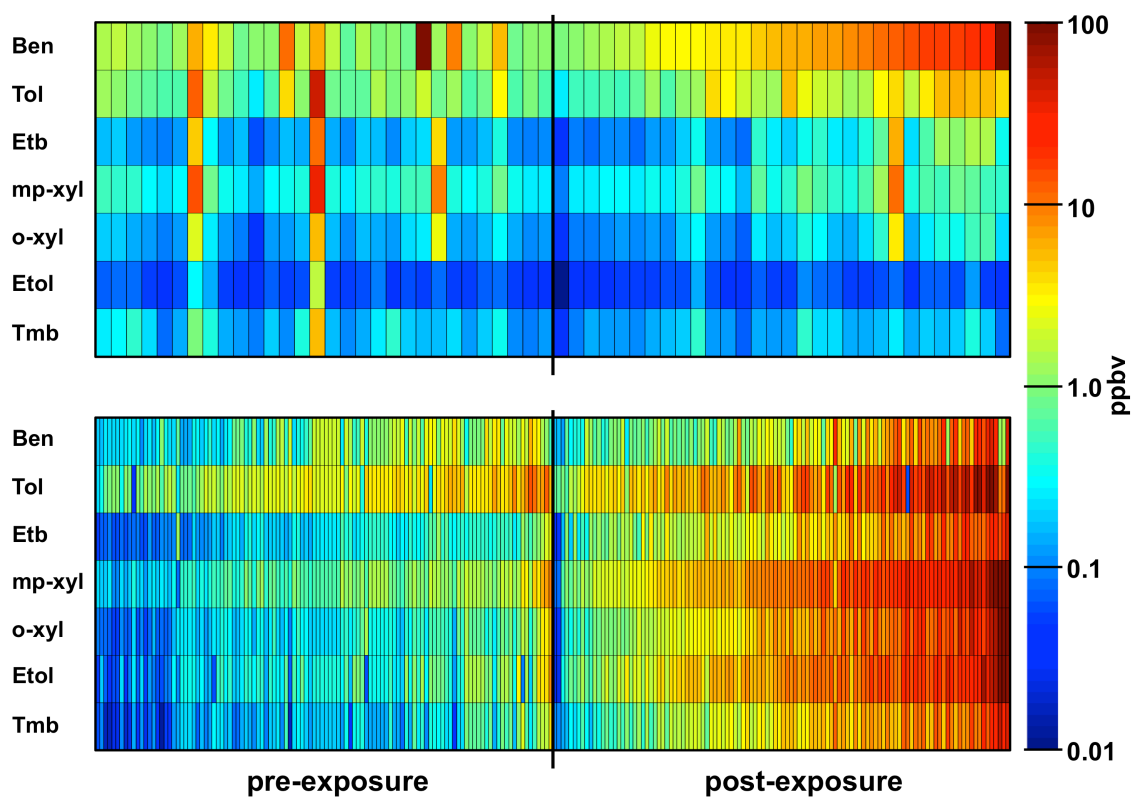


Figure 2. Direct heat map comparisons of occupational exposures to gas-phase hydrocarbons (single-ring aromatic compounds) as measured in exhaled breath. Upper section shows the heat map of fire fighters' exposures demonstrating a modest increase in benzene, and toluene immediately after the exposure. Lower section shows the corresponding heat map for Air Force subjects' exposures demonstrating across the board increases in exhaled aromatic hydrocarbons.

Figures 3a and b (below) show photographs of representative exposure scenarios for both studies and documents the personal protective gear. We see that the firefighters' turnout gear is much more substantive than the aircraft mechanics' gear (especially against dermal exposures) presumably because their workspace and general surroundings are much more hazardous and contaminated. These pictures are intended only to give the reader a comparative view of the occupational scenarios; due to the highly variable exposures in both cases, we cannot provide direct ambient air comparisons. However, we (NIOSH) had made a series of total PAHs measurements during the structural burns and found a median value of about 375 ppmv with an overall range of about 10 ppmv to 3,000 ppmv. We note that the Air Force environments were considered to be below levels of acute effects such as asphyxiation, inflammation, or neurological damage without protective gear, unlike the atmospheres encountered in structural fire.

a.

**Burning structure
and surroundings**



**Firefighters entering structure
for fire suppression in standard
turn-out gear**

b.

**Aircraft A10 “Warthog”
in maintenance hangar**



**Fuel systems maintenance
airman preparing for
tank entry in standard
protective gear**

Figures 3 a, b. Contrasting exposures and general surroundings from firefighter (a) and aircraft mechanics (b) studies. Visually, fire exposures appear more widespread and intense and firefighters' gear more heavily protective. Both occupations employ forced air respirators (albeit with different types); the difference may be that firefighters' gear is more effective against dermal exposures.

Statistical analysis – overall trends:

We calculated summary statistics for rounds 1 and 2 of all raw data for each of the 15 compounds and each of the three time points. As seen in the heat map in Fig. 1, there are a few inexplicable outliers in the measurements that might skew the mathematical results of the raw data; we subsequently removed these outliers from the data set if they exceeded the 99th percentile based on geometric mean (GM) and geometric standard deviation (GSD) calculations and recalculated the summary statistics. The summary statistics (n, GM, GSD, 95% value) are presented by rounds 1 and 2 and the three time points, pre-, post, and 6hr post exposure. As we saw in the qualitative patterns in the Figure 1 heatmap, the summary statistics confirm that there are no obvious overall trends. We present these results primarily to show the concentrations expected in such a scenario, and what could be considered “unremarkable” based on the pre- and 6hr post-exposure levels. We further note that all measurements were above the LoQs for their respective compounds.

Table 1. Summary statistics for exhaled breath concentrations (ppbv)

Concentration: ppbv	Pre- exposure				Post- exposre				6hr post-exposure			
Compound Round 1	n	GM	GSD	95%	n	GM	GSD	95%	n	GM	GSD	95%
benzene	14	1.469	0.620	5.262	14	7.713	0.722	36.551	9	4.152	1.090	42.027
toluene	14	0.869	0.449	2.330	15	2.323	0.580	9.975	15	1.580	0.471	4.622
ethylbenzene	13	0.152	0.366	0.364	14	0.447	0.528	2.095	14	0.180	0.292	0.284
m,p-xylene	13	0.355	0.344	0.759	14	0.554	0.354	1.245	14	0.287	0.367	0.689
styrene	14	0.114	0.420	0.345	15	0.826	0.593	4.728	14	0.167	0.357	0.376
o-xylene	13	0.149	0.329	0.291	14	0.278	0.382	0.717	14	0.163	0.323	0.311
4-ethyltoluene	14	0.055	0.306	0.119	15	0.070	0.282	0.131	15	0.037	0.312	0.084
1,3,5-trimethylbenzene	14	0.148	0.313	0.333	15	0.203	0.323	0.483	15	0.090	0.310	0.198
naphthalene	14	0.004	0.011	0.007	15	0.009	0.011	0.018	15	0.005	0.012	0.010
acenaphthalene	15	0.001	0.010	0.001	15	0.001	0.013	0.002	15	0.001	0.014	0.002
acenaphthene	15	0.001	0.014	0.004	15	0.001	0.015	0.004	15	0.001	0.018	0.006
fluorene	15	0.001	0.008	0.002	15	0.001	0.010	0.003	15	0.001	0.011	0.004
phenanthrene	15	0.004	0.007	0.005	15	0.003	0.008	0.006	15	0.003	0.010	0.009
anthracene	15	0.002	0.008	0.004	15	0.001	0.013	0.005	15	0.001	0.017	0.009
fluoranthene	15	0.004	0.007	0.007	15	0.002	0.010	0.008	15	0.003	0.015	0.025
pyrene	15	0.005	0.007	0.009	15	0.003	0.010	0.009	15	0.003	0.016	0.029
Concentration: ppbv	Pre- exposure				Post- exposre				6hr post-exposure			
Compound Round 2	n	GM	GSD	95%	n	GM	GSD	95%	n	GM	GSD	95%
benzene	15	1.779	0.708	8.131	15	3.280	0.733	16.002	14	1.696	0.427	3.064
toluene	14	0.954	0.482	2.912	15	1.264	0.596	5.708	13	0.966	0.376	1.868
ethylbenzene	14	0.133	0.308	0.230	15	0.136	0.445	0.464	14	0.143	0.352	0.318
m,p-xylene	14	0.365	0.324	0.696	15	0.318	0.375	0.792	14	0.380	0.321	0.715
styrene	14	0.126	0.437	0.411	15	0.215	0.613	1.305	14	0.159	0.379	0.400
o-xylene	14	0.138	0.327	0.268	15	0.123	0.358	0.281	14	0.143	0.319	0.265
4-ethyltoluene	15	0.066	0.318	0.154	15	0.045	0.307	0.098	14	0.064	0.274	0.112
1,3,5-trimethylbenzene	15	0.244	0.363	0.722	15	0.120	0.290	0.235	14	0.192	0.336	0.493
naphthalene	15	0.004	0.011	0.007	15	0.005	0.011	0.009	14	0.004	0.010	0.007
acenaphthalene	13	0.001	0.012	0.002	15	0.000	0.012	0.001	14	0.001	0.015	0.004
acenaphthene	14	0.001	0.015	0.006	15	0.001	0.010	0.002	14	0.001	0.013	0.005
fluorene	14	0.001	0.011	0.004	15	0.001	0.009	0.002	14	0.001	0.013	0.006
phenanthrene	14	0.004	0.008	0.008	15	0.003	0.008	0.005	14	0.004	0.010	0.012
anthracene	15	0.001	0.012	0.006	15	0.001	0.010	0.004	14	0.002	0.017	0.013
fluoranthene	15	0.003	0.009	0.008	15	0.003	0.008	0.006	14	0.004	0.012	0.020
pyrene	15	0.003	0.009	0.009	15	0.003	0.008	0.006	14	0.004	0.013	0.023

We subsequently reduced the composite data from Table 1 to assess statistical significance of any of the time point differences within the overall scatter of the

results. Table 2 shows one-sided significance ($\alpha = 0.05$) for an increase of the post-exposure value over the pre-exposure value (post>pre) and the post exposure value over the 6hr post exposure value (post>6hr). The assumption that 6-hrs are sufficient to eliminate exposures from the 1-hr firefighting activity is reasonable as breath uptake and elimination kinetics are generally symmetric (Pleil and Lindstrom 1998). Research has also shown that dermal exposures have similar kinetics for non-polar compounds and naphthalene has been used as the marker for fuels research (Kim et al. 2007). Results are presented for each round separately and as a compilation of both rounds. The measurements were treated as independent so we did not administer multiple-test corrections.

We see that overall there is a trend that most of the single-ring aromatics and naphthalene have a statistically significant exposure effect in at least one category. The 3- and 4-ring PAHs do not show any significant trends; this is expected, as short-term exposures are generally not sufficient to overcome the general background of systemic PAHs accumulated from other environmental sources, especially food. Previous studies have shown that short term (2-hr) scripted exposures to diesel exhaust, for example, do not statistically increase the higher PAHs, and that overall, blood and plasma samples in randomly selected healthy subjects have similar ranges of onboard PAHs (Sobus et al. 2010, Ghio et al. 2012). Detailed analyses with human meta-data and multiple studies have confirmed this observation (Lu et al 2014).

Table 2. Exposure significance by compound and experimental round ($\alpha = 0.05$)

Compound	Round 1		Round 2		Aggregate	
	post > pre	post > 6hr	post > pre	post > 6hr	post > pre	post > 6hr
benzene	yes	-	yes	yes	yes	yes
toluene	yes	-	-	-	yes	-
ethylbenzene	yes	yes	-	-	yes	yes
m,p-xylene	yes	yes	-	-	-	yes
styrene	yes	yes	yes	-	yes	yes
o-xylene	yes	yes	-	-	-	-
4-ethyltoluene	yes	yes	-	-	-	-
1,3,5-trimethylbenzene	yes	yes	-	-	yes	-
naphthalene	yes	yes	yes	-	yes	yes
acenaphthalene	-	-	-	-	-	-
acenaphthene	-	-	-	-	-	-
fluorene	-	-	-	-	-	-
phenanthrene	-	-	-	-	-	-
anthracene	-	-	-	-	-	-
fluoranthene	-	-	-	-	-	-
pyrene	-	-	-	-	-	-

Statistical analysis – individual (subject) trends:

We considered that the overall variability among subjects might have obscured individual (auto-correlated) changes. We explored this phenomenon by normalizing each post exposure measurement per individual by the average of their own pre-exposure and 6hr post-exposure values (under the assumption that 6-hrs is sufficient to wash out the immediate increments from the fire fighting activity). We used the data (Table 1) from which the extreme outliers had already been removed to harmonize the comparisons. We found that some firefighters had significant increases in breath biomarkers measured immediately post-work whereas others had no statistical changes. Table 3 shows the results at the individual level. For this exercise, we arbitrarily considered the individual's exposure to be of interest if the post-value is at least 50% higher than the established individual baseline.

Table 3. Individual firefighters, by round, exhibiting greater than 50% increase in exhaled breath concentration over their own baseline values.

Compounds	Round 1	Round 2
benzene	2, 4, 8, 11, 12, 13, 15	3, 4, 5, 7, 12, 13, 14
toluene	2, 7, 8, 9, 11, 13	3, 4, 14
ethylbenzene	2, 5, 7, 8, 9, 11, 13, 14	1, 5, 13, 14
m,p-xylene	2, 5, 6, 7, 11, 13	1, 14
styrene	1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 14	1, 3, 4, 5, 7, 12, 13, 14
o-xylene	2, 5, 7, 8, 11, 13, 14	1, 14
4-ethyltoluene	2, 4, 5, 6, 12	1
1,3,5-trimethylbenzene	2, 4, 5, 6, 7, 12	1
naphthalene	2, 3, 4, 6, 7, 8, 11, 12, 13, 14	5, 7
acenaphthalene	4, 8, 9, 10	6
acenaphthene	4, 9, 10	2, 5
fluorene	4, 9, 10	5
phenanthrene	8, 10	5
anthracene	4, 8	5
fluoranthene	4, 8	5
pyrene	4, 8	5

Here we see that certain firefighters were more consistently exposed than others. We also see that round 2 had appreciably fewer firefighters with relevant exposures. Overall, firefighters #'s 2, 4, 8, 11, and 13 in round 1, and #'s 1, 5, and 14 in round 2 exhibited significant exposure hits for 6 or more compounds, whereas #'s 1 and 3 in round 1, and #'s 2, 6, 7, 8, 9, 10, and 15 in round 2 had insignificant incremental exposures (1 or none exceedances). We note that firefighters numbered 1 through

12 are the same individuals in both rounds; these data indicate that exposures seem to be driven by the particular events, rather than by individual traits or behaviors. We also suggest that the specific training exercises, even within the same experimental round, are highly variable as fires (even under controlled burn conditions), are subject to many different factors. These observations are more robustly evaluated in the next section.

Statistical analysis – intra-class correlations:

An important feature of environmental and occupational exposure science is the assessment of within- and between subject variance components (σ_w^2 and σ_b^2 , respectively). This helps discern how to mitigate exposure exceedances and to get an estimate of average exposures that influence long-term risk. We have discussed this in detail elsewhere (Pleil and Sobus 2013), but briefly, when repeat measures are available, one can estimate the intra-class correlation co-efficient (ICC), which is defined as $ICC = \sigma_b^2 / (\sigma_w^2 + \sigma_b^2)$ and takes on a value between 0 and 1. When ICC is near 1, most of the variability is between subjects and implies that repeated measures for any individual yield approximately the same value; when ICC is closer to 0, repeated measurements will be fairly random regardless of the individual measured.

In this study, 12 of the 15 total subjects were common to rounds 1 and 2, and so we could treat them as having independent “repeat measures” one year apart. We arranged the data to pair round 1 to round 2 measurements for all compounds by sampling time point.

Table 4. ICC calculations using Round 1 and Round 2 as repeat measures

Compound	pre	post	6hr
benzene	0.000	0.811	0.840
toluene	0.030	0.505	0.175
ethylbenzene	0.000	0.899	0.674
m,p-xylene	0.000	0.789	0.000
styrene	0.011	0.876	0.168
o-xylene	0.000	0.881	0.620
4-ethyltoluene	0.252	0.789	0.733
1,3,5-trimethylbenzene	0.732	0.822	0.795
naphthalene	0.792	0.890	0.229
acenaphthalene	0.000	0.563	0.000
acenaphthene	0.000	0.331	0.000
fluorene	0.000	0.649	0.000
phenanthrene	0.000	0.000	0.000
anthracene	0.571	0.000	0.000
fluoranthene	0.731	0.134	0.000
pyrene	0.816	0.344	0.000

From Table 4, we interpret low ICC ($ICC < 0.4$) as indicating relatively weak year-to-year correlation, and high ICC ($ICC > 0.5$) as indicating stronger year-to-year correlation within the respective sampling time-points. These results are based on non-dimensional analysis (performed in log-space) and so absolute exposure levels

are divided out; what we can interpret is the relative influences from environmental and occupational (firefighting) sources. We see that the post-time point ICC values for the single-ring aromatics (benzene through 1,3,5-trimethylbenzene) and some of the PAHs (naphthalene, acenaphthalene and fluorine) are >0.5 indicating that these compounds affected firefighters similarly during rounds 1 and 2 exposures. The corresponding low ICC values in the pre column indicate that the environmental exposures are fairly random for these subjects. This is confirmed by the nature of exposure to volatiles (single-ring aromatics) wherein the elimination half-lives are relatively short. What is of particular interest is the handful of high ICC values in the 6-hr column; we would have expected these values to revert to their respective pre levels. These unexpected results indicate that there were likely some ongoing residual exposures of benzene, ethylbenzene, etc. from unknown sources that were too subtle to be seen in the qualitative heatmaps in Figure 1.

Conclusions

This study demonstrated that exhaled breath biomarkers represent a useful technology for assessing occupational exposures of firefighters to products of combustion, especially of toxic aromatic compounds. Of particular note is the novel application to the less volatile 3- and 4-ring PAHs (ranging from acenaphthalene to pyrene) that generally require blood or urine sampling. We found that background (pre-exposure and 6-hr post-exposure) levels are within the sensitivity of the methodology, that the single-ring compounds have overall higher levels in breath than the PAHs, and that the breath-borne concentrations can range from low parts per trillion (pptv) to 100's of parts per billion (ppbv) (Figure 1, Table 1).

We found that there are immediate post-exposure exceedances above an expected baseline of many of the gas-phase and semi-volatile aromatic compounds (Table 2). Furthermore, we found that individual firefighters have widely varying exposure profiles (Figure 1, Table 3) and we attribute this to specific situations, activities, timing and microenvironments from the inherent heterogeneity of fires. From the calculations of ICC's using round 1 and round 2 as repeat measures, we conclude that the exposures are most likely driven by situational (between-subject) rather than within-subject variance components, and that there were some residual exposures from unknown sources occurring during or just prior to the 6-hr post-work period.

In Figure 2 we demonstrate that the protective gear appears to be effective in protecting firefighters from toxic chemicals as shown in the contrast with jet-fuel maintenance workers protective gear (as illustrated in Figures 3 a, b.). Although the overall internal doses experienced from firefighting were shown to be relatively low, future work should focus on the particular events and situations that could give individuals an outlier exposure. Future tests should also be cognizant of the potential for post-work re-exposures, possibly from residual inhalation exposures or dermal exposures once the protective gear is removed.

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