University of Cincinnati

Date: 11/22/2011

I, Matthew V Jackson , 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:

The Relationship between urinary 1-Hydroxypyrene and DNA Adducts in the exfoliated Bladder Cells of Firefighters

Student's name: **Matthew V Jackson**

This work and its defense approved by:

Committee chair: Glenn Talaska, PhD

Committee member: Charles Stuart Baxter, PhD

Committee member: Paul Succop, PhD

2178

The Relationship between urinary 1-Hydroxypyrene and DNA Adducts in the exfoliated Bladder Cells of Firefighters

A thesis submitted to the
Graduate School
of the University of Cincinnati
in partial fulfillment of the
requirements for the degree of

Master of Science

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

Matthew V. Jackson

B.S., Eastern Kentucky University
May 2009

Committee Chair: Glenn Talaska, Ph.D.

Abstract

During this study, firefighters were examined based on their potential exposure to polycyclic aromatic hydrocarbons. Both pre-shift and post-shift urine samples were analyzed by measuring 1-hydroxypyrene levels and DNA adducts in the exfoliated bladder cells. 23 non clean catch urine samples were collected from 15 different firefighters, with 10 of the samples being post-shift samples.

We hypothesized that firefighters who had been exposed to PAH will have an increased level of 1-Hydroxypyrene and DNA adducts in their bladder cells. 1-HP levels along with DNA adducts in exfoliated bladder cells were compared between pre-shift and post-shift samples. 1-HP levels were measured using the Jongeneelen et al. method. DNA was first isolated using the Wizard Genomic DNA Kit, and adduct levels were quantified by ^{32}P-post labeling adenosine 5'triphosphate excess thin layer chromatography.

When comparing pre- and post-shift samples, three out of four were found to have an increase in 1-HP. The pre- shift samples 1-hydroxypyrene mean values was determined to be 0.48053, while the post-shift mean 1-hydroxypyrene values were 0.9736. The increase between pre- and post-shift 1-hydroxypyrene levels indicates a small exposure to PAH. Although all but one sample fell below the 1-HP ACGIH BEI value of 1 µg/l, the data suggests that these firefighters are exposed to PAH. A Pearson correlation coefficient was generated comparing 1-Hp levels and DNA adducts and a negative correlation was reported.

Acknowledgements

I would like to thank all the people that supported me during my master's education, especially my advisor Dr. Glenn Talaska who inspired and expanded my knowledge. I am thankful for the patience and advice he has given me over the last year.

I would also like to thank Brenda Schumann, who taught me the logistics behind my lab studies. Without her I would not have had such a pleasant experience while working in the laboratory.

My deepest gratitude goes to my family, who has shown continual support during my path to obtaining my master's degree. Without them this master's thesis would have never came to fruition. I feel that I am now forever in debt to my parents for their continual mental and financial support.

I would also like to thank the National Institute of Occupational Safety and Health for providing me the opportunity to attend graduate school on a scholarship. If not for this organization I would have been unable to complete my education.

Table of Contents

List of Tables

List of Figures

Abbreviations

1-HP – 1 Hydroxypyrene

PAH – Polycyclic Aromatic Hydrocarbon

PPE – Personal Protective Equipment

ACGIH – American Conference of Governmental Hygienists

BEI – Biological Exposure Index

ATP – Adenosine triphosphate

DNA – Deoxyribonucleic Acid

HPLC – High Performance Liquid Chromatography

SCBA – Self Contained Breathing Apparatus

gm - Grams

L - Liters

µg - Micrograms

µl - Microliters

mg – Milligrams

MNSPD - Micrococcal Endonuclease/Spleen Phophodiesterase

PNK - Polynucleotide Kinase

Introduction

Biological monitoring can give an estimation of internal dose by incorporating absorption, metabolism, distribution and other factors contributing to variation [3]. Since there are multiple ways a chemical can enter the body, biological monitoring may be the useful way of estimating a person's exposure [3]. Biological monitoring is useful in measuring internal dose by accounting for all routes of exposure which include inhalation, absorption and ingestion.

Air sampling evaluates the inhalation hazard, but the measurement of a person's urine can provide pertinent information on the contaminant itself or a metabolite of that contaminant. There are three primary categories of biological monitoring which are: the measurement of the primary contaminant, the measurement of the primary contaminant's metabolite and the measurement of altered structure or function [14]. In this study, a measurement of a contaminant's metabolite was studied. Pyrene, which is a polycyclic aromatic hydrocarbon produced during the incomplete combustion of organic materials, is metabolized to 1-hydroxypyrene (1-HP). 1-HP was measured over the course of this study in firefighters' urine. Frequently it is not possible to measure a primary contaminant by biological monitoring, since metabolism occurs rapidly, so it is very useful to measure its metabolite to gain a sense of exposure [14]. Biological monitoring can be viewed as an inexpensive, practical and effective way to accurately identify hazards in the workplace. However, some still have a negative view when it comes to the use of biological monitoring. It has been said that it interferes with confidentiality, invasiveness and has low worker participation rates [14].

Biological monitoring may be used in combination with air or dermal sampling to assess the different routes of exposure [14]. This information could then be used to potentially change the

view of the contaminant's safe level of exposure. Physiological processes and the understanding of biochemical dynamics must be well understood in order to perform BM [14]. Gaining knowledge of work practices along with the contaminants biochemical attributes is very important. The peak exposure of the contaminant present will pose the greatest quantifiable risk to the worker, and that value needs to be documented. Many contaminants and their metabolites have very different biological half-lifes, and it is very important to know this before using biological monitoring in order to capture the peak of elimination following exposure [14].

There are still many limitations of biological monitoring, such as it only measures contaminants after the exposure occurred, so it cannot be used as a preventative measure. Biological monitoring procedures should not be used alone, but along with dermal sampling and air sampling to gain a comprehensive knowledge of all potential exposures [14]. The goal of sampling is to determine how much of a contaminant is present, where the contaminant is coming from, and how it enters the body.

In this study, firefighters were examined based on their potential exposure to polycyclic aromatic hydrocarbons (PAH). There are several hazardous health risks in the field of firefighting. Common health risks include cardiovascular stressors, such as exposure to ultrafine particulates, heat stress due to high ambient heat and exposure to harmful gases and vapors [1]. Most injuries and deaths associated with fires are not directly related to burns, but smoke inhalation [7]. The inhalation of toxic gases may pose severe acute health risks to the exposed individual. Gases such as carbon monoxide, formaldehyde, benzene and acrolein have all been associated with building fires [8]. These exposures also include gases and particulates that are associated with the incomplete combustion of organic materials which may cause formation of polycyclic aromatic hydrocarbons (PAH).

Overhaul is the activity in which firefighters have suppressed the fire and are now looking for hidden fires or hot embers which have the potential to be a re-ignition source [9]. During this phase, the firefighter is likely to remove his/her personal protective equipment such as their SCBA, thus unknowingly exposing themselves to toxic gases which may not be visible to the naked eye.

The concerns with PAH exposure are long term health outcomes, including cancer and other chronic diseases. Several PAH are suspected or known human carcinogens [15]. PAH exposure has been linked to lung cancer, lymphoma and urinary bladder cancer [1]. There are three routes by which PAH can enter the body: dermal absorption, inhalation and ingestion. The main route of exposure associated with firefighting is inhalation and skin absorption. Previous studies have shown that firefighters may be at risk to certain cancers such as brain, bladder and kidney [2]. Since there are multiple ways of entry into the body, biological monitoring may be the most useful way of estimating a person's exposure to PAH. Determining PAH exposure through means of biological monitoring is useful in determining if the firefighter misuses or does not routinely wear personal protective equipment.

1-Hydroxypyrene is a metabolite of pyrene which can be found in the urine of a person exposed to PAH. Elevated levels of 1-hydroxypyrene are correlated with polycyclic aromatic hydrocarbon exposure [5]. The American Conference of Governmental Industrial Hygienists has a 1-hydroxypyrene (BEI) biological exposure index of 1 μg^{-1} [4]. Urinary 1-hydroxypyrene is a sensitive and specific marker when assessing exposure to polycyclic aromatic hydrocarbons (PAH) [5]. The formation of DNA adducts in urothelial cells may be used as a marker for the development of bladder cancer. Urothelial cells are epithelial cells that line the human urinary system, and if adducts are formed within these cells, the potential for the start of carcinogenesis

occurs. DNA adducts are DNA nucleotides covalently bonded to a carcinogen. Carcinogens bind to DNA (adducting), this causing a change in the DNA's shape and structure, which may alter the ability to repair damage, interrupting transcription [6]. If the adduct is not properly repaired before DNA replication occurs, nucleotide substitutions, deletions and chromosome rearrangements may take place [6]. Urothelial cells have an average lifespan of 50-100 days, which is very valuable to this procedure. The long lifespan of these cells allows DNA adducts to accumulate, increasing sensitivity and giving an ample amount of time for the analysis of adducts.

Materials

Materials for the Isolation of the DNA

Wizard Genomic DNA Purification Kit (Fisher Scientific)

DNA Rehydration Solution

Nuclei Lysis Solution

Protein Precipitation Solution

Ribonuclease A from Bovine Pancreas

Fisher Micro centrifuge Model 235B

500 ml Pyrex Glass Flask

Portuguese (Brazil) USA Scientific 1.5 ml Micro centrifuge Tubes

Gilson P20 Pipette

Gilson P1000 Pipette

Fisher Brand Redi-tips

70% Ethanol

Isopropanol

Ice

Fisher Iso Temp Dry Bath Model 145

Fisher Vortex Genie

Materials for DNA Postlabeling

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 [Supersaturated 1.5 M sodium phosphate- pH 6.0]

Deionized Water

Micrococcal Endonuclease/Calf Spleen Phosphodiesterase-MNSPD

5x Salts [50 mM CaCl2, 100 mM Sodium Succinate- pH 6.0]

Nuclease P1 [1 μg/μl] (Amersham/GE Health Care)

Polynucleotide Kinase Buffer [Bicine 200 mM- pH10, Dithiothreitol 100mM,

Spermidine 10mM, MgCl 100mM- pH 9.6] (Amersham/GE Health Care,)

Bicine (Amersham/GE Health Care)

32Phosphate (Perkin Elmer)

Apyrase

Forceps (Fisher Scientific)

Test Tube Holders (Fisher Scientific)

Fischer Scientific Micro-Centrifuge (Fisher Scientific)

Revco -80ºC Freezer

-20^{o}C Freezer

Fischer Scientific 630D 37º C Incubator (Fisher Scientific)

Hair Dryers

Micro centrifuge Tubes (USA Scientific)

Gilson Micropipettes [20ul, 100ul, 200ul, 500ul, 1000ul] (Fisher Scientific)

Glass Tanks (Fisher Scientific)

Kim Wipes (Fisher Scientific)

Scissors (Fisher Scientific)

Plastic bins (Fisher Scientific)

Safe Aire VA Ventilation Hoods

Cellulose Polyethyleneimine Plates (Alltech Associates)

Blue Basic Auto Rad 8x10 (Iso Bioexpress, Kaysville, Utah)

Kodak Film Developer

Tri-Carb19/2200CA Liquid Scintillation Counter (Perkin Elmer)

Autoradiography Cassettes (Fisher Scientific, Kodak)

Sharpie Marking Pens (Fisher Scientific)

Scintillation Vials (Fisher Scientific)

70% Ethanol (Fisher Scientific)

Fischer Scientific Vortex Genie (Fisher Scientific)

Ludlum Survey Meter (Ludlum)

Roper Refrigerator/Freezer (Fisher Scientific)

Methods

Sample Collection and Storage

Non-clean catch urine samples were collected from firefighters. 23 total samples were collected from 15 different firefighters, with 10 of the samples being post shift samples. The samples were stored on blocks of ice in a cooler at the fire station until they were transported to the lab where they were stored in a -20^{o} C freezer until analysis. Samples were collected pre-fire event, and post-fire event by Dr. Stuart Baxter.

Filtration and Isolation of the Cells

The collected urine samples were first thawed, mixed and allowed to settle. The sample was slowly poured through a 10 µm filter membrane. A 1000 ml vacuum flask with a 37mm attached filter holder was used. If the 10 µm filter membrane became clogged, a new filter was used and the process continued. Upon completion of proper filtration, the filtered urine was kept and stored in a 50ml sample tube for analysis of 1-hydroxypyrene (1-HP) in a -20^{o}C freezer. After the urine was filtered, the filter was placed in a petri dish and scraped using rubber policeman to isolate the urothelial cells. The filter was washed with a phosphate buffer solution to remove all cells from the filters surface and to collect any residual cells. The cells and phosphate buffer solution were then transferred into a 15ml sample tubes and labeled. The sample containing the urothelial cells and phosphate buffer solution was then centrifuged at 600rpm for 5 minutes to separate the pellet from the supernatant [(5)]. The pellet was washed 3 times in approximately 5 ml of the phosphate buffer solution. The next step was DNA isolation and this was completed using the Wizard Genomic DNA kit. 200µl of chilled Nuclei Lysis solution was added to the sample, and incubated for 20 minutes at 67^{o}C. 3 µl of RNase solution was then added to the sample

which was then incubated for 20 minutes at 37°C. The sample was cooled to room temperature and 200 µl of protein precipitation solution was added. Each sample was then vortexed and then chilled on ice for a total of 5 minutes. The sample was centrifuged for a total of 4 minutes to separate the proteins. The supernatant was carefully removed and added to 600 µl of isopropanol. The DNA was then isolated by gently inverting the tube. The tube was then vortexed at 1300rpm for 1 minute. The isopropanol was carefully aspirated from the pellet, and the pellet was washed with 600 µl of 70% EtOH. The EtOH was aspirated off, and the pellet was left to air dry for 15 minutes. The sample was then re-hydrated on solution and incubated at 65°C for 30 minutes. The samples were stored at -20°C overnight.

1-Hydroxypyrene Analysis

The urine which was previously filtered and stored in the -20°C freezer was used to measure the 1-hydroxypyrene levels in each sample by means of the Jongeneelen et al. method [(5)]. The samples were first thawed and fifteen milliliters of the filtered urine was transferred into a 50ml tube. The samples pH was adjusted to 5.00 ± .05. 1M HCL or NaOH was used to adjust the pH levels. The sample was hydrolyzed by adding 5ml of 0.1 M sodium acetate and 8.75µl of β-glucuronidase/arylsulfatase. The hydrolyzed samples were incubated at 37ºC for 4 hours by placing samples on a mixing platform to agitate the sample throughout the incubation process. After the samples were incubated, they were taken out and a Waters C18 Sep-Pak® was primed by filtering 5 ml HPLC grade methanol. 10ml of Milli-Q water was then pushed through the Waters C18 Sep-Pak®. The plunger was taken out of the syringe and roughly 60% of the urine sample was loaded. The sample was pushed through the Sep-Pak® plus C18 at a rate of less than 2.5 ml per minute. The remaining 40% of the urine sample was loaded and pushed through the Waters C18 Sep-Pak®. The filter was washed twice with 8 ml of fresh Milli-Q water, and this

was done to remove polar contaminants. Ten milliliters of HPLC grade methanol was pushed through the Sep-Pak® Plus C18 and was collected in a 25ml glass scintillation vial. The 25ml glass scintillation vial was placed in a 60^{o}C water bath and left under a gentle flow of nitrogen to dry. Upon complete evaporation of the solvent, 2ml of HPLC grade methanol was drawn by using a 3 ml syringe and added to re-suspend the sample in the 25ml glass scintillation vial. A 0.45 µm filter was placed on a 3 ml syringe and the re-suspended sample was loaded and then slowly pushed through into a 2 ml HPLC glass vial. The vial was positioned in the HPLC autoinjector system. A Waters Alliance HPLC system, which uses the 2695 solvent system, a column heater and a 474 fluorescence detector, was used to analyze the samples [(3)]. The fluorescence detector was set to an excitation and emission wavelengths of 242, and 387nm, respectively.

The initial 1-HP standard used was 53 pg/µl, and this was done to establish the retention time. 53 pg/µl resulted in a very large peak, so dilutions had to be made to the standard. A 1/10 dilution was first made which resulted in 5.3 pg/µl. The standard was ran and the peak was still much too large. A 1/5 dilution was made to the standard which resulted in 1.06 pg/µl. This amount was found to be in the expected range of the sample. Dilutions were made by mixing HPLC grade methanol with a lower concentration of the original standard.

^{32}P Postlabeling

Postlabeling was completed using a substantial variation of the Reddy and Randerath method [(10)] [(13)]. The ^{32}P-postlabeling adenosine 5'triphosphate (ATP) excess, thin layer chromatography and scintillation counting was used to analyze the DNA adducts. The relative adduct level (RAL) was used to count the carcinogenic DNA adducts. The RAL is determined by a ratio of counts

per minute in the adducted nucleotides to the counts per minute in the normal nucleotides relative to the concentrations of DNA adducts [9,10].

Hydrolysis

Hydrolysis was done one day in advance of the post labeling procedures. This was done to break the DNA into 3'Phosphonucleotides. 18 micro centrifuge tubes were set up and labeled 1A 1B, 2A and 2B, for a total of 16 samples (8 samples each with one duplicate.) A hydrolysis Micrococcal Endonuclease/Spleen Phophodiesterase (MNSPD) cocktail was made by adding 36ul of MNSPD, 36ul of 5X salts and 36ul of MilliQ H_2O. 6ul of the MNSPD cocktail was added to each micro centrifuge tube, which contained 4ul of the DNA sample. The tubes were mixed then centrifuged for a total of 1 minute. The micro centrifuge tubes now contained 4ul of sample and 6ul of MNSPD cocktail, totaling 10ul. The samples were placed in the 37°C incubator for 3 hours and then placed in the -70°C freezer overnight.

Postlabeling Procedure

The next morning the samples were taken out of the -70°C freezer and thawed. Plexiglas sheeting was used during this procedure to block harmful beta emitting ^{32}P. Along with the use of Plexiglas, safety glasses, arm shields, shoe covers, and a lab coat were donned.

The ^{32}P cocktail was then prepared by adding 27ul of PNK buffer, 5.4ul of PNK, and a calculated amount of bicine and ^{32}P-ATP. The total amount in the ^{32}P cocktail must add up to 72ul, so the amount of ^{32}P must first be calculated using this equation:

$$\frac{200\ Counts}{32P\ Activity\ x\ Decay\ Rate}\ x\ number\ of\ samples\ (18) = Amount\ of\ 32P\ needed$$

Bicine was then added to the cocktail to achieve the 72ul needed to complete the ^{32}P cocktail. An apyrase cocktail was also made at this time, which consisted of 144ul of bicine along with 36ul of Apyrase. While working in the radioactive work station, 4ul of the ^{32}P cocktail was added to each of the hydrolyzed samples. The samples were then centrifuged inside of the workstation for a total of 1 minute then transferred into the 37^{o}C incubator for 40 minutes. After the samples had incubated for 40 minutes, they were taken out and brought back to the workstation where 10ul of the Apyrase cocktail was added to each sample. The centrifuge tubes now contained a total of 24ul. The samples were centrifuged for a total of 1 minute and placed into the 37^{o}C incubator for a total of 40 minutes. During this time, duplicate tubes were labeled the same as before and 144ul of bicine was added to each tube. The duplicate samples represent the normals. After the original samples had incubated for 40 minutes, they were taken out and 4ul were added to the duplicate samples (normals) with the 144ul of bicine, in the workstation. The samples were then mixed and centrifuged for a total of 1 minute. The original tubes now contained a total of 20ul and were placed in the refrigerator while spotting the normals. Three 10X20 chromatograms were then marked using a template. Each chromatogram was labeled with initials, experiment number and identification of which solvent they will run in (LiCl (2) or PO_4 (1). A line was placed on each chromatogram representing, sample numbers 1A 1B, 2A 2B, etc. A 20ul pipette was then used to spot 5ul of the duplicate sample (normals) to each line (1A 1B) on each of the three chromatograms. A hair dryer located within the work station was used to dry each sample directly after the pipetting was completed. The 3 chromatograms were then placed in their respective solvents, 2 in the LiCl and 1 in the $NaPO_4$ solvent. 16 10X10 chromatograms were then properly labeled with initials, experiment name and id number (1A) and a dot representing the point of origin was placed on each 10X10. The point of origin is where the spotting of the

original samples took place. A long wick consisting of filter paper was placed onto the end of each 10X10's fastened with 2 hair pins. The original samples were then taken out of the refrigerator and 18ul of each sample was then spotted using a 20ul pipette at the point of origin of each 10X10 plate. After 8 samples were spotted by pipette, they were then transferred into the hood to minimize radioactive exposure, and the other 8 10X10 plates were then spotted by pipette and transferred into the hood with the others. Each 10X10 plate was dried by using the hairdryer in the workstation before they were placed into the hood. Inside of the hood were 4 large glass tanks in which the D1 solvent was located. Each chromatogram was carefully placed in the D1 solvent, making sure all sides and bottom of the chromatogram was touching the solvent. This process was repeated until all 16 10X10 plates were correctly placed in the D1 solvent. The chromatograms were then left overnight, allowing them to run up the wick in the D1 solvent. By this time, the normals in the LiCl and PO_4 solvent tanks should have completely run and they could be taken out and dried. After the 10X20 plates had completely dried, they were then marked with a sharpie marker dipped in diluted radioactive ^{32}P. This was done to identify where the chromatogram set on the film, so they can be easily matched up later in the process. The 3 chromatograms were put inside of a cassette and taken to the dark room where a piece of film was put inside of the cassette. The cassette was then placed in the -70^{o}C freezer for approximately 20 minutes to expose the film. The 2 cassettes were then taken back to the dark room and the films were developed. The next morning, the 10X10 plates were removed from the D1 solvent and placed in a tub of water. The hairpins were removed from the filter paper and placed in a small container. The plates were then washed using water and then dried by using the hairdryers behind the Plexiglas shielding. After the plates were completely dried, they were then dipped in deionized water and placed into the D3 solvent. The samples were then taken into the

37°C incubator and left to run until the entire plate becomes saturated with the solvent. After the plates had time to completely run, they were then taken to the sink where they were rinsed very well in deionized water. The plates were then transferred behind the Plexiglas, where hairdryers were used to once again dry the plates. After the plates were completely dried, they were dipped in deionized water and then placed into the D4 solvent. The samples were transferred into the 37°C incubator and left to run. After the plates have had a sufficient amount of time to completely run, they were rinsed then dried using the previous methods. Before the plates were put in the D5 solution, a small wick was attached to each plate and fastened using 2 hair pins. The plates were then dipped in deionized water and placed in the D5 solution in the 37°C incubator and left to run. Once the plates had completely ran in the D5 solvent, they were taken out then dried. A radioactive marking pen was used to id each sample. For example, 1A was marked once at the top left (indicating A) and then once in the top right corner (indicating sample 1), and 1B was marked twice in the top left corner (indicating B) and once in the top right (indicating sample 1.) The plates were then put in cassettes and taken into the dark room, where a film was applied to each cassette (4 cassettes total.) The cassettes were then placed in the -70°C freezer for 48-72 hours to expose the film. The cassettes were then taken to the dark room and the films were developed. The films were taken to Dr. Glenn Talaska and he circled the adducts and numbered each circle. The 10X10 plates were then placed over the films, circled and numbered. The circles on the plates were cut out and placed in scintillation vials which were numbered. 5ml of 70% ethanol solution was dispensed in each vial and a cap was placed on each. The scintillation vials were placed into the Packard Tri-Carb Liquid scintillation analyzer where they were counted. The relative adduct level (RAL) was then used to count the carcinogenic DNA adducts. The RAL is determined by a ratio of counts per minute in the

adducted nucleotides to the counts per minute in the normal nucleotides relative to the concentrations of DNA adducts [9,10].

Laboratory Safety and Qualifications

Radiation safety training was required prior to working in the laboratory with ^{32}P. The University of Cincinnati's radiation safety department taught a two day course on radiation safety. High activity ^{32}P is necessary to complete this experiment, so it is essential to acquire radiation safety training before handling ^{32}P. Personal protective equipment along with general radiation safety was taught in the coursework. At all times a lab coat, 2 pairs of nitrile gloves, safety glasses, tyvek sleeves and shoe covers were donned along with a radiation badge and finger ring. Plexiglas and lead shielding was used to reduce exposure throughout the process. Geiger Muller counters were also utilized while working in the radioactive area of the laboratory. Hands, shoes and coat were checked for radioactivity before leaving the laboratory at all times. A monthly dosimetery badge and ring was turned into UC radiation safety at the end of each month to ensure there was no overexposure.

Results

When comparing pre- and post-shift samples, three out of four were found to have an increase in 1-HP. The greatest increase between pre- and post-shift samples occurred with firefighter number 56. Firefighter number 56 had a pre-shift sample of 0.444 and a post shift sample of 2.64. Firefighter number 42 had a slight decrease in 1-HP when comparing the pre- to the post-shift sample. This could be due to a job task to where this particular individual had no contact with open flames or smoke. This is summarized in table 1. Although all but one sample are below the ACGIH BEI value of 1 μg/l, the data suggests that these firefighters are exposed to PAH.

The null hypothesis states the post-shift 1-hydroxypyrene levels and DNA adducts will be equal to or less than the pre-shift samples. The alternate hypothesis states the pre-shift samples will have a lesser amount of 1-hydroxypyrene and DNA adducts than the post-shift samples. The pre-shift samples 1-hydroxypyrene mean values was determined to be 0.48053, while the post-shift mean 1-hydroxypyrene values were 0.9736. The increase between pre- and post-shift 1-hydroxypyrene levels indicates a small exposure to PAH.

DNA adducts resulted the highest in adduct 1, followed by adduct 3. Out of the 29 samples that were examined, 19 samples had reportable RAL results associated with adduct 1. When comparing DNA adduct levels by pre or post shift, there was no statistical significance difference between the pre- and post-shift samples. Therefore the null hypothesis cannot be rejected and the alternative hypothesis cannot be accepted.

A Pearson correlation coefficient was generated comparing 1-Hp levels and DNA adducts and a negative correlation was reported. Unfortunately, the rather small sample size may have

influenced these findings. When comparing the pre- and post-shift 1-HP levels, DNA adduct levels could not be accurately predicted.

Discussion

The relationship between urinary 1-hydroxypyrene and DNA adducts was examined during this study. DNA adducts were studied as early biological effect markers for a group of firefighters that were potentially exposed to polycyclic aromatic hydrocarbons, and more specifically a metabolite of pyrene, 1-HP [9]. Urinary 1-hydroxypyrene levels were elevated in 3 out of 4 samples after a fire event, suggesting occupational exposure to PAH.

All firefighter recruits but one had 1-hydroxypyrene levels below the ACGIH recommendation of 1 μg/l for both pre and post shift samples. Firefighter 56 had an elevated 1-HP level of 2.64. There are several reasons why one could expect a low exposure to 1-HP. Several of the firefighters were very young. Young, new firefighters are compelled to closely follow the chief's training and decision making procedures. The fire chief oversees the health and safety of his/her crew, so young fresh recruits will have more of an obligation to closely follow strict instructions to please the chief.

Firefighters have the potential for dermal and inhalation exposures to PAH. If a firefighter does not have a correct seal on his/her SCBA, then exposure may occur. Also, dermal exposures to PAH resulting in the uptake of 1-HP into the body may occur when the skin comes into contact with ash or soot.

Unfortunately no data was collected regarding whether the firefighter smoked cigarettes, or was exposed to PAHs via diet. Cigarette smoking prior to a work shift can change the amount of 1-HP present in the urine, which would give an overestimation of the occupational PAH exposure. PAH are generated from smoking and are involved in the process of cooking various foods. A

previous study looked at the PAH exposure from cooking with different oils and it concluded that several carcinogenic PAH are created during this process [11].

Each fire event is different, so it is difficult to accurately gauge a firefighter's overall exposure to PAHs based solely on one fire event. Depending on the nature of the fire, special operations command firefighters have been shown to receive higher PAH metabolite exposure than firefighters in other departments [12].

The difference in the pre- and post-shift 1-HP samples are not statistically different when comparing the adduct levels. With an increase in 1-HP levels, it would seem an increase in adduct levels would follow, but this was not the case in all of the samples. A negative correlation was found when relating these two variables. All of the samples had a rather low level of 1-HP when compared to the ACGIH BEI. This could be a reason why a negative correlation Pearson correlation was found.

Future directions with this study needs to look at the firefighters' smoking, age and specific job tasks during the fire event. As noted in this paper, PAH are generated by cooking methods such as using different oils, so it would be of importance to document what types of food are being prepared in the firehouse.

The most important limitation in this study was the small sample size. A greater sample size is needed to obtain a strong core of values. The small sample size most likely influenced the negative correlation reported. A larger sample size is needed for a more powerful statistical analysis.

References

1. LeMasters GK, Genaidy AM, Succop P, Deddens J, Sobeih T, Barriera-Viruet H, Dunning K, Lockey J. Cancer risk among firefighters: a review and meta-analysisof 32 studies. *J Occup Environ Med.* 2006 Nov;48(11):1189-202.

2. Demers, P. A., Checkoway, H., Vaughan, T. L., Weiss, N. S., Heyer, N. J., Rosenstock, L. (1994) Cancer Incidence among firefighters in Seattle and Tacoma, Washington (United States). Cancer Causes and Control., 5. Pg 129 – 135.

3. Henn SA, Anderson K, Hecht SS, Gross M, Succop P, Talaska G. Carcinogen-DNA adducts are increased in the exfoliated urothelial cells of wives of smokers: biological monitoring of passive smoke exposure. *Polycyclic Aromatic Compounds* 24: 475-485 (2004).

4. ACGIH Biological Exposure Index (BEI): Polycyclic Aromatic Hydrocarbons (PAHs). (2005): 17.

5. Jongeneelen FJ, Bos RP, Anzion RB, Theuws JL, Henderson PT. Biological monitoring of polycyclic aromatic hydrocarbons. Metabolites in urine. *Scand J Work Environ Health*. 1986 Apr;12(2):137-43.

6. Glick, Glossary of Biochemistry and Molecular Biology, 1990; from Rieger et al., Glossary of Genetics: Classical and Molecular, 5th ed).

7. Stefanidou M, Athanaselis S. Toxicological aspects of fire. *Vet Hum Toxicol*. 2004 Oct;46(5):292.

8. National Institute for Occupational Safety and Health [NIOSH] (2007). *Preventing fire fighter fatalities due to heart attacks and other sudden cardiovascular events* Publication No. 2007-133. Cincinnati, OH

9. Dawn M. Bolstad-Johnson, MPH, CIH Jefferey L. Burgess, MD, MPH, MSClifton D. Crutchfield, Ph.D., CIH Chief Steve Storment, CSP Richard Gerkin, MD Jeffrey R. Wilson, Ph.D. *Characterization Of Firefighter Exposures During Fire Overhaul.*

10. Peters S, Talaska G, Jönsson BA, Kromhout H, Vermeulen R. Polycyclic aromatic hydrocarbon exposure, urinary mutagenicity, and DNA adducts in rubber manufacturing workers. *Cancer Epidemiol Biomarkers Prev.* 2008, 17(6):1452-9.

11. Li S, Pan D, Wang G. Analysis of polycyclic aromatic hydrocarbons in cooking oil fumes. Arch Environ Health. 1994 Mar-Apr ;49(2):119-22.

12. . Edelman, P., Osterloh, J., Pirkle, J., (2003) Biomonitoring of chemical exposure among New York City firefighters responding to the World Trade Center fire and collapse. Environ Health Perspect. 111:1906-1911.

13. Talaska, G., Dooley, K. B., and Kadlubar, F. F. Detection and characterization of carcinogen-DNA adducts in exfoliated urothelial cells from 4-aminobiphenyl-treated dogs by [32P]postlabelling and subsequent thin layer and high pressure liquid chromatography. Carcinogenesis 11: 639-646 (1990).

14. Fundamentals of Industrial Hygiene, 5th edition. Barbara A. Plog and Patricia J Quinlan. Natl. Safety Council, pg. 500-501.

15. Feunekes FD, Jongeneelen FJ, Laan H, Schoonhof FH. Uptake of polycyclic aromatic hydrocarbons among trainers in a fire-fighting training facility**.** *J Toxicol Environ Health.* 1993 Apr;38(4):355-68.

Table 1: 1-HP levels Pre and Post Shift

Sample ID	1-HP Level (µg/L)	
	Pre Shift	Post Shift
42	0.528	0.396
5	0.448	0.716
56	0.444	2.64
31	0.352	0.596
48	0.48	
17	0.448	
11	0.436	
34	0.808	
26		0.52
21	0.36	
51	0.348	
22	0.456	
92	0.512	
16	0.432	
96	0.584	
9	0.572	
n	15	5
mean	0.48053	0.9736
GM	0.46937	0.74661
SD	0.11544	0.93878

Table 2: Total DNA Adducts

Sample ID	Avg. Adducts
42	6.25
3	35.77
5	32.45
29	8.1
56	18.65
12	0.28
31	42.15
33	7.7
48	1
17	129.5
11	66.15
34	52.2
n	12
σ	37.11
mean	33.35
GM	13.72

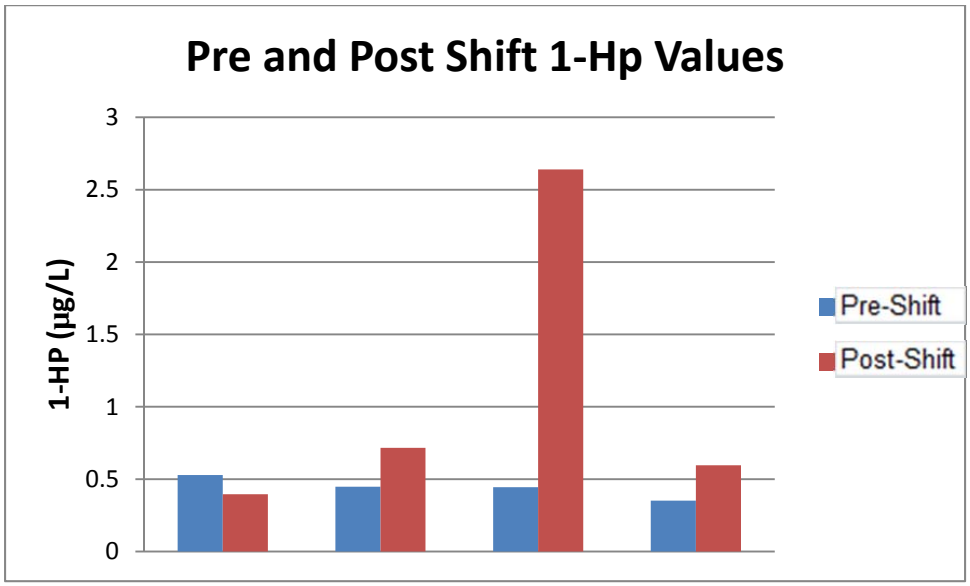


Figure 1: 1-HP values (µg/L) Values are displayed by pre and post shift.

Project Name HP_9_00
User Name System
Software Empower 2 Software Bui

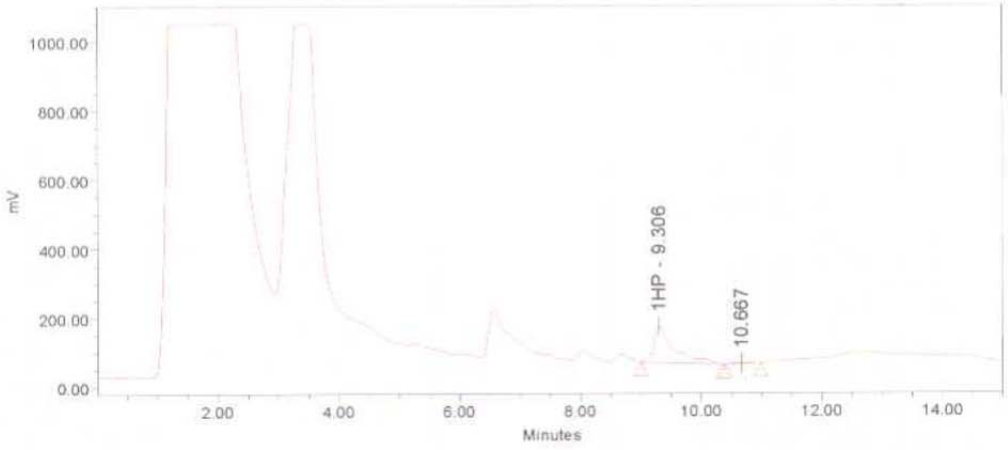
Report Method Name 1HP DATA_474
Current Date 6/28/2011

Sample Information

SampleName 12
Sample Set Name MVJ2
Vial 8
Injection 2
Injection Volume 25.00 ul
Channel SATIN
Run Time 15.0 Minutes

Sample Type Unknown
Date Acquired 6/28/2011 9:31:26 PM EDT
Acq Method Set 1HP_GT_2_3_8_00_rev
Processing Method NAP processing 6_10_10
Date Processed 6/28/2011 9:51:26 PM EDT

Auto-Scaled Chromatogram



Peak Results

	Name	RT	Area	Height	Amount	Units
1	2NAP	3.800				
2	1HP	9.306	2419740	106208	8.139	
3		10.667	46158	2246		

Figure 2 1-HP Peak and HLPC parameters