University of Cincinnati

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I, Tiffany R Beddoe, hereby submit this original work as part of the requirements for the degree of Master of Science in Industrial Hygiene (Environmental Health).

It is entitled:
Biomarkers of Polycyclic Aromatic Hydrocarbon (PAH) Exposure in Firefighters

Student's name: Tiffany R Beddoe

This work and its defense approved by:

Committee chair: Glenn Talaska, PhD
Committee member: Charles Stuart Baxter, PhD
Committee member: Tiina Reponen, PhD
Biomarkers of Polycyclic Aromatic Hydrocarbon (PAH) Exposure in Firefighters

Thesis submitted to the University of Cincinnati
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In partial fulfillment of the requirements for the degree of

Master of Science

In the Department of Environmental Health
of the College of Medicine
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By

Tiffany R. Beddoe
B.S., Eastern Kentucky University, 2007

Committee: Glenn Talaska, Ph.D. (Chair)
Tiina Reponen, Ph.D.
Charles Baxter, Ph.D.
Abstract

Firefighters are exposed to numerous hazards, including particulates and fumes, and physical, thermal and emotional stresses encountered during firefighting. The particulate and gaseous phases of smoke contain polycyclic aromatic hydrocarbons (PAHs), which are known carcinogens. A recent meta-analysis suggests that firefighting is linked to elevated risk of testicular, multiple myeloma, non-Hodgkin’s lymphoma, and prostate cancers.

We hypothesized that the urinary levels of 1-hydroxypyrene (1-HP) and DNA adducts in firefighters will be significantly greater than the Kelly baseline samples and that 1-HP levels in firefighters will be elevated following a fire suppression event. PAH exposures of firefighters after a fire event were assessed by measuring 1-HP levels and DNA adducts in exfoliated urothelial cells and compared to those in baseline Kelly day (5 consecutive days off work) samples.

The urine samples were analyzed for 1-HP according to the method by Jongeneelen et al. The DNA was isolated from the pellet using the Wizard Genomic DNA kit and then analyzed for DNA adducts by $^{32}$P-postlabelling adenosine 5'triphosphate (ATP) excess, thin layer chromatography and scintillation counting.

A total of 24 non-clean catch urine samples were collected from 15 firefighters after firefighters returned from their Kelly day (13 urine samples) and after a fire suppression
event (10 urine samples) and one sample was omitted because of labeling. Five firefighters gave both Kelly day and post fire samples. One gave 2 Kelly day samples and another gave 3 post fire samples. Results were averaged if more than one was collected.

One of the twelve 1-HP baseline samples exceeded the ACGIH recommendation of 1 μg/l. Three of the eight 1-HP post fire samples, exceeded the ACGIH recommendation. Four of the five post fire 1-HP levels were higher than the Kelly Day 1-HP levels, which suggest that the firefighters were exposed to PAHs during fire fighting. The fluctuation of 1-HP values between baseline and post-fire events can be explained by differences between fire events and subject variability between fire fighters.

Understanding firefighters PAH exposures will assist in future research in evaluation of the relationship between firefighter exposure and cancer incidence. The study also increased knowledge about exposure characterization, which could assist in decreasing cancer risk in firefighters or occupational groups who are exposed to similar agents.
Acknowledgement

I sincerely want to thank my advisor, Glenn Talaska, for having patience and faith in me throughout the year. I thank him for his ongoing encouragement, guidance, mentoring, humor, and availability. Brenda Schumann, thank you for having faith in me and always being there to help me in lab or talk to when things weren’t going as well as I was hoping. Don Goins and Jeff Thoroman, what would I have done without you guys. Thank you for always being there through the good and the bad. I will never forget any of you all. You have all contributed a part to who I have become this last year and always, I will miss you all!

Thank you to my committee for all their hard work, late hours revising my paper and patience.

To my family and friends, thank you for understanding that I was very busy. I would have never made it without each one of you. To my mom, thank you for always encouraging me, I love you! To my friends and classmates, thank you for always being available when I had a long week in lab. Best of luck to all of you in your future endeavors, I will miss all of you.
Table of Contents

Abstract.............................................................................................................................. ii
Acknowledgement........................................................................................................... v
List of Tables................................................................................................................... vii
List of Abbreviations....................................................................................................... viii
1.0 Introduction................................................................................................................. 1
2.0 Hypothesis.................................................................................................................... 3
3.0 Specific Aims............................................................................................................... 3
4.0 Materials & Methods................................................................................................. 4
   a. Materials.................................................................................................................. 4
   b. Sample Collection and Storage............................................................................. 4
   c. Preparation of Samples for Analysis................................................................. 5
   d. 1-HP Analysis....................................................................................................... 5
   e. Analysis of DNA Adducts.................................................................................... 6
   f. Human Subjects..................................................................................................... 9
   g. Laboratory Safety.................................................................................................. 10
   h. Statistical Analysis............................................................................................... 11
5.0 Results...................................................................................................................... 11
6.0 Discussion.................................................................................................................. 13
Tables.............................................................................................................................. 16
References....................................................................................................................... 19
Appendices...................................................................................................................... 21
List of Tables

Table 1: Baseline and Post-Fire Event 1-HP levels
Table 2: 1-HP Levels for Firefighter Baseline and Post-Fire Event Samples
Table 3: Total DNA Adducts for Firefighter Baseline and Post-Fire event Samples
List of Abbreviations

1-HP – 1 Hydroxypyrene
ACGIH – American Conference of Governmental Industrial Hygienists
ATP – Adenosine triphosphate
β-Gluc – β-glucuronidase/arylsulfatase
BEI – Biological Exposure Indices
Cpm - Counts per Minute
DNA – Deoxyribonucleic acid
GM - Geometric mean
g - Grams
L - Liters
μg - Micrograms
μl - Microliters
mg - Milligrams
HPLC – High performance liquid chromatography
LOD – Limit of detection
MNSPD - Micrococcal Endonuclease/Spleen Phosphodiesterase
Ng - Nanograms
NIOSH - National Institute for Occupational Safety and Health
PAH – Polycyclic aromatic hydrocarbon
PPE – Personal protective equipment
PNK - Polynucleotide Kinase
RAL - Relative Adduct Level
SCBA – Self-contained breathing apparatus
SD - Standard deviations
1.0 Introduction

The nature of firefighting puts unusually high demands on firefighters’ bodies. On-duty firefighters are exposed to numerous hazards including gaseous chemicals, particulates, gases, fumes and physical, thermal and emotional stresses \(^{(1, 2, 3)}\). The particulate and gaseous phases of smoke have both been shown to contain polycyclic aromatic hydrocarbons (PAHs), several of which are known carcinogens \(^{(4)}\). Exposures containing PAHs have been linked to lung, testicular, multiple myeloma, non-Hodgkin’s lymphoma, and prostate cancers \(^{(3, 5)}\). The burning of building materials produces a variety of different chemicals depending upon the type of material. The toxic chemicals produced during a fire include carbon monoxide, hydrogen cyanide, and others depending on the nature of the fire \(^{(13)}\). Particulate matter, including ultrafine particles, is also produced during the fire. A study by Bolstad-Johnson discovered that ceiling or short term exposure levels were exceeded during overhaul for acrolein, benzene, carbon monoxide, formaldehyde, glutaraldehyde, nitrogen dioxide, polynuclear aromatic hydrocarbons and sulfur dioxide \(^{(14)}\). The World Health Organization (WHO) classified occupational exposures of firefighters as “possibly carcinogenic to humans” as a result of being exposed to numerous carcinogens, including polycyclic aromatic hydrocarbons, benzene, styrene, and other materials and chemicals \(^{(15)}\).

PAHs can enter the body via inhalation, ingestion, and dermal absorption. Therefore it is more relevant to use biological rather than environmental monitoring to estimate the internal dose for all routes of exposure. Biomarkers can give more accurate estimations by encompassing absorption, metabolism, distribution, and individual differences.
including body fat, genetics, and respiratory rates (9). 1-hydroxypyrene (1-HP) is a metabolite of pyrene found in urine and is used as a biological marker of exposure (7). 1-HP urinary elimination is tri-phasic with half-lives of 5 hours, 22 hours and 17 days and urinary 1-HP levels in post shift urine samples mainly reflect an individual’s daily variable PAH exposure while pre-shift, before work week of urinary 1-HP levels reflect chronic exposures. Pyrene is used for PAH measurements because of its correlation with levels of total PAH and carcinogenic PAHs (9). Data concerning 1-HP use, as a biomarker in firefighters’ exposure is limited; however, it has been used in several studies (5, 10), with elevated 1-HP levels reported after exposure to burning diesel oil (4, 11).

DNA adduct levels integrate a longer exposure time span since urothelial cells have a life-span of 50 to 100 days (9). DNA adducts, covalent modifications to DNA, are formed after exposures to PAH’s in bladder cells. Bladder cells are sloughed off and excreted in urine daily. Dependent upon the amount of exposure and amount of cells that are in each sample, these daily samples may only contribute fractionally to adduct levels if exposures are not continuous.

The relationship between firefighting activities, 1-HP, and DNA adducts has not been completely reviewed in previous studies. Studies have linked PAH exposure to cancers and job tasks that can lead to potential exposures of PAH’s, including firefighting. This section is only part of a comprehensive firefighting study that will link PAH exposure to firefighting by reviewing blood, urine, and dermal analysis before and after a fire.
Understanding the effects of firefighters’ PAH exposures will assist in future research in evaluation of the relationship between exposure and cancer incidence. The study will also increase knowledge about exposure characterization, which could assist in decreasing cancer risk in firefighters or other occupational groups who are exposed to similar agents.

2.0 Hypothesis

The urinary 1-HP levels will exceed the American Conference of Governmental Industrial Hygienists (ACGIH) Biological Exposure Indices (BEI) of 1 μg following the fire event (6). The urinary 1-HP and DNA adducts levels will be higher after the fire suppression event compared to the Kelly day (5 days off work) baseline.

3.0 Specific Aims

Specific Aim 1: Determine the 1-hydroxypyrene concentration in urine in a cohort of experienced firefighters.

Specific Aim 2: Determine the DNA adduct concentration in urine in a cohort of experienced firefighters.

Specific Aim 3: Determine relationship between occupational exposure to PAH before and after a fire suppression event
4.0 Materials & Methods

a. Materials

Materials used in the procedure are listed in Appendix A.

b. Sample Collection and Storage

Non-clean catch urine samples were collected from 15 firefighters after returning from their Kelly day (5 days off work consecutively) and after returning from a fire suppression event. Twenty-four urine samples were collected; 13 were Kelly day samples, 10 were post-fire samples and one sample was omitted because of labeling. Of the 23 samples, 5 firefighters gave both Kelly day and post-fire samples, one gave two Kelly-day samples, and another gave 3 post fire day samples. Results that were collected in excess of more than one were averaged for this study. The samples were stored in a cooler with frozen ice blocks at the fire station until transport to the lab freezer. The urine samples were stored in the -20°F freezer until analysis. The samples were blinded prior to analysis.

Firefighter's age, sex, ethnicities and years of firefighting experience varied. The number of samples collected was small and variable. In total, twelve baseline Kelly day samples and eight post-fire event samples were collected (after averages).
c. Preparation of Samples for Analysis

The samples were thawed and passed through a 10μm pore filter to remove the exfoliated urothelial cells. The filtered urine was saved for analysis for 1-hydroxypyrene (1-HP). The cells were scraped from the filters with a rubber policeman and rinsed with phosphate buffered saline solution (1x PBS), the urothelial cells were then collected from the filters. The cells and saline solution were placed into a 15ml tube and centrifuged at 600rpm for 5 minutes to separate the pellet from the supernatant, which was removed by aspiration and the pellet washed 3 times in 3 to 5 ml of PBS and centrifuged for 5 minutes at 600rpm each time (7, 8). The DNA was isolated from the pellet using the Wizard Genomic DNA kit by following the kits specified directions for cell isolation. Once the DNA was isolated, its mass was determined using the Nanodrop instrument. A DNA concentration of 0.5μg/μL was considered acceptable for continuing with the $^{32}$P-Postlabeling.

d. 1-HP Analysis

The urine that was previously filtered was used to measure 1-HP concentrations based upon the method by Jongeneelen et al (7). Fifteen ml of urine were dispensed into a 50ml tube and the pH was adjusted to 5.00 ± 0.5 with 1 M HCL. The sample was hydrolyzed by adding 5ml of 0.1 M Sodium Acetate and 8.75μl of β-glucuronidase/arylsulfatase and then incubated at 37ºC and agitating for 4 hours using a mixing platform. A Waters C18 Sep-Pak® was primed by passing through 5ml HPLC grade methanol followed by 10ml of Milli-Q water. Sixty Percent of the urine sample
was then loaded and passed through the Sep-Pak® plus C18 at a rate of <2.5 ml/minute. After the remaining 40% was filtered through, the filter was washed with 8ml of Milli-Q water. Ten (10) ml of HPLC grade methanol was passed through the Sep-Pak® Plus C18 and collected into a 25ml glass scintillation vial. The methanol was evaporated under a gentle flow of nitrogen in a 60°C water bath and 2ml of HPLC grade methanol was added to re-suspend the sample. A 0.45μm syringe filter was rinsed with 5ml of HPLC grade methanol and dried. The sample was then filtered through the filter into a brown HPLC glass vial and placed into the HPLC autoinjector system. The HPLC system (Waters Alliance) used the 2695 solvent system, a column heater and a 474 fluorescence detector.⁶,⁸,⁹

e. Analysis of DNA Adducts

DNA was analyzed by ³²P-Postlabeling, developed by Reddy and Randerath. DNA adducts were quantitated in duplicate using excess adenosine 5’triphosphate (ATP), by thin layer chromatography and scintillation. The experiments were labeled FFS1 to FFS6. FFS1 and FFS2 were both discarded because the solvent was too strong, which caused the samples to run more normal. The day before postlabeling the DNA was hydrolysed into phosphonucleotides. The hydrolysis was completed by making a MN-SPD mixture and adding 6 μl of the mixture to 4μl of each duplicate DNA sample. The microcentrifuge vials containing 10 μl of the sample were centrifuged for 1 minute and placed in a 37°C degree incubator for 3 hours. The samples were then stored overnight at -70°F and allowed to thaw. After preparing the workstation (pipettes, Plexiglas rack, forceps, and placing a piece of work paper over the area) the P32 mixture was mixed. A
P32 mixture was made consisting of 27 μl of PNK buffer, 5.4 μl of PNK and calculated amounts of the bicine and synthesized P32-ATP. The calculation used to determine the amount of synthesized P32-ATP is as follows:

\[
\frac{200 \text{ counts}}{(\text{Start volume} \times \text{Decay rate})} \times 18 (\text{Number of samples}) = \text{Amount of P32 required}
\]

The volume of P32 mixture was adjusted to 72 μl by the addition of bicine. Four (4) ml of the P32 mixture were added to each sample tube, which was centrifuged for 1 minute and incubated for 40 minutes at 37ºC. Tubes were prepared and labeled 1A-8B with 746μl of bicine and refrigerated until needed. Thirty six (36) ml of Apyrase and 144μl of Bicine (Apyrase mixture) were added in a separate microcentrifuge tube and centrifuged to mix together. Ten (10) ml of Apyrase mixture was added to each tube after the 40-minute incubation and centrifuged for 1 minute. Duplicate tubes prepared earlier were removed from the refrigerator and 4ml solution from the original tubes were added by pipette to the duplicate tubes (original 1A to duplicate 1A) which were then centrifuged. The original tubes that contained 20μl were placed in the refrigerator. Three 10x20cm chromatograms, known as the normal, areas were marked for spotting 1A-8B and each chromatogram was labeled with LiCL (2) or PO4 (1). Sixteen 10x10 chromatograms were labeled with sample number (1A-8B) and an area was marked (point of origin) for the spotting of the sample. A long wick was placed on the top of the 10x10 with hairpins. From the duplicate tubes, 5μl of each sample were spotted by pipette (1A-8B) on the three 10x20 chromatograms normals. After making sure each spotted sample was dry, the designated (by label on chromatogram) chromatograms were placed in their
respective solvents (LiCl and PO4). The originals were removed from the refrigerator and 18µl spotted by pipette on the point of origin for each respective sample. After 8 of the 16 plates were completed, they were placed in the hood close by to decrease radioactive exposure, after checking for contamination on gloves. After spotting all 10x10's and making sure they were all dry, the chromatograms were placed in the first solvent, D1, making sure there was enough solvent in the tank to cover the bottom of the chromatogram. The wick of the chromatogram was draped over the back of the tank and allowed to run over night. The normals should run completely, they were then dried, marked with a hot pen, placed in cassettes and taken to the dark room. While working in the dark, the films were put on the plates, exposing for 5-15 minutes and the film developed. The films were labeled. 10x20's (normals) were saved in Plexiglas boxes and the cassettes were cleaned. The chromatograms in the D1 were checked to make sure they were running up the plates and wicks properly.

The following morning, the plates were removed from the D1 solvent. The 10x10's were put into a tub of deionized water and the plates were washed thoroughly and dried behind Plexiglas. They were then dipped in deionized water and placed in the D3 Solvent. The plates were placed in 37ºF incubator and solvent allowed completely run to the top of the plate. They were then rinsed in deionized water and allowed to dry. The 10x10's were dipped in deionized water then placed in the D4 solvent. Once the plates had run, they were rinsed in deionized water and allowed to dry. Short wicks were attached to the plate with hairpins and the 10x10's were dipped in deionized water then placed in the D5 solvent. Once they had run completely, the hairpins were removed and
the plates allowed to dry. Each plate was identified with a hot pen. Cassettes each containing four plates were placed in a dark room. While in the dark, film was placed on the plates inside the cassettes. The cassettes were stored at -70ºF for 48-72 hours. The cassettes were then taken to the dark room and developed. Each film was circled and numbered by Dr. Talaska to identify areas of DNA adducts on the plates and a standard to compare too. The circles were traced onto the plates, cut out and placed into scintillation vials with 5ml of 70% EtOH. The scintillation vials were placed into a Packard Tri-Carb liquid scintillation analyzer and counted.

Carcinogen DNA adducts where quantified by determining the relative adduct level (RAL). The RAL is the ratio of counts per minute in the adducted nucleotides to the counts per minute in the normal nucleotides relative to the concentrations of adducts/DNA normalized to adducts per $10^8$ nucleotides $^{(8,9,12)}$.

\[ f. \text{ Human Subjects} \]

This study was conducted as a partnership between the Cincinnati Fire Department (CFD) and the University of Cincinnati, Department of Environmental Health. The cohort of 25 firefighters was recruited and consented for the study. Each firefighter had to have 5 years of firefighting experience to be included in the study. The firefighter’s participation in the study consisted of giving urine, blood, and saliva samples, face and skin wipe samples and completing questionnaires. Potential risk to employees was deemed minimal as non-invasive biological samples were collected. The only cost to the
subjects was the time required to complete the questionnaires and to give the samples. Firefighters gave consent to participate in the study.

Recruitment and Informational sessions were provided two to three times to each of the three units in the fire house. The study was explained and emphasized by the investigators and the CFD that participation was strictly voluntary. Potential study subjects were given an opportunity to ask questions about the study and were given an extended amount of time to decide if they wanted to participate. Identifiable data was collected on each subject and measures were taken to ensure the confidentiality of the volunteers. These included: keeping the only copy of the questionnaires in a locked cabinet, allowing only selected investigators to open data, and keeping samples in a locked laboratory in the Kettering Laboratory. The analyzer of the samples was blinded and the samples were coded by a numbering system prior to the analyzer seeing the samples. The study received University of Cincinnati IRB approval (IRB# 8-11-12-01).

\(g. \text{ Laboratory Safety}\)

Prior to being able to begin sample analysis radiation training was conducted by the University of Cincinnati’s (UC) Radiation Department. Training was completed because the \(^{32}\)P postlabeling can use large amounts of \(^{32}\)P during the experiment. Personal protective equipment (PPE) used in the laboratory included laboratory coat, disposable laboratory coat, two pairs of nitrile gloves, safety glasses, disposable tyvex sleeves, and disposable booties. Workstations were equipped with lead or Plexiglas shielding materials to shield the analyst from \(^{32}\)P activity. Prior to and after the analysis Geiger
Muller Counters scanned the workstation and surrounding area, including the analyst. Each analyst’s exposure was measured by a monthly dosimetry badge and ring that was turned into UC’s radiation department.

Radioactive waste generated throughout the experiment was temporarily stored in a plastic lined glass container with lid positioned behind a lead shield in the workstation. UC Radiation Safety Department personnel removed the accumulated radioactive waste when notified.

h. Statistical Analysis

Mean values were determined for the Kelly day and post fire event samples along with a standard error for the mean values. The paired T-test on log-transformed data was used to examine if there was a statistically significant difference between the Kelly Day and Post Fire suppression event values. The Correlation between 1-HP and DNA adducts was assessed by using the Pearson Correlation Coefficient. P-values were determined for statistical significance between pre and post 1-HP and compared to the p<0.05 statistical criterion. The data were not tested for normality.

5.0 Results

Of the twelve 1-HP baseline samples analyzed, one exceeded the ACGIH recommendation of 1 μg/l. Out of the eight 1-HP post-exposure samples, 3 exceeded the ACGIH recommendation. Four of five 1-HP samples increased after exposure to a
fire event (Table 1). Baseline Kelly day 1-HP average was 0.506 μg/l and post-fire 1-HP event average 1.122 μg/l. The average 1-HP values doubled after exposure to a fire event compared to the average baseline Kelly day values (Table 2). The post fire event average was above the ACGIH BEI recommendation of 1 μg/l, thus being significant enough to warrant further studies. Firefighter #105 had the highest 1-HP level in the post fire event sample (2.540 μg/l).

Total DNA adduct levels in two of five firefighters increased after a fire event. Firefighter #101 had the highest total DNA adduct level after a fire event at 183.7. The average Baseline total DNA adducts level was 40.1. The average post-fire event total DNA adduct level was 39.2 (See Table 3).

The hypothesis for this study was that the 1-HP values would exceed the ACGIH BEI of 1μg/l following a fire event. The null hypothesis was that internal dose levels in firefighters would not exceed the ACGIH BEI of 1 μg l⁻¹ following a fire event. Three of the five firefighter samples were above the ACGIH BEI following a fire event. The other hypothesis stated that firefighters will have higher levels of urinary 1-HP and DNA adducts after the fire event compared to the Kelly day baseline (5 days off work consecutively). The null hypothesis was that firefighters will have lower levels of urinary 1-HP and DNA adducts after the fire event. Due to the variability of the sample group and small size of the group, some 1-HP and DNA adduct levels increased and some decreased after a fire event. No statistically significant correlation was found in the firefighters between levels of 1-HP and DNA Adducts (p=0.107 for 1-HP compared to
DNA Adducts). Therefore the null hypothesis was accepted and the alternate hypothesis was rejected.

6.0 Discussion

Biomarkers can give more accurate estimations of human internal exposure by encompassing absorption, metabolism, distribution, and individual differences including body mass index, genetics, and respiratory rates \(^{(9)}\). 1-hydroxypyrene (1-HP) is a metabolite of pyrene found in urine and is used as a biological marker of exposure \(^{(7)}\). Hydroxypyrene is used for PAH measurements because of its correlation with levels of total PAH and carcinogenic PAHs \(^{(9)}\). 1-HP urinary elimination is tri-phasic with half-lives of 5 hours, 22 hours and 17 days. DNA adduct levels reflect a longer time span of exposure since urothelial cells have a life span of 50 to 100 days \(^{(9)}\). The differences in the 1-HP and DNA adduct levels between both pre and post samples were not statistically significant. The 1-HP post-exposure averages exceeded the ACGIH BEI recommendation, thus warranting further review of this study group and analyze more samples to determine significance.

Four of the five post fire 1-HP levels were higher than pre –fire levels, which suggests that the firefighters were exposed to PAHs. The fluctuation of 1-HP values between baseline and post-fire events can be explained by differences in the timing of the sample collection. Certain samples were collected directly after the fire whereas others were collected hours later. 1-HP levels will be the highest a few hours after the exposure and then steadily decrease per the half-life of 1-HP \(^{(8,10)}\). Previous exposures
to fire, asphalt fumes, barbequed foods, and smoking can also skew the results. These exposures can increase the 1-HP value and total DNA adducts if the different exposures are continuous. DNA adducts in bladder cells, covalent modifications to DNA, are formed after exposures to PAHs. Bladder cells are sloughed off and excreted in urine daily. Dependent upon the amount of exposure and amount of cells that are in each sample, these daily samples may only contribute fractionally to adduct levels if different exposures are not continuous. The average 1-HP for post fire samples was 1.122 μg/l (Table 2), which is above the ACGIH BEI.

The variability of each fire and each firefighter’s task during the fire can also explain the variability of each sample. Each fire can be unique in its hazards and can affect each firefighter differently. The difference can cause a low correlation of the data because of the great variability of each sample that is received. Use of PPE can decrease the amount of potential hazards the firefighter is being exposed too, thus possibly causing a decrease from pre and post samples. However, each type of PPE can provide the firefighters with a false sense of security. The respirator may not seal tightly or slip during the firefighting task because of movement, heat, or moisture. If the personal protection is used properly, including gloves and fire protection suit that is free of soot and particulate, the intake of Polycyclic Aromatic Hydrocarbons can decrease. PPE contaminated with particulates and soot can lead to an increased potential to skin absorption. This can increase or decrease the1-HP levels, depending on PPE use and cleanliness. Each firefighter can have varied results because of the variability of how PAH’s get into the body and are dispersed. PAHs exposure from a fire event can be a
significant cause for the increase of 1-HP after the fire event as compared to the baseline.
### Tables

**Table 1: Baseline and Post-Fire Event 1-HP levels paired samples from the same worker**

<table>
<thead>
<tr>
<th></th>
<th>PreHP ug/l</th>
<th>PostHP ug/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>0.020</td>
<td>0.497</td>
</tr>
<tr>
<td>102</td>
<td>0.311</td>
<td>0.661</td>
</tr>
<tr>
<td>103</td>
<td>0.677</td>
<td>2.343</td>
</tr>
<tr>
<td>105</td>
<td>0.925</td>
<td>2.540</td>
</tr>
<tr>
<td>110</td>
<td>1.754</td>
<td>1.469</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td><strong>0.506</strong></td>
<td><strong>1.122</strong></td>
</tr>
<tr>
<td><strong>Std</strong></td>
<td><strong>0.458</strong></td>
<td><strong>0.899</strong></td>
</tr>
</tbody>
</table>
Table 2: 1-HP Levels for Firefighter Baseline and Post-Fire Event Samples

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Pre 1-HP (ug/l)</th>
<th>Post 1-HP (ug/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>101</td>
<td>0.020</td>
<td>0.497</td>
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<tr>
<td>102</td>
<td>0.311</td>
<td>0.661</td>
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<tr>
<td><strong>Std</strong></td>
<td><strong>0.458</strong></td>
<td><strong>0.899</strong></td>
</tr>
</tbody>
</table>
Table 3: Total DNA Adducts for Firefighter Baseline and Post-Fire event Samples

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Pre Total Adducts (per $10^8$ unadducted nucleotides)</th>
<th>Post Total Adducts (per $10^8$ unadducted nucleotides)</th>
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<td>101</td>
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<tr>
<td><strong>Std</strong></td>
<td><strong>33.9</strong></td>
<td><strong>59.5</strong></td>
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References


6. ACGIH. Documentation of the TLVs and BEIs. American Conference of Governmental Industrial Hygienists. Cincinnati (OH): American Conference of Governmental Industrial Hygienists; 2007.


Appendix A

Materials used to perform DNA Adduct analysis of the urine samples included:

- Micrococcal Endonuclease/Calf Spleen Phosphodiesterase-MNSPD (Sigma, St. Louis, Missouri)
- 5x Salts [50 mM CaCl2, 100 mM Sodium Succinate- pH 6.0]
- Deionized Water
- Tap Water
- Wizard® Genomic DNA Purification Kit (Fisher Scientific, Pittsburgh, Pennsylvania)
- Beckman DU 640 Spectrophotometer (Fisher Scientific, Pittsburgh, Pennsylvania)
- Nuclease P1 [1 μg/μl] (Amersham/GE Health Care, Piscataway, New Jersey)
- Zinc Chloride [0.3 mM] (Fisher Scientific, Pittsburgh, Pennsylvania)
- Sodium Acetate (Fisher Scientific, Pittsburgh, Pennsylvania)
- Sodium Hydroxide [0.25 M, pH 5.0] (Fisher Scientific, Pittsburgh, Pennsylvania)
- Polynucleotide Kinase Buffer [Bicine 200 mM- pH10, Dithiothreitol 100mM, Spermidine 10mM, MgCl 100mM- pH 9.6] (Amersham/GE Health Care, Piscataway, New Jersey)
- Bicine (Amersham/GE Health Care, Piscataway, New Jersey)
- 32Phosphate (Perkin Elmer, Billerica, Massachusetts)
- Apyrase
- Weigh Boats (Fisher Scientific, Pittsburgh, Pennsylvania)
- Tweezers (Fisher Scientific, Pittsburgh, Pennsylvania)
- Test Tubes (Fisher Scientific, Pittsburgh, Pennsylvania)
- Test Tube Holders (Fisher Scientific, Pittsburgh, Pennsylvania)
- Denver Instruments TR403 Balance (Fisher Scientific, Pittsburgh, Pennsylvania)
- Fischer Scientific Micro-Centrifuge (Fisher Scientific, Pittsburgh, Pennsylvania)
- Revco -80°C Freezer
- Brinkman Homogenizer (Fisher Scientific, Pittsburgh, Pennsylvania)
- 500 ml Beakers (Fisher Scientific, Pittsburgh, Pennsylvania)
- Fischer Scientific 630D 37º C Incubator (Fisher Scientific, Pittsburgh, Pennsylvania)
- Blow Dryers
- Microcentrifuge Tubes (USA Scientific, Ocala, Florida)
- Micropipette Holders (Fisher Scientific, Pittsburgh, Pennsylvania)
- Glass Tanks (Fisher Scientific, Pittsburgh, Pennsylvania)
- Chucks Pads (Fisher Scientific, Pittsburgh, Pennsylvania)
- Kim Wipes (Fisher Scientific, Pittsburgh, Pennsylvania)
- Scissors (Fisher Scientific, Pittsburgh, Pennsylvania)
- Plastic Trays (Fisher Scientific, Pittsburgh, Pennsylvania)
- Safe Aire VA Ventilation Hoods
- D1 Solvent [0.65 M sodium phosphate- pH 6.0]
- D3 Solvent [3.6 M lithium formate, 8.5 M urea- pH 3.5]
- D4 Solvent [0.8 M lithium chloride, .5 M tris HCl, 8.5 M urea- pH 8.0]
- D5 Solvent [1.5 M sodium phosphate- pH 6.0]
- Whatman Paper (Fisher Scientific, Pittsburgh, Pennsylvania)
- Cellulose Polyethyleneimine Plates (Alltech Associates, Deerfield, Illinois)
- Blue Basic Auto Rad 8x10 (Iso Bioexpress, Kaysville, Utah)
- Kodak Film Developer
- Tri-Carb19/2200CA Liquid Scintillation Counter (Perkin Elmer, Billerica, Massachusetts)
- Autoradiography Cassettes (Fisher Scientific, Pittsburgh, Pennsylvania)
- Sharpie Markers (Fisher Scientific, Pittsburgh, Pennsylvania)
- Scintillation Vials (Fisher Scientific, Pittsburgh, Pennsylvania)
- 70% Ethanol (Fisher Scientific, Pittsburgh, Pennsylvania)
- Fischer Scientific Vortex Genie (Fisher Scientific, Pittsburgh, Pennsylvania)
- Ludlum Survey Meter (Ludlum, Sweetwater, Texas)
- Roper Refrigerator/Freezer (Fisher Scientific, Pittsburgh, Pennsylvania)

Materials used to perform 1-Hydroxypyrene analyses of the urine samples included:

- 0.1M Sodium Acetate (Pure Sodium Acetate (1M solution =82.03g/l) was diluted 1:10 by adding 8.203 g and adding to 100ml water to the graduated cylinder yielding a 0.1M solution=8.203g/100ml) (Fisher Scientific, Pittsburgh, Pennsylvania)
- β-glucuronidase/arylsulfatase (G-0876 Type h-2 from Helix pomatia, 105,000 β-glucuronidase units/ml and 4,300 arylsulfatase units/ml, Sigma)
- HPLC Grade Methanol (CAS#67-56-1, Fisher Scientific, Pittsburgh, Pennsylvania)
- Milli-Q (ultra pure) water (double deionized water)
- Analytical nitrogen evaporator with nitrogen tank with nitrogen regulator turned to pressure of approximately 50psi
- Water bath (contained at bottom of analytical nitrogen evaporator)
- Roper Refrigerator/Freezer (Fisher Scientific, Pittsburgh, Pennsylvania)
- 37°C Incubator (Fischer Scientific Model 630D, Fisher Scientific, Pittsburgh, Pennsylvania)
- Shaking platform
- High Performance Liquid Chromatograph (HPLC) (2695 separation module, Waters) with Fluorescence detector (730 Water Data Module fluorescence detector) connected to a PC with Empower 2 HPLC software
- Jack Berberich’s Milk crate
- 50ml Screw cap tubes (samples were received in these)
- 25ml glass Scintillation vials (Fisher Scientific, Pittsburgh, Pennsylvania)
- Gilson Micropipettes (20μl, 5ml) (Fisher Scientific, Pittsburgh, Pennsylvania)
- Pipette tips (Fisher Scientific, Pittsburgh, Pennsylvania)
- Test tube racks (Fisher Scientific, Pittsburgh, Pennsylvania)
- 20ml syringes (Fisher Scientific, Pittsburgh, Pennsylvania)
- 0.45 um filters (Fisher Scientific, Pittsburgh, Pennsylvania)
- 3ml syringes (Fisher Scientific, Pittsburgh, Pennsylvania)
- C18 Sep Pak Cartridges (Part No. WAT020515 Waters Corporation, Milford, Massachusetts)
- Tape (Fisher Scientific, Pittsburgh, Pennsylvania)
- Sharpie Markers (Fisher Scientific, Pittsburgh, Pennsylvania)
- Plastic tray (Fisher Scientific, Pittsburgh, Pennsylvania)
- Weigh boat (Fisher Scientific, Pittsburgh, Pennsylvania)
- Balance (Denver Instruments model TR403, Fisher Scientific, Pittsburgh, Pennsylvania)