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Assessment of the toxicity of firefighter exposures using the PAH CALUX bioassay

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ABSTRACT

Firefighters can be exposed to a complex set of contaminants while at a fire scene. Identifying new ways to monitor and assess exposure, particularly relating to toxicity is essential to determine the effectiveness of intervention techniques to reduce exposure. This study investigated the use of the polycyclic aromatic hydrocarbon (PAH) CALUX® bioassay for the assessment of exposure and associated toxicity firefighters might encounter. This was done through analysis of extracts of dermal wipes and urine samples collected from firefighters before and after a controlled fire. An increased bioassay response was observed from post-fire neck and calf samples, indicating a greater concentration of PAH-like compounds on the skin. The use of a baby wipe to clean the face and neck during rehab resulted in the attenuation of the observed bioassay response from the neck post-fire. Though a correlation was observed between the bioassay response and hydroxylated PAH concentrations found in the urine, the increased bioassay response from the post-fire urine samples was likely due to unknown compounds other than the hydroxylated PAHs tested. Our results suggest that this bioassay provides a useful measure of firefighter exposure, particularly relating to the potential toxicity of contaminants.

1. Introduction

There are many products of combustion with known toxic effects, including but not limited to carbon monoxide, hydrogen cyanide, benzene, formaldehyde, and polycyclic aromatic hydrocarbons (PAHs) (IARC, 2010; Fabian et al., 2014; Kirk and Logan, 2015). Firefighters and associated personnel at a fire scene may be exposed to these contaminants through inhalation, dermal, and ingestion routes. Numerous studies have concluded a greater cancer incidence and mortality in firefighters overall and/or for specific cancers compared to the general population (Daniels et al., 2014; Monash University, 2014; Pukkala et al., 2014; Daniels et al., 2015; Glass et al., 2016), and the International Agency for Research on Cancer (IARC) has classified firefighting as possibly carcinogenic to humans (Group 2B) (IARC, 2010).

PAHs are formed as a result of incomplete combustion of organic material and include chemicals known to be mutagenic and/or carcinogenic (Boffetta et al., 1997; Papa et al., 2008; IARC 2010 vol 92; Fernando et al., 2016; Andersen et al., 2017). Previous studies have quantified the concentrations of different PAHs in smoke from both training and active fires, along with extracts from swabs of the gear and skin (Baxter et al., 2014; Fabian et al., 2014; Fernando et al., 2016; Fent et al., 2014; Keir et al., 2017; Wingfors et al., 2017; Stec et al., 2018). Dermal exposure is thought to be an important route of exposure of PAH-like compounds to firefighters particularly if inhalation of these compounds is minimized due to the use of a self-contained breathing apparatus (SCBA) (VanRooij et al., 1993; Stec et al., 2018). Exposure to these compounds have been observed not only while actively fighting fires, but also during overhaul and at the firehouse (Baxter et al., 2014; Oliveira et al., 2017). In addition to ambient exposure to PAHs, researchers have quantified PAH metabolites in the urine of firefighters before and after fires as biomarkers of exposure (Edelman et al., 2003; Fent et al., 2014; Fernando et al., 2016). The primary method of quantification of both PAHs and metabolites has been through the use of targeted mass spectroscopy, which is a strong tool when investigators are interested in specific PAHs or specific metabolites. However, it can be very difficult to assess the potential toxicity or overall exposure when a limited number of PAHs and related compounds are being quantified, as each PAH can have a different toxic potential and

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exposures often involve complex mixtures.

Toxicity of PAHs, along with other dioxin-like compounds, is primarily caused through the binding to the aryl hydrocarbon receptor (AhR), induction of AhR-related genes and subsequent transformation to toxic metabolites (Behnisch et al., 2001; Tuyen et al., 2014). The *in vitro* PAH CALUX® bioassay can be used to assess the overall AhR mediated toxicity from PAHs and related compounds. A shorter incubation time is used to maximize the observed response from compounds metabolized at a faster rate, such as PAHs. This bioassay reports the total AhR mediated toxicity from dioxins, furans, PAHs, polychlorinated biphenyls, among others, in the form of Benzo[a]Pyrene (B[a]P) equivalence. The assay has been used to assess the presence and associated toxicity of PAH-like compounds from sediment, crude oil and from particulate matter from wood combustion (Pieterse et al., 2013; Gauggel-Lewandowski et al., 2013; Radovic et al., 2014). To date, the PAH CALUX® assay has not previously been used to assess the potency of hydroxylated PAHs or the overall exposures that firefighters encounter.

This study investigated whether the PAH CALUX® bioassay could be used to aid in the assessment of toxicity of exposure that firefighters and associated personnel encounter at a fire scene. This bioassay was used to assess the overall AhR activity from extracts of skin wipes before and after a controlled burn to identify the overall load of AhR active compounds on the skin that have the potential to enter the body through dermal absorption. Additional endpoints within the dermal sampling campaign included investigation into whether a prototype particulate blocking hood could help decrease exposure to PAHs and other AhR active compounds, and investigation into whether the use of a baby wipe to clean the face and neck post-fire could remove AhR active compounds from the skin. Urine samples collected before and after the control fire were also tested with this bioassay. Investigation into the bioactivity of a suite of hydroxylated PAHs and related compounds commonly used as predictors of exposure was conducted to identify if hydroxylated PAHs responded in this bioassay and what proportion of the bioactivity observed in the urine extracts can be explained. Understanding if other unmeasured compounds are primarily responsible for the bioactivity related to fire exposure will help determine the need for evaluation of other toxic contaminants in urine.

2. Materials and methods

2.1. Test subjects

The study was approved by the University of Arizona Institutional Review Board (approval No. 1509137073, and all subjects signed informed consent forms prior to participation in the research. A total of 11 non-smoking male Tucson Fire Department incumbent firefighters participated in this study involving one controlled fire. The average age, weight and height of the participants ± standard deviation were 39 ± 9 yr, 84.4 ± 7.3 kg and 175 ± 5 cm, respectively. To reduce PAH exposure from dietary sources, participants were asked not to eat grilled or charred food 12 hrs before the control fire and until after their final urine collection post-fire.

2.2. Test chemicals

Benzo[a]Pyrene (BaP) was supplied by BioDetection Systems (Amsterdam, The Netherlands). 1-Hydroxyphenanthrene, 2-Hydroxyphenanthrene, 3-Hydroxyphenanthrene, 4-Hydroxyphenanthrene, 3-Hydroxychrysene, and 3-Hydroxy-Benzo[a]Pyrene were purchased from Toronto Research Chemicals Inc. (Toronto, Ontario, Canada). 9-Hydroxyphenanthrene, Eugenol, 4-Ethylguiacol, 2–6-Dimethoxyphenol, 2-Methoxy-4-methylphenol, 2-Methoxy-4-propylphenol, 2-Hydroxyfluorene, 1-Hydroxypyrene, 2-Hydroxynaphthalene and dimethyl sulfoxide (DMSO) were purchased from Sigma Aldrich (Milwaukee, WI). 3-Hydroxyfluorene was purchased from Cambridge Isotope Laboratories Inc. (Tewksbury, MA). 6-Hydroxycrysene was purchased from Crescent Chemical Co. Inc. (Islandia, NY). All chemicals tested for response in the bioassay were dissolved in DMSO.

2.3. Controlled fire

The controlled fire took place at the Tucson Public Safety Academy in Tucson, AZ, USA. The building used for this test fire had a total of 3 rooms: a burn room and maze room on the ground floor with 10 ft ceilings, and one room on the second floor containing two windows and two doors (all closed during the burn). Ten firefighters were in the building, with 5 in either the maze room or burn room, and 1 individual outside of the building in full gear who did not enter the building. Half way through the fire, the individuals switched rooms and activities. The burn room was heated to 425–500 °F, with the maze room having smoke and residual heat ranging from 100 to 175 °F. The burn time was 14 min to resemble the average time of response activities during a basic residential structural fire. The items burned were chosen to represent a room and contents fire and consisted of wood with a pallet base, a padded arm chair, particle board shelving, a 4′ × 6′ carpet and padding, and miscellaneous objects (books, a clock radio, and a plastic vase). When smoke production in the room decreased, additional materials were added to the fire to maintain 14 min of smoke production. During the fire, firefighters simulated firefighting activities. They carried a hose up and down the 19 steps to the second floor, swung a sledge hammer against a tire, and crawled around the maze to simulate search and rescue. Upon exiting the fire, the firefighters did not remove their SCBA until they were away from the structure, and they received assistance removing their turnout gear and hood to avoid cross contamination from their gear to their skin. New turnout gear and hoods (never before used) were utilized for this study to avoid contamination from past fires. Two different hood types were used within this study. Five participants wore prototype particulate blocking hoods (not commercially available) meant to provide improved protection against particulates while the other 5 participants wore a traditional non-particulate hood comprised of blended PBI, Kevlar and Lenzing fibers.

2.4. Sample collection

Dermal wipes were collected to measure the PAHs and other AhR active compounds present on the neck and calf. Dermal samples were collected on the right side of the body at each location pre-fire, and the left side of the body at each location post-fire. Texwipe™ AlphaWipe™ polyester wipes (Fisher Scientific, Denver, CO) were prepared for use by being cut to 2″ × 4″ in size, submerged in GC grade Methylene Chloride (DCM) (Fisher Scientific, Chino, CA) and sonicated for 30 min, removed from DCM, and allowed to dry. Once dry, the wipes were sonicated for a second time in fresh DCM for 30 min, dried and placed into scintillation vials (Fisher Scientific, Chino, CA). Prior to the sampling campaign, 15 mL of LC/MS grade isopropanol (IPA) (Fisher Scientific, Chino, CA) was added to each scintillation vial to saturate the wipe prior to use. For dermal sampling, wipes were removed from the scintillation vial with forceps and a 3″ × 3″ section of skin at each sample site was wiped in a circular motion 10 times. The IPA in the vial was removed, and the processed wipe was placed back into the vial. Wipes were stored at 4 °C prior to extraction. Dermal sampling was collected first from the neck, followed by the calf for each sampling event. An additional dermal wipe of the neck was taken after each subject cleaned their neck and face with a baby wipe containing no alcohol or aloe. This final neck sample was collected from the right side of the body.

Urine samples were collected in 120 mL Coviden urine collection cups (Fisher Scientific, Pittsburgh, PA) to measure exposure to PAHs and other AhR active compounds. Urine was collected just before the control fire and 2, 4, and 6 hrs post-fire. Urine samples were held on ice until transported according to ADOT guidelines to the laboratory where they were processed immediately.

2.5. Sample preparation and extraction

Dermal wipes were extracted to be analyzed using *in vitro* bioassays. Wipes were sonicated with 20 mL of DCM for 30 min, followed by the DCM being transferred through a sodium sulfate (Sigma Aldrich) packed column. This sonication and transfer step was completed a second time, with the extracts being combined. The extract was then evaporated under a stream of nitrogen below 1 mL, then filtered through a Millex 13 mm PFTE 0.45 μm filter (Fisher Scientific) using a glass syringe. Finally, 85 μL of DMSO was added to the extract, evaporated under nitrogen, and brought up to 100 μL DMSO by weight for analysis with *in vitro* bioassays. All extracts were stored at −20 °C until analyzed.

Urine samples were well mixed prior to processing. One 10 mL vial of each urine sample was kept in neat form, specific gravity (S.G.) was measured using an Atago urine specific gravity refractometer (Atago USA, Inc., Bellvue, WA), and the remaining urine was centrifuged for 10 min at 1500–2000 rpm (400–600 × g). The supernatant was aliquoted into 10 mL aliquots. All 10 mL aliquots were stored at −20 °C.

Extraction of urines for bioassay analysis began with a deconjugation of the urine which was conducted in a similar manner as (Fernando et al., 2016). Briefly, 10 mL of pre-centrifuged urine was added to 16.5 mL of 0.100 M sodium acetate buffer (pH 5.5) and 33.3 μL of β-glucuronidase from *Helix pomatia* (Sigma Aldrich, Milwaukee, WI). This mixture was incubated at 37 °C for 16–18 hrs. Waters HLB cartridges (150 mg, 6 mL) (Waters, Milford MA) were conditioned with 5 mL of HPLC grade methyl-tert butyl ether (MtBE) (Fisher Scientific) followed by 5 mL of HPLC grade Methanol (MeOH) (Fisher Scientific) and then 5 mL of ultra-pure water. The sample was loaded onto the cartridge followed by 5 mL of water. The cartridge was dried by aspirating nitrogen through to remove all residual water. Elution was done with 5 mL of MeOH followed by 5 mL of a solution containing 90% MtBE and 10% MeOH. Eighty five microliters of DMSO was added to the eluent, MtBE and MeOH evaporated under nitrogen, and then brought up to 100 μL DMSO by weight. Extracts were stored at −20 °C until analyzed.

Extraction of urines for analytical analysis of hydroxylated PAHs on an Agilent Gas Chromatography-Triple Quadrupole Mass Spectrometer (GC-QQQ) followed a similar extraction procedure as above. A detailed description is provided in the supplemental material.

2.6. In vitro bioassay culture and exposure

PAH CALUX® assays were cultured and exposed according to the manufacturer's guidelines (BioDetection Systems). Briefly, cells were seeded in 96-well plates at a density of 400,000 cells/mL, incubated at 37 °C for 16–18 hrs prior to adding the test chemicals and reference compound at a final concentration of 0.8% DMSO. Benzo[a]pyrene was used as the model PAH for this assay and for all relative potency (REP) calculations. All concentrations were tested in triplicate on each plate. Once exposed, cells were incubated for 4 hrs, then washed, lysed, luminescence reagent added and luminescence read using a Molecular Devices FlexStation 3 multi-mode microplate reader (Molecular Devices, Sunnyvale, CA). Relative potency of the PAHs and hydroxylated PAHs were calculated by first subtracting the solvent control (DMSO), then the maximum signal from the reference compound (B[a]P) was set to 100%, with the signal observed from the compounds of interest being illustrated as percentage of max response. Response curves are based on testing the compounds a total of 2 times for compounds with response < 15% the max response and 3 times for compounds with responses > 15% max response. When analyzing urine and dermal wipe samples, results were calculated as B[a]P equivalence in order to allow for comparison among samples, and method blank samples subtracted. S.G. was used to calculate a concentration factor to standardize the urine samples for how hydrated the subjects were at the time of collection. The concentration factor was calculated as follows: Concentration factor = (1.02 – 1.0)/(S.G. – 1.0).

2.7. Statistical analysis

All statistical analyses were performed using SPSS version 20.0 (SPSS, Chicago, IL, USA) and data expressed as mean ± standard deviation. Data were analyzed by one-sample Kolvogrov-Smirnov test for normality, and by Levene's test for homogeneity of variance. Data from skin wipes of the calf were analyzed by a student's *t*-test for comparison of samples within individuals, and a paired *t*-test when individuals were grouped. Data from skin wipes of the neck were analyzed by analysis of variance (ANOVA), followed by a Tukey's test. Data from the urine samples were analyzed by ANOVA, followed by a one-tailed Dunnett's test, comparing 0 hr to the 4 post-fire time points. A one-way ANCOVA was conducted to determine if there was a statistically significant difference between the type of hood on the relative PAH concentration on the skin post-fire, with the pre-fire response being the covariate. Correlations were assessed using the Spearman correlation coefficient. Levels below the limit of detection (LOD) were substituted with half the LOD for statistical analyses.

3. Results and discussion

3.1. Dermal wipes

Dermal wipes were collected before and after the fire at locations of the body found previously to have soot post-fire to address the hypothesis that dermal exposure contributes to the overall exposure of PAHs and related compounds to firefighters, and for the neck to investigate whether a prototype particulate blocking hood helped decrease exposure to PAHs and other AhR active compounds. Analysis of the dermal calf wipes showed a statistically greater response from the calf post-fire (M = 5.58 ng/cm^2, SD = 2.76 ng/cm^2) compared to pre-fire (M = 2.64 ng/cm^2, SD = 2.61 ng/cm^2) for the group of 10 firefighters who entered the control fire ($t(9) = -2.690$, $p = 0.025$), (Fig. 1). As expected, there was no statistical difference in the observed bioassay response post-fire between the group wearing the hood with specialized particulate blocking material and the group wearing a hood without specialized particulate blocking material for calf wipes ($F(1,7) = 0.704$, $p = 0.429$). The variability observed among the individuals pre-fire could be due to any number of activities they might have been involved in that would cause deposition of compounds on their skin. This could include use of skin care products or standing near any type of exhaust, among others. It is hypothesized the variability observed post-fire is likely due to how fitted the turnout gear was for each individual, providing more or less space for the particulates in the smoke to get underneath the turnout gear while they were simulating firefighting activities. It is important to note that the majority of individuals showed a statistical increase in deposition of compounds on the skin post-fire that interact with the AhR, regardless of the inherent variability of human participants.

The increased bioassay response observed from the calf wipe extracts post-fire indicates that PAHs and other AhR active compounds from the fire were deposited on the skin. This is in alignment with previous studies that demonstrated that a select number of PAHs were found at greater concentrations on the skin underneath PPE post-fire, and contribute to the overall exposure of PAHs and related compounds to firefighters (Laitinen et al., 2010; Baxter et al., 2014; Fent et al., 2014; Fernando et al., 2016; Stec et al., 2018). Laitinen et al (2010) illustrated that the hands are one area that PAHs can be deposited, and that wearing undergloves can decrease the amount of PAHs on the hands by up to 80%. Fernando et al (2016) found an increase in PAHs post-fire on the wrist, neck, forehead, back, and fingers of firefighters exposed to wood smoke. Stec et al (2018) found an increase in PAHs post-fire on the front of the neck, back of the neck, the jaw and the hands; and through a cancer risk characterization using PAH concentrations concluded that there is an elevated risk primarily through dermal exposure. Fent et al (2014) looked at the forearms, hands, neck,

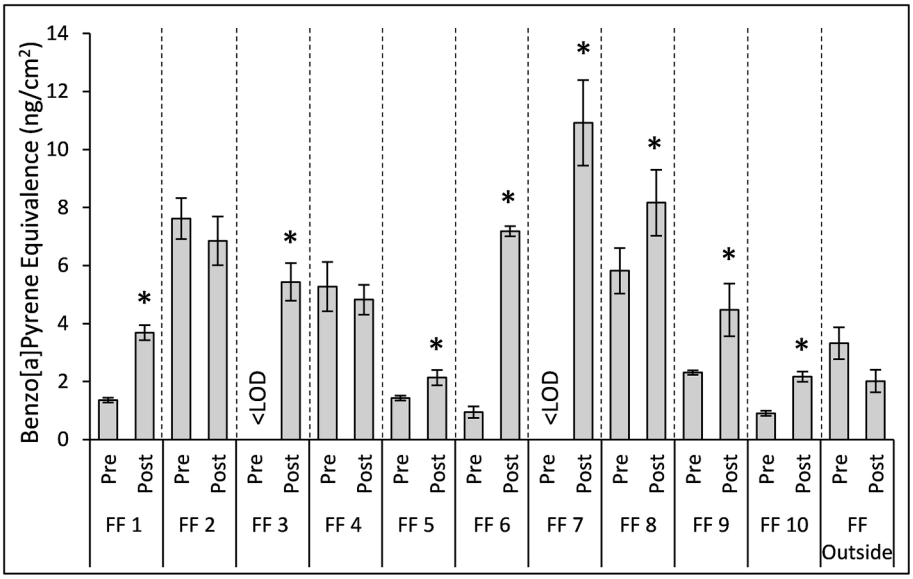

Fig. 1. Benzo[a]pyrene equivalence of dermal wipe samples taken pre-fire and post-fire from the calf of firefighters. '*' represents a post-fire sample being statistically greater than pre-fire (*t*-test, $p < 0.05$).

face, and scrotum as areas of concern, with the neck being the only site that was statistically greater post-fire in one of the conducted rounds. The neck is also of increased concern as the skin is thinner and in general, sites with thinner skin tend to have faster absorption rates (VanRooij et al., 1993). Finally, the neck is also the only location in which the PPE does not have a vapor barrier built into it, and therefore could lead to a greater deposition of contaminants and increased exposure compared to other areas. With this in mind, two different hood types were tested within this study, one with and one without specialized particulate blocking material.

When evaluating the neck for the presence of AhR active compounds using the *in vitro* bioassay, it was found that the majority of individuals saw greater concentrations of AhR active compounds post-fire (Fig. 2). Four of the five individuals wearing the particulate blocking hoods had greater bioassay response post-fire (Fig. 2A), as did 4 of the 5 individuals wearing the hoods without particulate blocking material (Fig. 2B). The individual who did not enter the fire was also wearing the non-particulate blocking hood and was found to have elevated AhR active compounds on the neck post-fire. It is uncertain if this increased bioassay response resulted from exposure to smoke within the vicinity of the fire, or if this was due to contamination during sampling. When comparing the two types of hoods, it was found that there was no statistically significant difference of post-fire bioassay response from the neck between the group wearing the hood with particulate blocking material and the group wearing a hood without particulate blocking material ($F(1,7) = 1.948$, $p = 0.206$). When this study was conducted, the hood containing the particulate blocking material was a prototype design which was ultimately not commercially produced.

The use of a baby wipe to wash the head and neck area during rehab was tested to see if this type of intervention would result in decreasing the concentration of PAHs and other AhR active compounds on the neck. It was found that there was a statistically significant decrease in the majority of individuals that had increased AhR active compounds on their skin post-fire (Fig. 2). This illustrates that the use of a baby wipe is beneficial to remove AhR active compounds from the skin soon after a fire as there is usually a time delay before the firefighters are able to shower and clean their skin. Additional studies designed to evaluate how effective skin wipes are at reducing the concentrations of PAHs, other AhR active compounds, and their metabolites in blood and/or urine would be beneficial to link the observed decrease of concentrations on the skin to what could be absorbed and enter the body.

3.2. Urine extracts

Urine collected pre- and post-fire was analyzed to investigate the degree of exposure firefighters received from this fire in the form of the AhR response in the PAH CALUX® assay. While dermal wipes give an estimation of skin deposition, measuring metabolites in the urine can help measure absorbed dose from inhalation, dermal and ingestion exposure. Two of the 5 individuals that wore the hood containing the particulate blocking material had a greater AhR-mediated response post-fire when compared to pre-fire (Fig. 3A), while only 1 of the 5 individuals who wore the hood without particulate blocking material had a greater AhR-mediated response post-fire compared to pre-fire (Fig. 3B). It was found that 2 to 4 hr post-fire was the optimal time to observe an increase in AhR active compounds in the urine (Fig. 3A and B). This is in accordance with a previous pilot study that was conducted by this research group, where urine samples were collected before and after a similar training fire (Fig. S.1) and from urine samples collected from firefighters responding to structural fires in the community (Fig. S.2). It is hypothesized that the variability in the intensity of the fire could be one reason only a few firefighters in this current study showed a significant increase in bioassay response in 2–4 hr post-fire urine extracts compared to the majority of firefighters from the pilot study and structural fires in the community.

3.3. Relative potency of PAH and hydroxylated PAHs standards in PAH CALUX bioassay

Of the 20 different compounds tested on the PAH CALUX® assay, 7 were found to have a quantifiable agonistic response with relative potencies (REPs) less than that of B[a]P (Table 1). The particular isoform of the compound was found to be important with respect to the observed response. Although metabolism usually leads to a decrease in biological activity of a compound, this was not the case for 4-hydroxyphenanthrene and 3-hydroxyfluorene. In both of these cases, the potency of the metabolite was greater than that of the parent compound (Table 1). It should be noted that the potency of some of the parent compounds could not be determined and therefore the REPs are being based off of the maximum concentration tested.

Since an agonistic response was observed from some of the hydroxylated PAHs, it is likely that the response observed from the urine extract has a proportion of the response coming from the hydroxylated PAHs in the mixture. In order to determine how much of the bioactivity

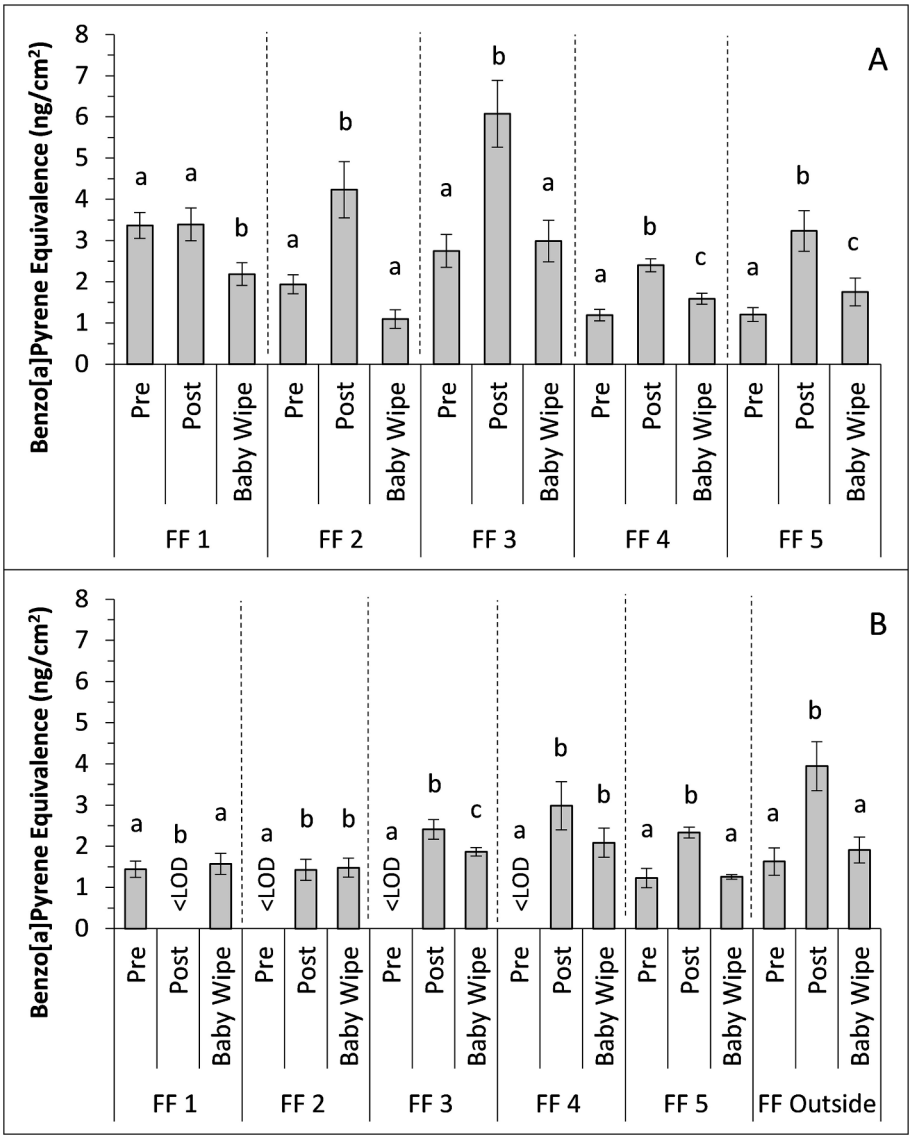

Fig. 2. Benzo[a]pyrene equivalence of dermal wipe samples taken pre-fire, post-fire and post-baby wipe from the neck of individuals wearing A) hoods with prototype particulate blocking material and B) hoods without particulate blocking material. A different letter represents a statistical difference ($p < 0.05$) among the different dermal samples collected pre-fire, post-fire or post-baby wipe for each individual when analyzed with ANOVA followed by a Tukey's test.

in the urine extracts was from the known hydroxylated PAHs, and how much was attributed to unknown metabolites, analytical analysis of these 20 hydroxylated PAHs was conducted on extracts of the same urine sample. Understanding what proportion of biological response was from the commonly quantified hydroxylated PAHs is important as current analytical tests use a select number of hydroxylated PAHs to represent the complete mixture of all PAH metabolites in the urine. Although correlations have been found with increased concentrations of some hydroxylated PAHs and exposure to fires (Fent et al., 2014; Keir et al., 2017; Wingfors et al., 2017), being able to get a measurement of the complete mixture is of great importance in evaluating overall exposure. This study also looked at the correlation of the sum of the quantified hydroxylated PAHs to the bioassay response from a set of urine samples collected from firefighters who responded to structural fires in the community. Baseline urine samples were collected to represent a no-exposure sample; and 2 hr post-fire urine samples were collected after responding to the structural fire. The select set of samples was chosen in order to provide a range of quantified hydroxylated PAHs post-fire, to identify if there is a correlation between quantifiable PAHs and bioassay response. It was shown that there was a statistically significant relationship between the hydroxylated PAHs known to be elevated post-fire and the *in vitro* bioassay response ($r(14) = 0.638$, $p = 0.008$).

Concentrations of the hydroxylated PAHs were quantified in extracts of the urine samples from the control fire (Table S.1) and were used along with the REPs to predict a B[a]P equivalence response. This predicted B[a]P equivalence was compared to the response observed from the urine samples using the PAH CALUX® assay. Of the PAH-OHs that were responsive in the bioassay and used to calculate the predicted B[a]P equivalence, 2-hydroxyphenanthrene, 3-hydroxyfluorene, 1-hyroxypyrene, 6-hydroxychrysene and 2-hydroxynaphthalene were detected in the urine samples, where 4-hydroxyphenanthrene and 3-hydroxychrysene were below the detection limit in all samples. This comparison between predicted- and observed B[a]P equivalence showed that less than 1% of the response was able to be accounted for by the quantified hydroxylated PAHs, and therefore greater than 99% is from unknown compounds. This is not surprising as only a few of the likely vast number of metabolites in the urine were tested, and the AhR is known to interact with a diverse set of compounds. It must be noted that only a few of the hydroxylated metabolites of PAHs were

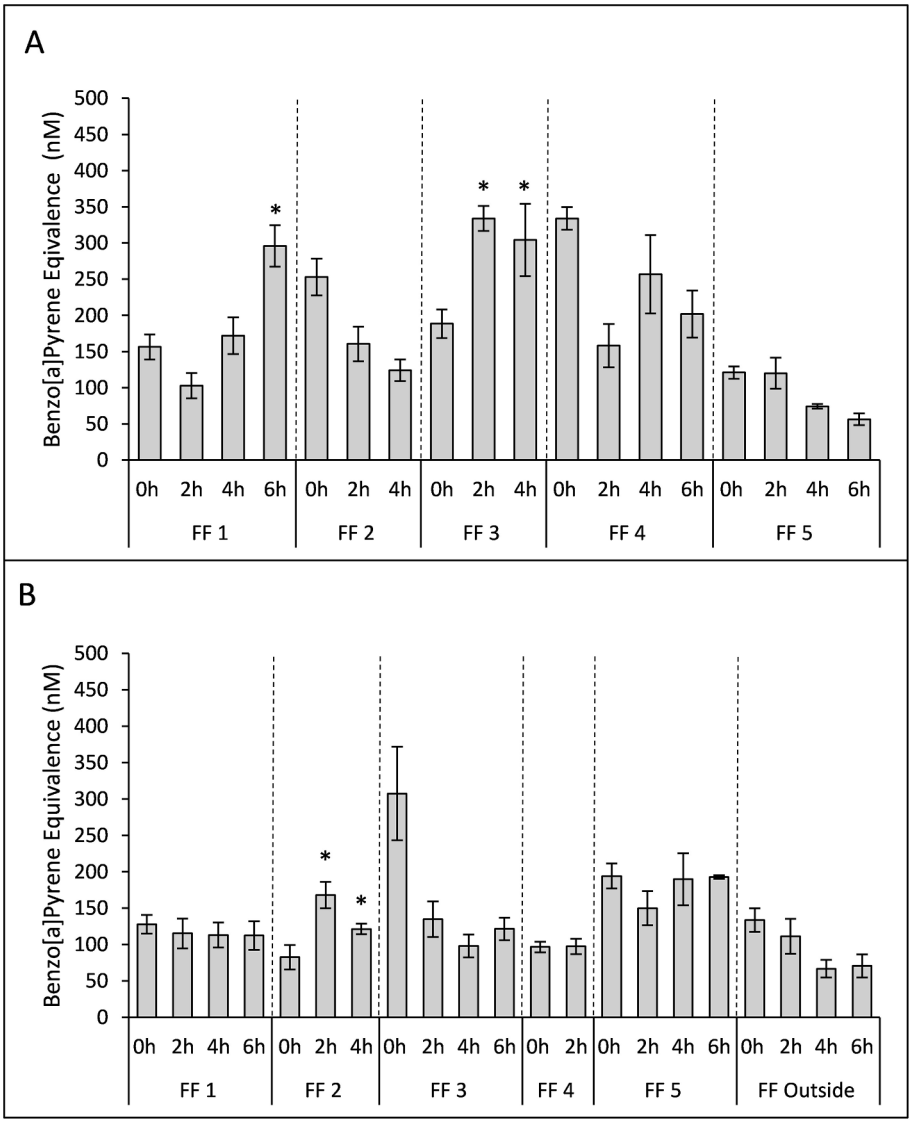

Fig. 3. Benzo[a]pyrene equivalence of urine samples taken pre-fire (0 hr) and at 2, 4, and 6 hr post-fire for individuals wearing A) hoods with prototype particulate blocking material and B) hoods without particulate blocking material. '*' represents a post-fire sample being statistically greater than 0 hr (ANOVA, $p < 0.05$).

quantified in the urine and the hydroxylated metabolites are a result of only one metabolic pathway, of which there are multiple.

There are sub classes of PAHs and related compounds which include alkylated-, heterocyclic- and nitro-PAHs that have been shown to be important groups to analyze in order not to underestimate the overall load of PAH contamination in environmental samples (Talaska et al., 1996; Titaley et al., 2016; Lam et al., 2018). Specifically, methylated PAHs have been shown to have greater potency in terms of AhR response than parent PAHs (Myers and Flesher, 1991; Pieterse et al., 2013; Lam et al., 2018) and have been shown to be mutagenic and carcinogenic. One study found that 5-methylchrysene was responsive in the PAH CALUX® assay, having a REP of 1.4 compared to B[a]P (Pieterse et al., 2013), which is between 280× and 170,000× of greater potency compared to the responsive hydroxylated PAHs in this study. Nitro PAHs and other unsubstituted PAHs have AhR-mediated activity, and have also been shown to have mutagenic activity (Pitts, 1987; Talaska et al., 1996; Ciganek et al., 2004; Amakura et al., 2016). In fact, some of these PAHs have been shown to respond in the PAH CALUX® bioassay with greater REPs than B[a]P. These include benzo[*j*] fluoranthene, dibenz[*a,h*]anthracene, indeno[1,2,3-*cd*]pyrene, benzo [*k*]fluoranthene, and benzo[*b*]fluoranthene, having REPs of 1.3, 1.3, 1.3, 3.7, and 5.0, respectively (Pieterse et al., 2013). Finally, poly aromatic ketones are a class of polar compounds found in wood smoke at equal abundances as PAHs that also have been shown to cause mutagenicity (Ramdahl, 1985). Unfortunately, the relative potencies of the metabolites of these compounds are not known and would need to be investigated to determine if they are present in the urine post-fire and to what extent they would respond. In addition to PAHs, some metabolites of PCBs have been shown to have agonist responses with the AhR (Machala et al., 2004). These are all metabolites of the variety of compounds that firefighters are exposed to and therefore could be in the urine and responsible for some of the observed bioassay response. Unfortunately, quantification of all metabolites in order to gain a comprehensive view of the exposure profile is nearly impossible, which is why the bioassay is advantageous as its strength lies in assessing mixture effects. Additional research should be conducted to identify the compounds present in the urine that are responsible for the majority of the bioassay activity, which could result in new, more prominent biomarkers of exposure. It is suggested that future research use an effect directed analysis approach to aid in the separation and identification of possible bioactive compounds.

Additional *in vitro* bioassays designed to assess toxicity endpoints such as genotoxicity or oxidative stress could also be used in addition to the PAH CALUX® assay to characterize the toxicity of the exposures that

Table 1
Overview of relative potencies (REP) of PAHs, hydroxylated PAHs and methoxyphenols in relation to Benzo[a]pyrene in the PAH CALUX® assay.

PAH derivative	EC20 ± S.D (M)	EC50 ± S.D (M)	REP EC20	REP EC50	Reference
1-Hydroxyphenanthrene	> 1.00E−5	> 1.00E−5	N/A	N/A	This Study
2-Hydroxyphenanthrene	3.93E−5 ± 4.57E−6	8.11E−5 ± 4.00E−6	2.69E−5	5.63E−5	This Study
3-Hydroxyphenanthrene	> 1.00E−5	> 1.00E−5	N/A	N/A	This Study
4-Hydroxyphenanthrene	1.83E−6 ± 2.19E−7	7.75E−6 ± 1.26E−6	5.78E−4	5.89E−4	This Study
9-Hydroxyphenanthrene	> 1.00E−5	> 1.00E−5	N/A	N/A	This Study
Eugenol	> 1.00E−4	> 1.00E−4	N/A	N/A	This Study
Guaiacol	> 1.00E−4	> 1.00E−4	N/A	N/A	This Study
4-Ethylguaiacol	> 1.00E−4	> 1.00E−4	N/A	N/A	This Study
2,6-Dimethoxyphenol	> 1.00E−3	> 1.00E−3	N/A	N/A	This Study
2-Hydroxyfluorene	> 8.00E−4	> 8.00E−4	N/A	N/A	This Study
3-Hydroxyfluorene	2.38E−6 ± 8.7E−7	8.43E−6 ± 2.75E−6	4.44E−4	5.41E−4	This Study
4-Hydroxyfluorene	> 3.00E−5	> 3.00E−5	N/A	N/A	This Study
1-Hydroxypyrene	1.09E−4 ± 1.38E−5	> 1.00E−4	9.70E−6	N/A	This Study
2-Methoxy-4-Methylphenol	> 1.00E−4	> 1.00E−4	N/A	N/A	This Study
2-Methoxy-4-Propylphenol	> 1.00E−4	> 1.00E−4	N/A	N/A	This Study
3-Hydroxychrysene	2.46E−7 ± 4.72E−9	1.03E−6 ± 1.31E−7	4.30E−3	4.43E−3	This Study
6-Hydroxychrysene	3.06E−6 ± 3.71E−7	1.34E−5 ± 1.42E−6	3.45E−4	3.40E−4	This Study
1-Hydroxynaphthalene	> 2.40E−4	> 2.40E−4	N/A	N/A	This Study
2-Hydroxynaphthalene	3.54E−5 ± 4.56E−6	7.50E−5 ± 1.25E−5	2.99E−5	6.08E−5	This Study
3-Hydroxybenzo[a]pyrene	> 8.00E−6	> 8.00E−6	N/A	N/A	This Study
Benzo[a]pyrene	1.06E−9 ± 1.31E−10	4.56E−9 ± 8.66E−10	1.00	1.00	This Study
2,3,7,8-Tetrachlorodibenzodioxin				5.0	Pieterse et al, 2013
5-Methylchrysene				1.4	Pieterse et al, 2013
Chrysene				0.8	Pieterse et al, 2013
Naphthalene				< 1.00E−4	Pieterse et al, 2013
Fluorene				< 1.00E−4	Pieterse et al, 2013
Phenanthrene				< 1.00E−4	Pieterse et al, 2013
Pyrene				< 1.00E−4	Pieterse et al, 2013

firefighters receive. For example, a recent study found increased mutagenic potential of post-fire urine compared to pre-fire urine collected from firefighters (Keir et al., 2017). These endpoints would be of importance in addressing the targeted toxicity pathways that contaminants entering the body might take. This would add to the comprehensive view of the exposure firefighters receive, and ultimately these endpoints could be used to monitor and assess intervention techniques with the primary goal of decreasing the exposure firefighters and associated personnel receive.

4. Conclusion

The PAH CALUX® bioassay was shown to be useful in assessing firefighter's exposure to PAHs and other AhR active compounds by quantifying bioassay responses from extracts of dermal wipes and of urine samples collected before and after a fire. The majority of individuals had an increase of AhR active compounds on the skin of the calf and neck post-fire. Wearing a prototype hood with particulate blocking materials as compared to a standard hood did not affect the increase in AhR active compounds on the neck post-fire. The use of a baby wipe to wipe the skin of the face and neck during rehab post-fire decreased the observed bioactivity and therefore the amount of AhR active compounds on the skin. Although some hydroxylated PAHs were found to be agonistic of the AhR, the majority of bioassay response observed in the urine extracts was likely from compounds other than the hydroxylated PAHs quantified. More research is needed to identify which compounds are primarily responsible for the increased bioassay response in the urine post-fire, which might lead to a new biomarker of exposure.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105207.

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