University of Cincinnati

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I, Felix K Boachie , 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:

Evaluation of Polycyclic Aromatic Hydrocarbons as Biomarkers of Exposure to Diesel Exhaust in Tunnel Workers

Student's name: **Felix K Boachie**

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Committee chair: Glenn Talaska, PhD

Committee member: Mary Beth Genter, PhD

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Evaluation of Polycyclic Aromatic Hydrocarbons as Biomarkers of Exposure to Diesel Exhaust in Tunnel Workers

Thesis submitted to the University of Cincinnati
Division of Graduate Studies
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

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Bachelor of Science, Georgia Institute of Technology, 2007

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Abstract

Diesel exhaust emissions include levels of volatile organic compounds and particulates that can be harmful to the health of both humans and the environment. Polycyclic aromatic hydrocarbons (PAH) are often associated with particulates and can be absorbed through dermal contact, inhalation or ingestion. Exposures to excess occupational amounts of diesel exhaust have been linked to an increased risk of lung cancer; however, research supporting a possible connection between diesel exhaust and bladder cancer has yet to be agreed upon.

The current study measured diesel exhaust exposure of construction workers in the Elbe Tunnel in Hamburg, Germany. The study examined the validity of using urinary 1-hydroxypyrene (1-HP) as a biomarker of exposure to diesel exhaust by determining a statistical association between levels of 1-HP and the presence of DNA adducts which may contribute to tumor induction.

Thirty-five urine samples from construction workers were measured by analyzing the levels of 1-HP in worker's pre- and post-shift urine samples collected over a six week period. Urinary 1-HP was detected using HPLC fluorescence techniques. DNA adduct levels were measured in exfoliated urothelial cells using a ^{32}P-postlabelling assay. Each sample was double-blinded and the identifiers were not released until the results were completed.

All thirty-five samples analyzed for urinary 1-HP contained levels below both the ACGIH recommendation of 1 µg/L and the population background level of 0.2 µg/L. The pre- and post-shift data for urinary 1-HP levels showed no statistical significance. There was no observed statistical significant association between urinary 1-HP and DNA adducts. A weak negative association between 1-HP and DNA adducts was observed (Pearson r=-0.313). Due to the variation of the results in this study combined with the lack of additional information concerning confounding data, no conclusions can be made that support the relationship between urinary 1-HP and DNA adduct levels.

This study was a precursor to a larger study that will evaluate PAH breathing zone exposure, hand wipes from exposed construction workers, DNA adduct levels and 1-HP levels to find a correlation between cumulative external dose and internal effect. These baseline data will be a precursor to further epidemiological studies that attempt to determine the potential connection between diesel exhaust exposure and risk of bladder cancer.

Acknowledgements

First and foremost, I would like to give thanks to my Lord and Savior for guiding me down this avenue of my life and helping me achieve heights I never imagined. I could spend a great deal of time naming individuals that helped me get to this point, but it would be negligent not to mention my advisor, Dr. Glenn Talaska, who was an ally even before I entered the program. "Dr. T" has been much more than just a scholastic advisor, as we have had many of conversations about life and general happenings. He has been a great friend and for that I say thanks.

Thanks to my committee members, Dr. Mary Beth Genter and Dr. Paul Succop, who showed great spirits in helping me to reach my deadlines while providing guidance that proved invaluable. I would also like to thank my partners in the trenches: Brenda Schumann, Shalini Bhatnagar, and Adrienne Eastlake. Those times in lab would have been a lot less enjoyable without the three of you.

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List of Abbreviations

β-Gluc	B-glucuronidase/arylsulfatase
µCi	Microcurie
1-HP	1-hydroxypyrene
^{32}P	Phosphorus 32
ACGIH	American Conference of Governmental Industrial Hygienist
ATP	Adenosine triphosphate
BEI	Biological Exposure Indices
CPM	Counts per minute
D1	Sodium monohydrate phosphate solvent used during D1 chromatography
D3	Lithium formate solvent used during D3 chromatography
D4	Lithium chloride-Tris HCl-urea solvent used during D4 chromatography
D5	Sodium monohydrate phosphate solvent used during D5 chromatography
DE	Diesel exhaust
DEP	Diesel exhaust particulates
DNA	Deoxyribonucleic acid
HPLC	High performance liquid chromatography
IARC	International Agency for Research Cancer
LiCl	Lithium Chloride
MeOH	Methanol
mM	Millimolar
MNSPD	Micrococcal endonuclease and calf spleen phoshodiesterase

NIOSH	National Institute for Occupational Safety and Health
OSHA	Occupational Safety and Health Administration
PAH	Polycyclic aromatic hydrocarbon
PBS	Phosphate buffer solution
PEI	Polyethyleneimine
PEL	Permissible exposure limit
PPMC	Pearson product moment correlation
Pg	Picogram
PNK	Polynucleotide kinase
RAL	Relative adduct labeling
QNS	Quantity not sufficient

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1 Introduction

Particulate matter ($PM_{2.5}$) is particulate air pollution consisting of particles that are two and one half micron or less. Diesel exhaust (DE) contributes approximately 6% of all ambient $PM_{2.5}$ but reaches as high as 36% in some urban areas throughout the United States [21]. Diesel exhaust is a mixture of thousands of particulates and gases that may be inhaled deep into the lung [7]. Elemental carbon comprises the majority of the particles present in diesel engine exhaust with trace organic metals and other organic materials comprising the rest [8]. Carbon dioxide, water and sulfur dioxide are just some of the gaseous derivatives produced during the combustion of diesel fuel. Other gaseous products include low molecular weight hydrocarbons and their derivatives, including low level polycyclic aromatic hydrocarbons (PAH) and nitro-polycyclic aromatic hydrocarbons [8, 25].

Diesel exhaust has been described by the International Agency for Research on Cancer (IARC) as a probable carcinogen to humans [8]. The National Institute for Occupational Safety and Health (NIOSH) recommends the reduction of diesel exhaust exposure to the lowest feasible amount [11]. Various epidemiological studies have been completed that support the link between occupational exposure to diesel exhaust and its potential contribution to lung cancer [3, 6, 17]. Even for these studies, there are complications with potential confounders including cigarette smoking [16]. This has made it ever so difficult to attempt to find a relationship between occupational exposure to diesel exhaust and cancers affecting other target organs. Evidence has been lacking in connecting occupational diesel exhaust exposure to bladder cancer although some preliminary studies have findings that support an increased risk of bladder cancer with

little confidence due to bias and confounding [4]. It has been estimated in the United States, that approximately 46,000 new cases of bladder cancer and 11,000 resultant deaths occur annually [9]. Overall, the current link between diesel exhaust exposure and its effect on the risk of bladder cancer is weak.

Currently, there is no regulatory standard for diesel exhaust particulate exposure. There is an exposure limit for PAH and other main components of diesel exhaust. The American Conference of Governmental Industrial Hygienists (ACGIH) has developed Biological Exposure Indices (BEI) as measurements of an individual's biological level of a contaminant [1]. ACGIH utilizes 1-hydroxypyrene (1-HP) as the marker of choice for measuring levels of PAH. 1-HP is a metabolite of pyrene which is abundant in most PAH mixtures and is commonly used as a biomarker of exposure. It should be noted that 1-HP is not itself carcinogenic and is not a direct measurement of carcinogen exposure and must be combined with knowledge of the breakdown of actual PAH air contamination to determine the carcinogenic potential.

Previous research has supported the association between the increasing presence of leukocyte DNA adducts and increased cancer risk [22]. DNA adducts are chemicals that are covalently bound to the genomic DNA. They have been found to interfere with DNA methylation, activate DNA repair and lead to upregulation of genes [12, 14, 15].

Occupations routinely encountering exposure to diesel exhaust include: truck drivers, underground workers, railroad workers, ship yard workers, and bus garage workers [3, 6, 16, 17]. These jobs all include individuals who are likely to be consistently exposed to diesel powered vehicles in closed quarters with limited ventilation in certain cases. Individuals with occupations located in tunnels with high diesel powered vehicle

traffic could also be considered a part of this group [5]. It is currently unclear just how much exposure tunnel workers have to diesel exhaust and if an increased risk of lung and urinary bladder cancer is a result of this exposure.

During this study, we evaluated the effects of occupational diesel exhaust exposure by measuring the levels of 1-hydroxypyrene in urine and carcinogen-DNA adducts found in exfoliated urothelial cells of construction workers at the Elbe tunnel in Hamburg, Germany [2]. We investigated the potential relationship between the biomarker of exposure (1-HP) and the biomarker of effect (DNA adducts) in the bladder. This study was completed to support a larger study that will include data gathered in this study together with hand wipe and personal breathing zone samples. Using these data and the data from other supporting studies, we hope to update current worker safe exposure guidelines.

2 Statement of Purpose

2.1 Objective

The purpose of this study was to evaluate: 1) the feasibility of using 1-hydroxypyrene as a biomarker of exposure for diesel exhaust; 2) the detection of DNA adducts found in exfoliated urothelial cells of DEP-exposed workers; and 3) the association between levels of 1-HP and DNA adducts as a product of diesel exhaust exposure. This study is just one component of a larger study that includes the measurement of total PAH in worker's breathing zones and dermal PAH contamination measurements via the use of hand wipes.

The significance of this study is that it will provide further information regarding the use of 1-HP as a biomarker for diesel exposure and provide an avenue for further research to determine if a relationship exists between occupational exposure to diesel exhaust and bladder cancer.

2.2 Hypothesis

Occupational exposure to diesel exhaust will lead to a measurable elevation in levels of 1-hydroxypyrene in urine and DNA adducts in exfoliated bladder epithelial cells. There will be a statistical association between levels of 1-HP and levels of DNA adducts in construction workers.

2.3 Specific Aims

The specific aims of this study are the:

1. Measurement of urinary levels of 1-hydroxypyrene in tunnel workers;
2. Measurement of DNA adducts in exfoliated urothelial cells in the target organ;
3. Determination of a correlation between levels of 1-HP and amount of DNA adducts in exfoliated urothelial cells.

3 Materials & Methods

3.1 Materials

The following items were obtained from these companies:

Fisher Scientific, Pittsburgh, PA: lithium hydroxide, sodium hydroxide, sodium phosphate monobasic monohydrate, bicine, buffer solution pH 4.0, buffer solution pH 7.0, HPLC grade methanol, isopropanol, sodium acetate, denatured ethanol, lithium

formate, 1X phosphate buffered saline (pH 7.4), 5X salts (50 mM CaCl2, 100 mM Sodium Succinate- pH 6.0), micro-centrifuge, incubator (37°C).

Sigma Chemical Company, St. Louis, MO: β-glucoronidase, urea, micrococcal endonuclease and calf spleen phosphodiesterase (MNSPD), glycogen, apyrase.

Waters, Milford, MA: separations module, photodiode array detector, scanning fluorescence detector, Sep-Pak cartridges.

Other materials: Wizard® Genomic DNA Purification Kit (Promega Corporation, Madison WI), 345 mm Whatman paper, Meyer N-Evap analytical evaporator, Photo developer and cassettes (Kodak, Rochester, NY), deionized water, Milli-Q water, polynucleotide kinase buffer, phosphorus 32 [^{32}P] (Perkin Elmer, Billerica, MA), blow dryers, Macherey Nagel polyethylenimine (PEI) cellulose thin layer chromatography plastic backed sheets (Alltech, Deerfield, IL).

3.2 Sample Preparation

3.2.1 Sample Collection

Pre- and post- shift urine samples were collected from 28 construction workers over a 6 week period from 2010-2011 by research collaborators in Hamburg, Germany. Nine samples, representing nine different time points, were collected from each worker. The urine samples were suspended with 20 % glycerol and frozen at -80ºC. One hundred and forty samples were received for analysis. Thirty of those samples were analyzed for this study. The samples were coded and contained no personal identifiers allowing for the study to be completely blinded. The participants signed an informed consent prior to participation in the study. The Institutional Review Board (IRB) determined that the study did not involve human subjects. As a result, IRB exemption was given in a letter to Dr. Glenn Talaska (Principal Investigator) dated July 21, 2009.

3.2.2 Isolation of Exfoliated Urothelial Cells

Isolation of exfoliated urothelial cells were performed as described in the method of Talaska et al [18]. Urine was thawed in a warm water bath while being mixed to uniformly defrost the sample. Once thawed, the urine was filtered by slowly pouring the sample through a 1000 ml vacuumed flask containing a 10 µm, 37 mm filter. Once the sample was completely exhausted, the loaded filter was placed on a petri dish containing about 5 ml of 1X phosphate buffer saline (PBS) solution. The remaining filtered urine in the 1000 ml flask was collected in 50 ml conical tubes and placed in a -20ºC freezer for 1-hydroxypyrene (1-HP) analysis. A rubber policeman was used to scrape off the exfoliated urothelial cells from the filter into the PBS solution. The filters were scraped in a uniform motion rotating the filter clockwise to ensure all of the cells were collected from the filter. The collective cell-PBS solution was removed from the petri dish and placed in 15 ml conical tubes and centrifuged for 10 minutes at 28 x g. Upon removal from the centrifuge, the supernatant was removed from the tube using an aspirator while being careful not to disturb the pellet. The pellet was washed by being resuspended in PBS solution and centrifuged for 10 minutes at 600 rpm. The supernatant was removed from the tube again and the 400 µl of PBS was added to the pellet to resuspend, using a pipet. The remaining solution was transferred to microcentrifuge tubes and centrifuged for 1 minute. The washed cells were used for DNA isolation.

3.3 DNA Isolation

DNA isolation was completed using a Wizard® Genomic DNA kit. The pellet-PBS solution was mixed on the vortex. After mixing, 600 µl of chilled nuclei lysis solution and

3 µl of RNase solution were added to the pellet and mixed and incubated for 30 minutes at 37ºC. After incubation, 200 µl of protein precipitation solution was added to the mixture, mixed with a vortex and chilled on ice for 5 minutes. The mixture was centrifuged for 4 minutes. The resulting supernatant was transferred to a new tube containing 600 µl of isopropanol and mixed by inverting and tapping the tube. The mixture was centrifuged for 1 minute and the supernatant was removed with an aspirator. The pellet was washed with 600 µl of 70% ethanol solution and centrifuged for 1 minute. The supernatant was aspirated and the pellet was allowed to air dry for 15 minutes. Once dry, the pellet was rehydrated with 20 µl of DNA rehydration solution and placed in an incubator at 65ºC for 1 hour. The rehydrated samples were removed from the incubator and placed in the -20ºC freezer for storage for the ^{32}P-postlabeling process.

3.4 Urinary 1-Hydroxypyrene

1-hydroxypyrene (1-HP) was measured in 35 urine samples using a modification of the method used in Jongeneelen [10]. Prior to performing the procedure, Waters Sep-Pak C18 cartridges were primed by performing two cycles of filtering 5 ml of HPLC grade methanol followed by 10 ml of Milli-Q water. Each 0.45 µm filter was rinsed with 5 ml of HPLC grade methanol and placed aside to dry before use. The frozen samples of filtered urine obtained previously from the isolation of exfoliated urothelial cells were thawed in a warm water bath while stirring. Once thawed, 15 ml of the samples were transferred to 50 ml conical tubes and were adjusted to pH 5.0 (with ± 0.05 error) using 1N HCl and NaOH. Once properly adjusted for pH, 8.75 µl of β-glucuronidase and 5 ml of 0.1 M sodium acetate were added to each sample and the samples were placed on a

mixing platform to agitate in an incubator at 37ºC for 4 hours. After incubation, primed Sep-Pak cartridges were added to 10 ml syringes with the plungers removed and 60% of the samples were poured in the syringes. The samples were then slowly pushed through the cartridges at a rate of 2.5 ml per minute to allow the 1-HP to be collected on the Sep-Pak. Once the syringe was empty, the rest of the samples were added and the step repeated to finish loading the cartridges. Once loaded, the cartridges were washed with 8 ml of Milli-Q water by slowly pushing it through the Sep-Pak. After all contaminants were removed from the cartridge, 10 ml of HPLC grade methanol was added to the syringe and the 1-HP was eluted at a rate of 2.5 ml per minute into 25 ml glass scintillation vials. The vials were placed in a warm water bath nitrogen evaporator and placed under a gentle flow of nitrogen until all of the solvent evaporated. The samples were resuspended by adding 2 ml of HPLC grade methanol to each scintillation vial. A 3 ml syringe was used to draw up the remaining methanol solution and a 0.45 µm filter was added to each syringe before filtering the sample into 2 ml HPLC vials [10]. The samples were injected in a Waters Alliance HPLC system where the characterization of 1-HP and the reference by fluorescence excitation (242nm) and emission (388 nm) spectra showed the levels of the metabolite mentioned [19, 20]. Standards were placed every tenth sample to validate results. A standard calibration curve was determined using a 5.3 pg standard injected in the following amounts: 2, 3, 4, 6, 7, 8, and 10 µl. Using this curve, the amounts of 1-hydroxypyrene in the samples were determined using linear interpolation. The percentage of 1-HP recovered was determined comparing the amount of detection of the standards to the expected amount

with known amounts of 1-HP. This recovery percentage was used to adjust the samples accordingly.

3.5 DNA Adducts

3.5.1 DNA Hydrolysis

The DNA samples that were isolated as described earlier, were hydrolyzed with the addition of a micrococcal endonuclease and spleen phosphodiesterase (MNSPD) cocktail. The cocktail was composed of 36 µl of Milli-Q water, 36 µl of chilled 5X salts, and 36 µl of MNSPD that was mixed using a vortex and centrifuged for 1 minute. New sample tubes were used to add 4 µl of the isolated DNA samples and 6 µl of the MNSPD cocktail for a total volume of 10 µl. These samples were then mixed and centrifuged before being placed in an incubator at 37ºC for 3 hours. This process produced both adducted and unadducted 3'-phosphodeoxynucleotides. The hydrolyzed samples were then placed for storage over night at -80ºC.

3.5.2 ^{32}P-postlabeling

32P-postlabeling was performed as described by Randarath et al [13]. A polynucleotide kinase (PNK) cocktail was used to label the 5' position of the hydrolysates with ^{32}P-ATP. The PNK cocktail consisted of 27 µl of PNK buffer, 5.4 µl of PNK, and calculated amounts of ^{32}P and 10 mM bicine for a total of 72 µl. The amount of ^{32}P added was dependent on the day that the ^{32}P was synthesized and the decay of radioactivity that occurred prior to its use. Approximately 250 µCi of activity were used per sample. Once the amount of ^{32}P was determined, the amount of bicine was calculated by subtracting the difference of the total volume of PNK, PNK buffer, and ^{32}P from 72 µl. It was necessary to add the ^{32}P to the cocktail prior to adding the PNK to

ensure that labeling occurred. Once the cocktail was prepared, 4 µl were added to each hydrolyzed sample, mixed, centrifuged, and placed in the incubator at 37ºC for 40 minutes. During the incubation, an apyrase-bicine (A-B) mixture was prepared consisting of 36 µl of apyrase and 144 µl of bicine. Following the incubation, 10 µl of the A-B mixture was added to each sample, mixed, centrifuged and placed in the incubator at 37ºC for 40 minutes. Following the incubation, 4 µl of the ^{32}P labeled samples were transferred into tubes containing 746 µl of bicine to use for spotting normalized plates that would be utilized to adjust samples for variability (for a total volume of 750 µl). The remaining of the ^{32}P labeled samples were placed in the refrigerator for storage while the normals were spotted. Three 10 by 20 cm polyethyleneimine (PEI) cellulose sheets were prepared by labeling points of origin for each sample (total of 16; 1-8 with both “A” and “B” samples [1A,1B…]) and 5 µl of the ^{32}P-bicine mixture were spotted on each samples respective point of origin. Once completed, the PEI plates were dried using a blow dryer and prepared by dipping the spotted ends of the PEI plates in deionized water. Once prepped, two of the plates (labeled LiCl) were placed in a 0.5 M lithium chloride solvent while the last plate (labeled PO4) was placed in a 0.15 M sodium monophosphate solvent until the solvent ran all the way up the plates causing complete saturation. After running, the plates were dried, placed into cassettes, and exposed to film. Both plates were exposed for a period of 90 minutes with the PO4 plate being stored at -20ºC and the LiCl plate stored at a room temperature. After the exposure time elapsed, the films were developed and kept for determination of adducts from the ^{32}P labeled samples.

Sixteen 10 x 10 cm PEI plates were prepared and labeled with a point of origin that was 1.5 cm away from the bottom and the left side of the sheet. A 30 cm Whatman paper wick was attached to the bottom of the 10 x 10 cm plates and held in place by hair pins. The ^{32}P labeled samples were removed from the refrigerator and 18 µl of sample was spotted on its respective 10 x 10 cm plate. Once spotting was completed, the plates were dried and placed in D1 solvent (0.65M NaH2PO4 Monohydrate) to run overnight. The next day the plates were taken out of the D1 solvent, the pins and wicks were removed, and the plates were placed in a deionized water bath for washing. Once all of the residual D1 solvent was rinsed off of the plates, they were dried with blow dryers. Once dried, the plates were dipped in deionized water with the point of origin on the bottom left of the plate, placed in D3 solvent (3.6M Lithium formate, 8.5M Urea), and placed in the incubator at 37ºC in a hot water bath until complete saturation was achieved. The D2 process was omitted from the procedure due to its lack of necessity. Following complete saturation of D3, the plates were removed from the incubator, washed and dried as before, dipped in deionized water with the point of origin on the bottom right of the plate, placed in D4 solvent (0.8M LiCl, 0.5M tris HCL,8.5M Urea), and incubated as described earlier. Following complete saturation of D4, the same process was repeated for D5 with the only difference being the addition of a 2 cm Whatman paper wick to the top of the plate attached with hair pins. After the D5 process the plates were washed and dried, placed in cassettes, exposed to films, and stored at -20ºC. The positive controls were exposed for 72 hours and the samples were exposed for 96 hours. Once films were adequately exposed, they were developed and the resulting films were used to determine the presence of adducts. The films were

circled for adducts and background levels for each sample. The corresponding 10 x 10 cm PEI plates were then circled and numbered using the films as a guide. The circles were then cut out of the plates and placed in 25 ml scintillation vials with 5 ml of 70% ethanol. The vials were then placed in a scintillation counter and radioactivity was measured. DNA adduct levels were calculated by using the counts per minute data provided by the scintillation counter to determine the relative adduct labeling (RAL).

4 Results

4.1 Analysis of 1-Hydroxypyrene in Urine

The limit of detection (LOD= 0.0343 µg/L) was determined based on the standard deviation of the response and the slope of the calibration curve (Table 1). The regression data for the calibration curve can be seen in Table 2. Four out of the thirty-five samples were below the LOD and given the value 0.0172 µg/L (or one half the value of the LOD). 1-HP concentration in urine of construction workers ranged from 0.0172 to 0.1514 µg/L with the geometric mean 0.0564 µg/L (GSD ± 0.0463 µg/L) (Table 3). All of the thirty-five samples analyzed were below the ACGIH recommended BEI of 1 µg/L. All of the thirty-five samples analyzed were below the population background level of 0.2 µg/L.

Pre- and post-shift urinary 1-HP levels collected from four workers at two different time points (1: December 28-31, 2010 and 2: February 2, 2011) were analyzed using a paired t-test (Table 4, Table 5). There was a significant difference between pre- and post-shift levels of 1-HP found during the first time point (p=0.0115, Pearson r=-0.207) (Figure 1). However, there was no significant difference between pre- and post-shift

levels of 1-HP found during the second time point (p=0.4377, Pearson r=0.550) (Figure 2).

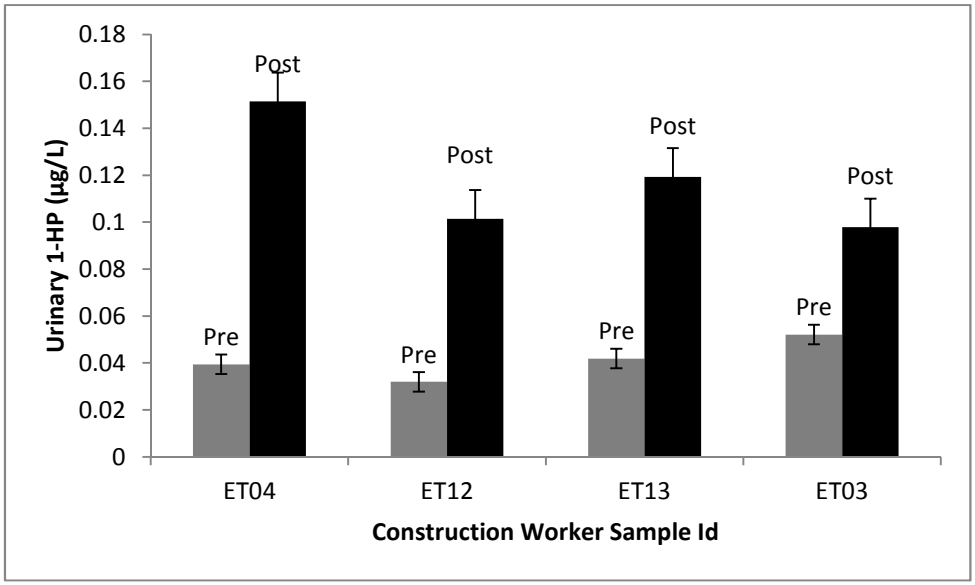

Figure 1: 1-HP levels in pre- and post- shift urine of construction workers exposed to diesel exhaust. There is a significant difference in 1-HP concentrations between pre- and post-shift samples (p=0.0115, Paired T-test) collected between December 28th and 31st of 2010 (Table 5).

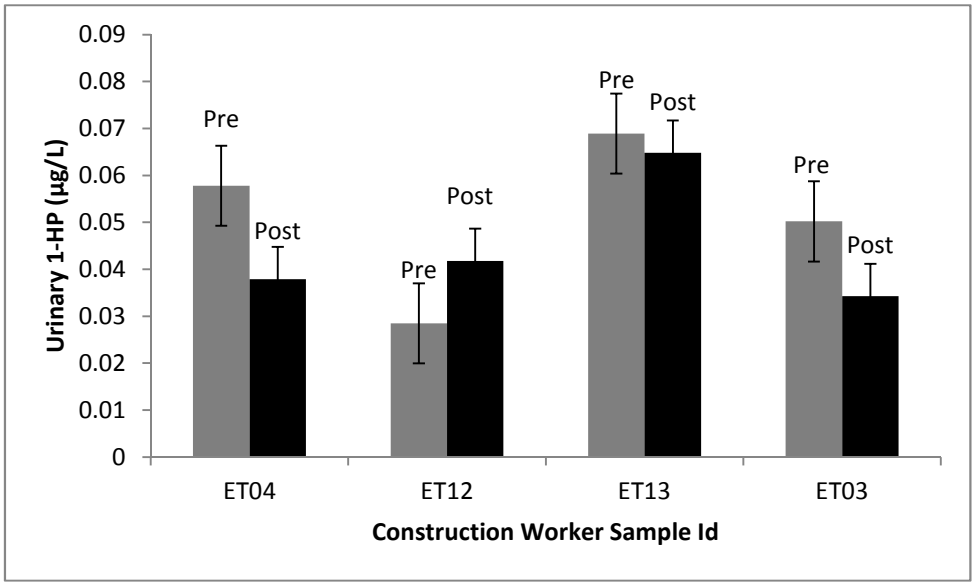

Figure 2: 1-HP levels in pre- and post-shift urine of construction workers exposed to diesel exhaust. There is no significant difference in 1-HP concentrations between pre- and post-shift samples (p=0.4377, Paired T-test) collected on February 2, 2011 (Table 6).

4.2 Quantification of DNA Adducts

Twenty-nine samples of exfoliated urothelial cells from construction workers were analyzed for DNA adducts. Six different DNA adducts were identified and categorized as shown in Table 7. Three samples were determined to lack a sufficient amount of DNA, and were labeled "QNS" (Quantity Not Sufficient) [23]. None of the samples contained all of the adduct types; however, one sample had four of six adducts. Adducts were identified by noting the position of each adduct on the chromatographs. Figure 3 shows the mean values of each DNA adduct type. Total DNA adduct levels ranged from 0 to 2521 adducts per 10^8 nucleotides with the geometric mean 24.6 (GSD ± 2.19). Adduct 3 was the most prevalent adduct found, being present in 21 of 29 samples. Three workers were analyzed for pre- and post-shift DNA adduct levels (Table 8). No statistical difference between pre- and post-shift DNA adduct levels was observed (p= 0.453, Pearson's r=-0.638) (Table 9).

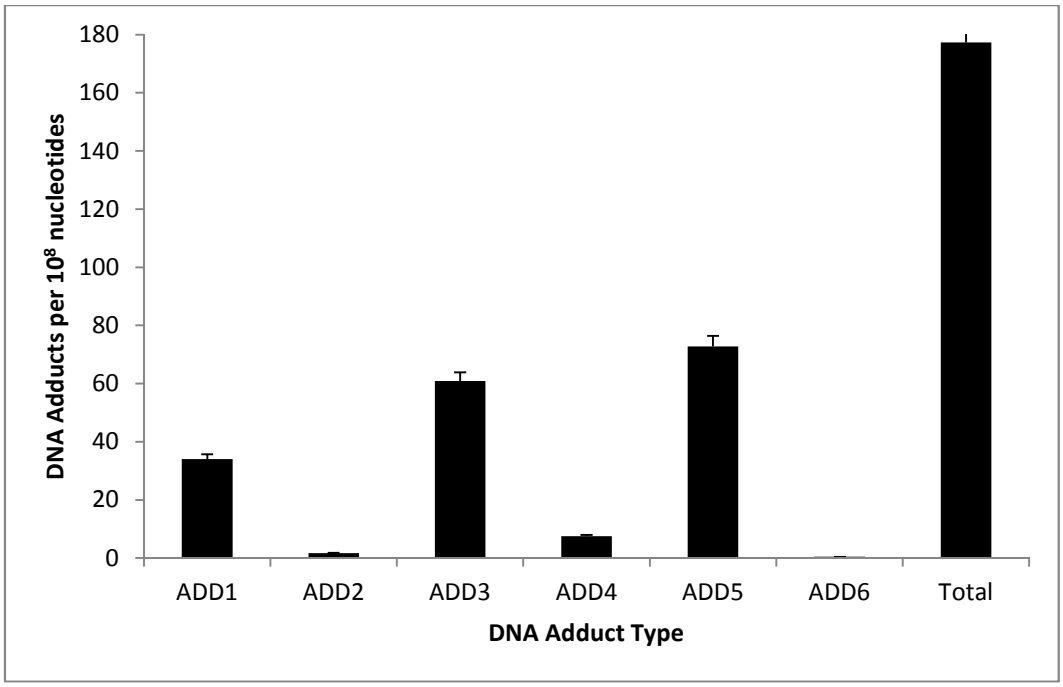

Figure 3: Counts of DNA adducts per 10^8 nucleotides by adduct type.

4.3 Characterization of Association between 1-HP and DNA adducts

The correlation between urinary 1-HP and DNA adduct levels was analyzed using the Pearson Product Moment Correlation (PPMC) test in the computer statistical program R (Figure 4). Initially, a positive correlation between urinary 1-HP and DNA adducts was found to be statistically significant (p=0.0417, Pearson r=0.419). Upon further inspection, it was determined that one specific adduct count (F91, ADD5) was extremely high and could be contributing to the positive correlation. This data point was excluded as an outlier due to it being greater that two standard deviations above the man. With its exclusion, the correlation between urinary 1-HP and DNA adduct levels remained statistically insignificant (p=0.1464, Pearson r=-0.313).

Each adduct type was evaluated to determine if there was a statistical significant relationship with urinary 1-HP (Table 10). There was no significant relationship between

urinary 1-HP and any of the specific adduct types. These results support the null hypothesis that there is no statistical association between urinary 1-HP and DNA adduct levels in construction workers that were exposed to diesel exhaust.

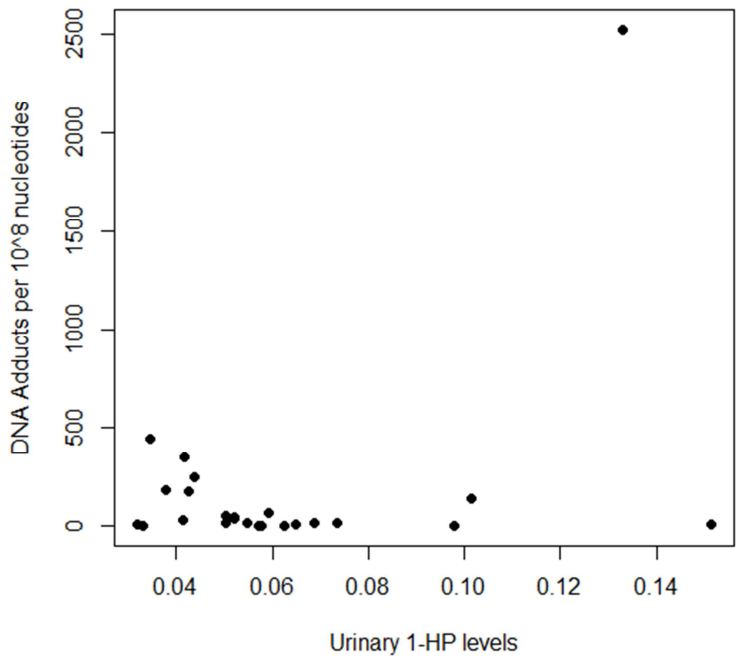

Figure 4: Correlation between levels of urinary 1-HP and total DNA adducts in urine samples collected from construction workers exposed to diesel exhaust (df=22, Pearson r=0.419, p=0.0417). One data point was identified as an outlier and the analysis was repeated without inclusion of the outlier.

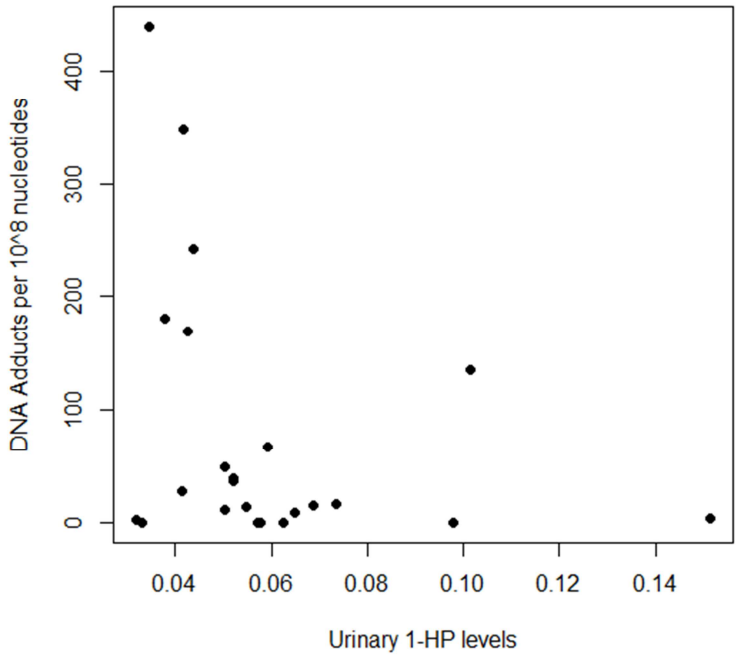

Figure 5: Correlation between levels of urinary 1-HP and total DNA adducts in urine samples collected from construction workers exposed to diesel exhaust excluding the outlier identified in Figure 3 (df=21, Pearson r=-0.313, p=0.1464).

5 Discussion

In this study we investigated the potential relationship between urinary 1-hyroxypyrene and DNA adducts as a way to characterize the health effects of diesel exhaust exposure. Construction is one of the occupations identified to have increased occupational exposure to diesel exhaust and provided an opportunity to examine DE exposure [4]. Ultimately, with enough evidence, urinary 1-HP may be used as a valid biomarker for DE exposure. Further knowledge of the relationship between urinary 1-HP and DNA adducts can provide additional insight into the potential connection between occupational DE exposure and bladder cancer.

All 1-HP levels were measured to be below the population background and much below the ACGIH BEI. With the test population consisting of an occupation observed to have high DE exposure, one would expect the minimum values to be above background levels [4]. In this study, the low levels of urinary 1-HP measured were a cause of concern and may be attributed to the standard calibration curve. It is possible that there were not enough calibration points to adequately account for the regression error. The addition of more calibration points could better define the scatter and potentially increase the confidence bands on either side of the curve [24]. Unfortunately, the standard curve did not evenly represent the analytical range.

There are additional potential reasons for the low 1-HP levels. The first being that, the workers may have not been exposed to the increased levels of DE initially thought. At the time of this study, environmental data was not available and could not be utilized as a resource. Secondly, personal protection equipment (PPE) could have been worn to help reduce worker's potential exposure. Without full knowledge of the level of PPE

available and identification of the workers who donned their PPE properly, there is no way to account for its effect. Lastly, there were no unexposed workers included in this study. The addition of unexposed workers could have provided some insight into why the results were so off base with general convention. Smoking status was unknown and could not be eliminated as a confounder. These results could have severely altered the outcome of the study and could have had a negative effect on the correlation with DNA adducts. This is disturbing due to the fact that PAH exposure occurs in many walks of life. An individual can have elevated levels of PAH in their system due to consumption of charbroiled foods or exposure to cigarette smoke.

Four out of four 1-HP post-shift levels were higher than their respective pre-shift levels during the first time point collection in December (Table 4). These values support the hypothesis that construction workers were exposed to PAH throughout the shift. However, the second time point collection in February showed three out of four 1-HP pre-shift levels to be higher than their respective post-shift levels (Table 5). These conflicting findings give little insight into the worker's exposure. The limited sample size could a contributing factor to this phenomenon. It would have been advantageous to obtain pre- and post-shift values on each day of collection to provide additional power to the study. These values should be revisited once the remaining twenty-four workers pre- and post-shift samples have been analyzed.

The three DNA adduct samples determined to be "QNS" could be a result of insufficient amounts of DNA present in the samples analyzed. The results show there was no statistical significant association between urinary 1-HP and DNA adduct levels. In fact, a negative correlation was observed between 1-HP and DNA adduct levels

(Table 10). The lack of a relationship between 1-HP and DNA adduct levels shows the limitations in using this method in determining the effects of diesel exhaust exposure. It is possible, that with alterations a better correlation can be determined with more analytical and population controls.

Potential confounding factors for levels of 1-HP were not accounted for. Creatinine was not measured in this study. Previous studies have measured creatinine in conjunction with 1-HP to normalize the data [10]. This lessens the variability of the urine sample collected. Ideally, smoking status would have been tracked along with job function, PPE utilized, and shift durations. The completion of the analysis of the remaining worker' samples combined with the environmental data gathered from the study collaborators should provide further insight into the relationships examined in this study. Further research is still needed in evaluating DE exposure in workers and the biological outcome of that exposure. Future studies will be necessary to confirm or deny the link between diesel exhaust and bladder cancer.

6 References

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7 Appendix

Table 1: 1HP Standard levels utilized for calibration curve data

Standard (5.3 pg/µl)	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Vol. Inj	2	3	4	6	7	8
Amt. of 1-HP (pg)	10.6	15.9	21.2	31.8	37.1	42.4
Area	4833986	7686659	10723533	16226350	19303494	20902095
Estimated 1-HP (pg)	10.21096	15.71306	21.57045	32.18404	38.1191	41.20241
Est. 1-HP (pg/µl)	5.105478	5.237688	5.392613	5.364007	5.445586	5.150301
% Difference	3.7%	1.2%	-1.7%	-1.2%	-2.7%	2.8%

A 5.3 pg/µl standard prepared by Dr. Glenn Talaska was used to create a calibration curve. There were ten total standards recorded. The remaining four not included in this table were excluded due to the increased noise obtained at higher concentrated levels of 1-HP.

Table 2: Regression statistics for the 1HP standard curve

Regression Statistics	
Multiple R	0.998134
R Square	0.996271
Adjusted R Square	-1.5
Standard Error	444782.4
Observations	1

ANOVA

	df	*SS*	*MS*	*F*
Regression	6	2.11425E+14	3.52E+13	1068.711
Residual	4	7.91325E+11	1.98E+11	
Total	10	2.12216E+14		

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	1068.7113	0.996271131	1072.711	4.53E-12	1065.945205	1071.47739

Table 3: 1-HP Levels for Construction Workers

Sample Id	Area	1-HP (ug/L)
F2	224535	<LOD
F21	613003	0.0521
F38	2607274	0.0978
F91	2275915	0.1328
F103	670638	0.0549
F4	614263	0.0522
F19	440520	0.0437
F1	414757	0.0425
F23	824387	0.0624
F46	1055646	0.0736
F12	391133	0.0413
F48	907147	0.0664
F24	718529	0.0572
F10	351993	0.0394
F94	758374	0.0592
F76	578973	0.0504
F81	173817	<LOD
F3	126225	<LOD
F8	400594	0.0418
F26	254778	0.0347
F40	2141080	0.1263
F16	2050345	0.1219
F88	1997890	0.1193
F39	958260	0.0689
F43	402031	0.0419
F96	874125	0.0648
F36	319770	0.0379
F5	983042	0.0701
F25	573036	0.0502
F75	254737	0.0347
F22	2657763	0.1514
F78	729661	0.0578
F31	198324	<LOD
F33	1628928	0.1014

Table 4: Comparison between 1-HP Pre- and Post- shift levels

Worker	**12/28-31/2010**		**02/02/2011**	
Sample #	Pre	Post	Pre	Post
ET04	0.0394	0.1514	0.0578	0.0379
ET12	0.0320	0.1014	0.0285	0.0418
ET13	0.0419	0.1193	0.0689	0.0648
ET03	0.0521	0.0978	0.0502	0.0343

Table 5: T-test performed for 1-HP pre- and post-shift urine collected from construction workers between December 28th and 31st, 2010.

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	0.04135	0.117475
Variance	6.9E-05	0.0006
Observations	4	4
Pearson Correlation	-0.20697	
Hypothesized Mean Difference	0	
df	3	
t Stat	-5.54763	
P(T<=t) one-tail	0.005775	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.011549	
t Critical two-tail	3.182446	

Table 6: T-test performed for 1-HP pre- and post-shift urine collected from construction workers on Feb. 2, 2011.

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	0.05135	0.0447
Variance	0.000291	0.000189
Observations	4	4
Pearson Correlation	0.550305	
Hypothesized Mean Difference	0	
df	3	
t Stat	0.892886	
P(T<=t) one-tail	0.218852	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.437704	
t Critical two-tail	3.182446	

Table 7: DNA Adduct Levels (per 10^8 nucleotides) in Construction Workers

Sample Id	Adduct 1	Adduct 2	Adduct 3	Adduct 4	Adduct 5	Adduct 6	Total Adducts
F1	156.4	0	12.8	0	0	0	169.2
F10	3.7	0.8	0	0	0	0	4.5
F103	0	0	14.1	0	0	0	14.1
F11	62.8	23.1	9	0	0	0	94.9
F12	8.4	19.4	0	0	0	0	27.8
F14	8.3	0	3.5	0	0	0	11.8
F19	98.7	0	102.2	29.3	0	12.7	242.9
F2	0	0	0	0	0	0	0
F20	171.3	0	441.1	0	0	0	612.4
F21	3.2	0	32.75	0	0	0	35.95
F22	0.6	0	3.4	0	0	0	4
F23	QNS	0	0	0	0	0	0
F24	QNS	0	0	0	0	0	0
F25	0	0	48.7	0	0	0	48.7
F31	0.7	0	1.3	0	0	0	2
F33	129.1	0	5.3	0	0	0	134.4
F36	100.45	0	80.25	0	0	0	180.7
F38	0	0	0	0	0	0	0
F39	4.8	0	9.4	0	0	0	14.2
F4	0	0	38.7	0	0	0	38.7
F46	8.8	0	6.6	0	0	0	15.4
F6	31.5	8.4	41.4	6	4.8	0	92.1
F75	0	0	439.4	0	0	0	439.4
F76	0	0	10.9	0	0	0	10.9
F78	QNS	0	0	0	0	0	0
F8	126.3	0	221.9	0	0	0	348.2
F91	0	0	220	124	2177	0	2521
F94	0	0	0	66.5	0	0	66.5
F96	5.1	0	2.8	0	0	0	7.9

Table 8: Comparison between DNA adduct levels (per 10^8 nucleotides) in Pre- and Post- shift samples

Sample #	Pre	Post
ET04	4.5	4.0
ET12	2.0	134.4
ET13	14.2	7.9

Table 9: T-test performed on DNA adduct levels from pre-and post-shift samples collected from construction workers.

t-Test: Paired Two Sample for Means

	Variable 1	*Variable 2*
Mean	6.9	48.76667
Variance	41.53	5503.603
Observations	3	3
Pearson Correlation	-0.63848	
Hypothesized Mean Difference	0	
Df	2	
t Stat	-0.92426	
P(T<=t) one-tail	0.226463	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.452925	
t Critical two-tail	4.302653	

Table 10: Pearson's correlation results between urinary 1-HP and DNA adduct levels

X-Value	Y-Value	Pearson's r value	p-value
1-HP	ADD1	-0.060	0.784
1-HP	ADD2	-0.147	0.493
1-HP	ADD3	-0.088	0.684
1-HP	ADD4	0.387	0.061
1-HP	ADD5*	-	-
1-HP	ADD6	-0.147	0.493
1-HP	Total	-0.313	0.146

* Note: The correlation with 1-HP and ADD5 had a standard deviation of zero.