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Biomarkers of exposure to polycyclic aromatic hydrocarbons (PAHs) and DNA damage: a cross-sectional pilot study among roofers in South Florida

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ABSTRACT

Objective: The main goal of this pilot study was to assess the technical and logistic feasibility of a future study. The research hypothesis is that occupational exposures to polycyclic aromatic hydrocarbons (PAHs) are associated with increased risk of DNA damage among roofers who work with hot asphalt.

Design: This is a cross-sectional pilot study.

Setting: The study included roofers from four different construction sites in Miami-Dade County, Florida.

Participants: 19 roofers were recruited (six Hispanics and 13 African–Americans, all male), all of whom were eligible (no history of cancer and no history of chronic diseases of kidneys or liver). All participants provided pre-shift samples and 18 provided post-shift samples. Samples of one participant were excluded from the final analyses as they were considered unreliable.

Results: Levels of urinary PAH metabolites increased during 6 h of work. Linear regression models of post-shift metabolites included their pre-shift levels, post-shift urinary creatinine levels (for models of 1-OHPyr and 9-OHPhe), and skin burn due to contact with hot asphalt (for models of 1-OHPyr and 1-OHNap). Pre-shift levels of urinary 8-OHdG were not associated with any of the variables considered. For post-shift levels of 8-OHdG, however, post-shift 1-OHPyr (95% CI 0.091 to 0.788) and use of protective gloves (95% CI −1.57 to −0.61) during work explained 86.8% of its variation. Overall, highest levels of urinary PAH metabolites and of 8-OHdG were observed among workers who reported having skin burn and who did not use gloves during work.

Conclusions: Urinary 1-OHPyr is a promising predictor of oxidative DNA damage among roofers. Work-related skin burn and use of protective gloves appear to influence PAH exposure and DNA damage levels in this group, suggesting the importance of dermal absorption.

ARTICLE SUMMARY

Article focus

- Studies reported increased risks of various cancers among roofers. PAHs are considered to be the main carcinogenic exposures in this worker group.
- This pilot study was conducted to assess the feasibility of a future study that will investigate potential predictors for PAH biomarkers and DNA damage among roofers.

Key messages

- Cigarette smoking is a significant predictor of pre-shift levels of PAH metabolites.
- Skin burn due to contact with hot asphalt and use of gloves during work appear to be possible modifiers of PAH exposures and DNA damage among roofers who work with hot asphalt. Preventive strategies in this population should include efforts to reduce skin contact with hot asphalt.

Strengths and limitations of this study

- This pilot study simultaneously examines biomarkers of PAH exposure and oxidative DNA damage among roofers in South Florida. It is the first study to suggest a link between skin burn due to contact with hot asphalt and increased risk of DNA damage during work. Main limitations of the study are the lack of personal exposure data and the small sample size.

INTRODUCTION

Construction work has been repeatedly linked to occupational cancers, accounting for more than 30% of all work-related cancers in some studies.[1–3] Within construction workers, roofers are a particularly vulnerable group with increased risks of lung, bladder, stomach, skin and buccal cavity cancers, and leukaemia.[1 4–10] Asphalt is considered to be the main source of carcinogenic exposures in this industry, mainly due to the release of carcinogenic polycyclic aromatic hydrocarbons (PAHs).[1 5] It has been estimated that there are about 50 000 on-roof workers in the USA exposed to asphalt fumes during approximately 40% of their working hours.[11 12] According to the US Department of Labor, Florida had the second

highest concentration of roofers in 2010 accounting for almost 10% (9910) of roofers in the entire USA.[13] Despite the size of this worker population and the significance of the health concerns, workplace exposures in roofers have not been investigated widely and no studies so far have presented exposure and health risks among roofers in Florida.

Epidemiology studies investigating cancer risk among roofers have been criticised for mainly two problems: lack of specific personal exposure data and inadequate consideration of confounding factors such as cigarette smoking.[14–16] Indeed, many studies reconstructed historical exposure scenarios using qualitative data obtained from questionnaires, company records or industrial hygiene measurements, while others have relied on job histories or job-exposure matrices.[1 3 4 6–8 14 15] Furthermore, behavioural risk factors of cancer, such as cigarette smoking and alcohol consumption, are prevalent in this worker population.[17] Studies of the National Health Interview Survey reported that among all occupations covered, construction workers, and roofers in particular, have the highest smoking rates in the US workforce.[18] Currently, there are many research gaps, such as the quantification of organ-specific cancer risks among roofers, identifying the relative contribution of occupational exposures and work-related factors, as well as behavioural risk factors.[14–16 19]

Asphalt fumes have been shown to be mutagenic, genotoxic[20 21] and carcinogenic[22] in animals. While fumes created in the laboratory appeared to be more mutagenic than those collected from actual work sites,[21] studies among highway maintenance workers consistently reported increased DNA strand breaks, DNA adducts and sister chromatid exchanges.[10 23–25] So far, two studies assessed biological changes related to work in roofers, with only one quantifying individual PAH exposures and biomarker levels.[26 27] Recent studies support the link between insufficient cellular defence towards oxidative DNA damage and increased susceptibility to cancer development.[28–31] Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) is a widely accepted marker of oxidative DNA damage and repair,[29 32] as well as a biomarker of DNA damage related to occupational and environmental exposures.[33] The main goal of this pilot study was to assess the feasibility of a future study that investigates the association between urinary PAH metabolites and markers of DNA damage in roofers exposed to asphalt.

METHODS

Study population and sample collection

Study participants consisted of roofers in the Miami-Dade area who were recruited with informed consent under Institutional Review Board-approved protocols from the Florida International University. All participants were eligible based on the criteria that they had no history of cancer and chronic diseases of the kidneys or liver. Sample size was based on available funding and access to asphalt roofing sites during the study period. Urine samples were collected from 19 roofers at four different construction sites during December 2008 (two sites, n=5), January 2009 (one site, n=4) and June 2009 (one site, n=9). Samples were collected before and after 6 h of roofing work. There were one to nine roofers per site. Separate questionnaires were applied by trained interviewers before and after the work shift and were available in both English and Spanish. Information on personal characteristics (eg, age, height, weight, body mass index (BMI)), history of known medical problems, lifestyle factors (frequency and amount of smoking and alcohol consumption), dietary PAH exposures (consumption of grilled, barbequed or smoked meat/chicken/fish within the 24 h), use of protective equipment (gloves, overalls, masks, protective shoes) and specific work tasks performed during the day (tearing off old roofing, application of new roofing materials, hot asphalt work) was collected via the questionnaires. Participants were also asked of skin contact with asphalt during work, extraordinary events (accidents) during the workday and the existence of skin irritation. Additionally, roofers were asked if they had any skin burns as a result of contact with hot asphalt.

Quantification of urinary analytes

Urine samples were aliquoted into 15 ml polypropylene tubes in the field, immediately frozen in dry ice and kept frozen at −80°C until analysis. Samples were thawed at room temperature followed by vortex mixing. Aliquots of urine were spiked with the internal standards (1-hydroxypyrene-d_9 for PAH metabolites and creatinine-d_3 for creatinine). Sample analysis for both PAH metabolites and creatinine was performed on an API 3000 mass spectrometer (Applied Biosystems/MDS Sciex, Toronto, Canada) coupled with a Shimadzu model 10-ADvp and SIL-HTc HPLC system (Shimadzu, Columbia, Maryland, USA) using electrospray ionisation. The area under the mass ion peaks of the analytes and internal standard were integrated using Analyst software (version 1.4.2; Applied Biosystems/MDS, Sciex, Toronto, Canada).

Biomarkers of PAH exposure

Urinary PAH metabolites were enzymatically deconjugated using β-glucuronidase/aryl sulphatase, followed by solid-phase extraction using C_{18} cartridges as described by Onyemauwa *et al*.[34] Chromatographic separation was conducted on a Develosil C_{30} HPLC column (4.6×50 mm, 3 µm). Mass spectrometric detection was performed in negative mode and multiple reaction monitoring was used to detect 2-OHNap, 1-OHNap, 1-OHPyr, 2-OHFlu, 9-OHPhe and the internal standard at *m/z* 143/115, 143/115, 217/189, 181/180, 193/165 and 226/198, respectively. Quantitation was based on peak area ratios relative to the internal standard. The lowest limits of quantitation (signal to noise ratio of 10) were 25 ng/l for 1-OHPyr and 50 ng/l for all other PAH metabolites. The linearity dynamic ranges were from 25 to 20 000 ng/l for 1-OHPyr and 50−20 000 ng/l for all other PAH metabolites. To estimate the recoveries of

PAH metabolites from urine, 2-OHNap, 1-OHNap, 1-OHPyr, 2-OHFlu and 9-OHPhe were spiked to control urine (0.5, 1, 5 and 15 µg/l, four replicates) followed by enzymatic hydrolysis, extraction and liquid chromatography - tandem mass spectrometry analysis. Recovery rates ranged from 88.1% to 112.1%, 87.2% to 109.3%, 89.5% to 106.4%, 90.1% to 113.7% and 87.9% to 113.4% for 2-OHNap, 1-OHNap, 1-OHPyr, 2-OHFlu and 9-OHPhe, respectively. The intra- and inter-day accuracy was 87.2%−113.7%, and the intra- and inter-day precision was 5.2%−14.1% for all metabolites.

Urinary creatinine

Creatinine was dissolved in methanol for the preparation of standard samples. Urine samples were diluted 10 000-fold with methanol and chromatographic separation was conducted on a Develosil C_{30} HPLC column (4.6×50 mm, 3 µm). Mass spectrometric detection was performed in positive mode and multiple reaction monitoring was used to detect creatinine and the internal standard at *m/z* 114/44 and 117/47, respectively. The lowest limit of quantitation of creatinine diluted in methanol was 10 µg/l (signal to noise ratio of 10), equivalent to 10 mg/dl in urine samples. The linearity dynamic ranges were from 10 to 2000 µg/l in methanol. Recovery rates were measured using spiked control urine (50, 100, 500 and 1000 mg/dl, four replicates) and ranged from 85.7% to 112.1%. The intra- and inter-day accuracy was 88.4%−112.9%, and the intra- and inter-day precision was 4.5%−13.2%.

Biomarkers collected in spot urine samples are many times reported in respect to levels of urinary creatinine to adjust for differences in dilution. The WHO guidelines for biomonitoring recommend excluding urine samples with creatinine levels below 30 mg/dl or above 300 mg/dl.[35] However, Barr *et al*[36] recently reported that the WHO criteria are too restrictive and would have led to a loss of up to 19% of the samples from their study in the general population. In this study, measurements from one individual were excluded from analyses, based on concerns that this participant may have added water to the samples before giving them to the investigators. Overall, we observed higher levels of urinary creatinine following work hours (98.9−586.0 mg/dl) compared with measurements before work (39.1−382.0 mg/dl). This may reflect water loss during work since outdoor temperatures were high throughout the study (22−29°C, highest at 33°C). Race and BMI were significant predictors of urinary creatinine, explaining 74.8% of the variation in post-shift samples (higher values among African−Americans and those with higher BMI). Thus, urinary creatinine was only added as an independent predictor of urinary biomarkers in linear regression models as suggested by others.[36 37]

Biomarker of oxidative DNA damage

Levels of urinary 8-OHdG were determined via ELISA using the New 8-OHdG Check Kit (Japan Institute for the Control of Aging, Shizuoka, Japan).

Statistical analyses

All statistical tests were performed after natural logarithmic transformation of the biomarker data at a significance level of 0.05 using the SAS version 9.2 system software (SAS). Normality was tested using the Shapiro−Wilks test (Proc CAPABILITY of SAS). Geometric mean levels of urinary analytes in samples collected before and after work were compared using Student t test of the logged values (Proc *t* test of SAS). Median levels of urinary metabolites were also compared by the Wilcoxon rank-sum test (using NPAR1WAY procedure of SAS) with respect to sampling time. Very consistent results were obtained with the Wilcoxon rank-sum test and the analysis of variance. Multiple comparisons were applied to investigate the effect of sampling time on concentrations of urinary metabolites categorised by cigarette smoking status (two-way analysis of variance using Proc GLM of SAS). Correlation coefficients for pairs of urinary analytes were calculated for samples collected before and after the work using Proc CORR of SAS. Very consistent results were obtained with both Spearman and Pearson (with log-transformed data) correlation tests. Multiple linear regression (PROC REG of SAS) was used to investigate the sources of variation of urinary biomarkers. Independent variables included demographic and lifestyle factors, diet, work-related factors, protective equipment use, skin contact with asphalt during the work, self-reported skin irritation or skin burn (due to contact with hot asphalt). Univariate analyses were conducted first to select the most likely candidates ($p<0.10$); the remaining variables were then examined by backward elimination to achieve final models. Only variables with significant contributions ($p<0.05$) are presented in the final models.

RESULTS

Of the 19 roofers, six were Hispanics and 13 were African−Americans. All were men with an average age of 38.4 (22−55). Nine of the roofers (47.4%) were smokers and six of them reported having smoked within the past 24 h. While 10 (52.6%) reported never consuming alcoholic beverages, seven (36.9%) reported consuming them at least 3 days a week. Among those who consumed alcohol, four reported having 12−24 drinks in one sitting.

Roofing sites during this study involved built-up roofing where several layers of roof felts were laminated together by hot asphalt mopped in between the layers. All roofers were involved in a number of different tasks during the workday, but most of them reported 'tearing old roof' (n=18) and 'applying new roofing' (n=12) as their main tasks. Nine of the roofers reported working with hot asphalt during the study day. There were also nine workers who reported having skin burn (located on hands and forearms) due to contact with hot asphalt; four of these roofers did not work with hot asphalt during the study. Protective gloves were used by 10 workers (52.6%). No other protective clothing was used.

When asked how frequently they changed into clean work clothing, 15 workers reported changing them daily, while four reported changing them once a week.

Urinary biomarkers of exposure and DNA damage

Measurements of urinary metabolites, except for 9-OHPhe, were above the lowest limit of quantitation in all samples. Pre-shift urinary 9-OHPhe was below the lowest quantitation limit (LLOQ =0.05 µg/l) for one individual and was replaced with LOD/$\sqrt{2}$ as described by Hornung and Reed.[38] Tables 1 and 2 present geometric mean levels of urinary biomarkers by sampling time and smoking status. Smokers had higher levels of all urinary PAH metabolites, but 2-OHFlu showed the strongest associations with smoking status (4.18-fold higher pre-shift levels observed in smokers, table 2). Measurements of urinary metabolites were higher in post-shift samples, with the largest increases observed among non-smokers (table 2). Levels of urinary 8-OHdG increased by 4.3-fold during the work (table 1). Correlations among all PAH metabolites were strong before the work (Pearson r between 0.716 and 0.888, $p<0.05$ for all, table 3) but became weaker or not significant after the work (table 3) with the exception of urinary 1-OHPyr. None of the urinary PAH metabolites correlated with urinary 8-OHdG before the work (table 3). In samples collected after the work, however, 8-OHdG significantly correlated with the levels of 1-OHNap, 9-OHPhe and 1-OHPyr (table 3).

Regression models of urinary biomarkers

Table 4 presents results of final linear regression models of urinary markers. While a number of factors were investigated, only the significant predictors ($p<0.05$) were left in the final models. None of the variables explained 8-OHdG before the work. However, post-shift levels of urinary 1-OHPyr and use of gloves explained 86.8% of its variation after the work (table 4). For pre-shift levels of PAH metabolites, urinary creatinine and being a smoker were the only significant variables explaining 30.0%–66.9% of their variation (table 4). In samples collected after the work, 'skin burn related to work' was an important contributor of urinary 1-OHPyr and 2-OHNap along with their pre-shift measurements (table 4). Skin burn, pre-shift levels and urinary creatinine explained 92.0% of the variation of post-shift 1-OHPyr; skin burn and pre-shift levels explained 61.8% of the variation of 2-OHNap (table 4). The use of gloves during work was also inversely associated with urinary 1-OHPyr but was excluded from the final models since it was not significant ($p=0.09$) based on our criterion. Urinary creatinine was a significant predictor for pre-shift levels of all PAH metabolites but remained significant for only 1-OHPyr and 9-OHPhe in post-shift models (table 4).

Table 5 provides an ad hoc analysis of urinary analytes by self-reported glove use and work-related skin burn. Overall, the highest levels of urinary PAH biomarkers were observed among workers who reported having skin burn and who did not use gloves during work hours (table 5). Similarly, levels of urinary 8-OHdG were highest among workers who had skin burn and did not use gloves (105.6 µg/l). In fact, these levels were 6.7-fold higher than those observed among workers who did not have skin burn and used gloves during the work (15.8 µg/l).

Table 1 Geometric means (and geometric SD) of urinary analytes before and after 6 h of work

Levels of urinary analytes (ng/l)	Before work (n=18)	After work (n=17)
1-OH-napthalene	992 (4)	2553 (2)*
2-OH-naphthalene	2670 (2)	3984 (2)
9-OH-phenanthrene	441 (4)	1033 (2)*
2-OH-fluorene	672 (3)	1541 (3)*
1-OH-pyrene	361 (3)	692 (2)*
8-OHd-guanosine (µg/l)	9 (4)	40 (2)*

*$p<0.05$ when compared with samples collected before work (using Student t test).

DISCUSSION

Levels of 1-OHPyr in this pilot study were higher than those observed among the general population in the USA (which varied between 50.0[39] and 74.8[40] ng/l, table 6) but were lower than the levels reported in other roofer groups (range from 1.74 to 10.65 µg/l in post-shift samples,[27 43] table 6). Table 7 presents published values of urinary 8-OHdG. Pre-shift measurements of 8-OHdG in our study were similar to those observed among healthy Japanese volunteers[49] and controls from Taiwan[52] but were lower than those reported in China.[50] While 8-OHdG is a widely accepted marker of oxidative DNA damage, there are uncertainties regarding its use in population-based studies. A dose–response relationship with various exposures is not well established. The analytical method has an impact on the results, with higher levels reported using ELISA compared with HPLC.[33 49] A significant correlation was observed between urinary 8-OHdG and 1-OHPyr among coke oven workers.[53] A study in roofers, however, failed to show such increase of urinary 8-OHdG over a work week, except among those roofers who were exposed to coal tar.[27] We observed significant increases of urinary 8-OHdG levels during the work shift and strong correlations between this oxidative DNA damage marker and 1-OHNap, 9-OHPhe and 1-OHPyr, after 6 h of roofing work. 1-OHPyr and use of gloves were the only predictors of post-shift urinary 8-OHdG. Urinary 1-OHPyr levels strongly correlated with 8-OHdG after the work, suggesting an association between exposure to pyrene and oxidative DNA damage during work hours. This may indirectly reflect other potential exposures that are correlated with pyrene and are responsible for oxidative DNA damage (such as unmeasured carcinogenic PAHs). A striking finding of this pilot study was that both

Table 2 Geometric means (and geometric standard deviations) of urinary analytes before and after 6 h of work by self-reported smoking status

Levels of urinary analytes (ng/l)	Before work (n=18)		After work (n=17)	
	Non-smokers (n=10)	Smokers (n=8)	Non-smokers (n=9)	Smokers (n=8)
1-OH-naphthalene	672 (4)	1604 (3)	1978 (2)*	3395 (2)*
2-OH-naphthalene	1808 (2)	4316 (2)*	2670 (2)	6186 (2)* ‡
9-OH-phenanthrene	265 (4)	846 (2)*	713 (3)*	1572 (2)*
2-OH-fluorene	358 (3)	1495 (2)*	925 (3)*	2724 (2)* ‡
1-OH-pyrene	213 (3)	692 (2)*	498 (2)*	1002 (2)*
8-OHd-guanosine (μg/l)	8 (6)	12 (2)	29 (2)*	59 (2)* †

Using multiple comparisons: *p<0.05 when compared with non-smokers before work; †p<0.05 when compared with smokers before work; ‡p<0.05 when compared with non-smokers after work.

8-OHdG and 1-OHPyr could be significantly predicted by variables that potentially alter dermal absorption of PAHs. A negative association between glove use during work and levels of 8-OHdG supports the importance of this simple protective clothing. Skin burn, mostly located in forearms or hands of the roofers, was a significant predictor of post-shift levels of 1-OHPyr. Furthermore, a post-hoc analysis showed that highest levels of 1-OHPyr and 8-OHdG appear to be among roofers who had skin burn and did not use gloves, while the lowest levels were among those who did not have skin burn and used gloves during work (table 5). Hot asphalt work poses significant risks for skin burn and injury since the kettles are usually maintained around 288–316°C (550–600°F).[12] In a previous study among Finnish workers, skin irritation was reported by 44% of the roofers and 31% of the road pavers who worked with bitumen.[54] However, no studies have thus far looked into the relationship between skin burn and levels of biomarkers of exposure in roofers. In our analyses, the existence of skin burn was an important predictor of PAH biomarkers. This may reflect increased exposures to PAHs during hot asphalt work. However, work with hot asphalt was not a significant predictor in any of the regression models. We speculate that skin burn increases dermal absorption of PAHs through injured skin. Alternatively, skin burn could also be a cumulative marker of exposure through work with hot asphalt, with the assumption that incidence of skin burn is correlated with number of hours of hot asphalt work. This needs to be investigated in future studies.

Two important limitations of our study were the small sample size and the lack of PAH exposure data. Because of the small sample size, single observations in this study may have large potential influence on our findings. Our results need to be interpreted cautiously until consistent findings are observed in similar studies with larger populations. We observed an increase in PAH metabolite levels during work, but we could not determine the relative contribution of occupational exposure sources for this increase. A future study with personal exposure measures would allow us to identify different sources of variation of PAH exposures and possibly strengthen the association between occupational exposure and DNA damage. Despite these important limitations, we were able to observe statistically significant associations between 8-OHdG and 1-OHPyr after 6 h of work. 1-OHPyr is the main metabolite of pyrene and has been widely accepted as the gold standard of biomarkers of exposure to PAHs.[55 56] In this study, average level of 1-OHPyr increased during the work hours, after which it showed strong associations with post-shift urinary 8-OHdG. When compared with the smaller molecular PAHs, which are almost exclusively in gaseous phase, approximately 40% of pyrene is estimated to be in particulate phase.[57] While this provides a major challenge in assessing total inhalation exposures, pyrene may be a better surrogate of dermal exposures when compared with the smaller PAHs. As a contrast, metabolites of the smaller molecular PAHs appear to have stronger associations with cigarette smoking in this study, particularly urinary 2-OHFlu.

Table 3 Pearson correlation coefficients (and p values) among urinary measurements observed in roofers

	Post-shift					
	1-OHNap	2-OHNap	9-OHPhe	2-OHFlu	1-OHPyr	8-OHdG
Pre-shift						
1-OHNap	1.0	0.343 (0.177)	0.457 (0.065)	0.340 (0.182)	0.640 (0.006)	0.492 (0.045)
2-OHNap	**0.785 (0.0001)**	1.0	0.617 (0.008)	0.715 (0.001)	0.578 (0.015)	0.235 (0.365)
9-OHPhe	**0.839 (<0.0001)**	**0.785 (0.0001)**	1.0	0.814 (<0.0001)	0.848 (<0.0001)	0.603 (0.010)
2-OHFlu	**0.716 (0.0008)**	**0.888 (<0.0001)**	**0.839 (<0.0001)**	1.0	0.698 (0.002)	0.401 (0.111)
1-OHPyr	**0.772 (0.0002)**	**0.727 (0.0006)**	**0.792 (<0.0001)**	**0.814 (<0.0001)**	1.0	0.802 (0.0001)
8-OHdG	**0.316 (0.202)**	**0.030 (0.905)**	**0.228 (0.362)**	**−0.004 (0.988)**	**0.171 (0.497)**	1.0

Values in bold represent pre-shift correlations, and values that are not in bold represent post-shift correlations.

Table 4 Linear regression models of urinary biomarkers

	Overall R^2	Predictor variables	Estimates	95% CI	p Value
Ln (8-OHdG)*					
Post-shift	0.868	Intercept	1.34	−1.11 to 3.78	0.261
		Ln (post-shift 1-OHPyr)	0.440	0.091 to 0.788	0.017
		Gloves	−1.09	−1.57 to −0.61	0.0002
Ln (1-OHPyr)					
Pre-shift	0.569	Intercept	4.91	4.30 to 5.52	<0.0001
		Ln (pre-shift creatinine)	1.10	0.333 to 1.87	0.008
		Smoker	1.09	0.309 to 1.87	0.009
Post-shift	0.920	Intercept	3.04	2.34 to 3.74	<0.0001
		Ln (pre-shift 1-OHPyr)	0.416	0.295 to 0.537	<0.0001
		Ln (post-shift creatinine)	0.712	0.459 to 0.964	<0.0001
		Skin burn history	0.528	0.262 to 0.794	0.001
Ln (1-OHNap)					
Pre-shift	0.300	Intercept	6.30	5.56 to 7.03	<0.0001
		Ln (pre-shift creatinine)	1.35	0.256 to 2.44	0.019
Post-shift	0.742	Intercept	4.25	3.07 to 5.44	<0.0001
		Ln (pre-shift 1-OHNap)	0.519	0.351 to 0.688	<0.0001
Ln (2-OHNap)					
Pre-shift	0.629	Intercept	7.15	6.74 to 7.55	<0.0001
		Ln (pre-shift creatinine)	0.853	0.340 to 1.37	0.003
		Smoker	0.802	0.282 to 1.32	0.005
Post-shift	0.618	Intercept	2.19	−0.672 to 5.05	0.123
		Ln (pre-shift 2-OHNap)	0.730	0.378 to 1.08	0.0006
		Skin burn history	0.590	0.042 to 1.14	0.037
Ln (2-OHFlu)					
Pre-shift	0.669	Intercept	5.33	4.74 to 5.93	<0.0001
		Ln (pre-shift creatinine)	1.32	0.570 to 2.07	0.002
		Smoker	1.32	0.562 to 2.08	0.002
Post-shift	0.775	Intercept	2.76	1.38 to 4.14	0.0007
		Ln (pre-shift 2-OHFlu)	0.700	0.493 to 0.908	<0.0001
Ln (9-Phe)					
Pre-shift	0.591	Intercept	4.90	4.18 to 5.63	<0.0001
		Ln (pre-shift creatinine)	1.64	0.725 to 2.56	0.002
		Smoker	1.02	0.094 to 1.96	0.033
Post-shift	0.650	Intercept	4.02	2.56 to 5.48	<0.0001
		Ln (pre-shift 9-OHPhe)	0.355	0.103 to 0.607	0.009
		Ln (post-shift creatinine)	0.707	0.123 to 1.29	0.021

*No significant predictors identified for pre-shift levels.

In their recent work among road pavers, Sobus *et al*[44] noticed a work-related effect on urinary metabolites of phenanthrene and pyrene but not for metabolites of naphthalene, which the authors linked to the close association between naphthalene and cigarette smoking.[44] The authors also observed an increase in urinary biomarker levels over the workweek, with higher levels observed towards the end of the week.[44] Results from this pilot study support the closer link between work-related factors and urinary 1-OHPyr levels. Since we only collected urine samples after 6 h of work, we could not compare urinary biomarker levels during the week.

Table 5 Geometric mean levels (and geometric SDs) of urinary biomarkers after the work by skin burn and glove use

After work levels of urinary PAH metabolites (ng/l)	No skin burn		With skin burn	
	No gloves, n=6	With gloves, n=2	No gloves, n=3	With gloves, n=6
1-OH-naphthalene	2416 (2)	1808 (5)	5014 (2)	2143 (2)
2-OH-naphthalene	2969 (2)	3752 (2)	8866 (1)	3605 (2)
9-OH-phenanthrene	1163 (3)	1119 (1)	2864 (1)	534 (1)
2-OH-fluorene	1408 (2)	3011 (3)	4447 (1)	788 (2)
1-OH-pyrene	804 (2)	388 (1)	1959 (1)	437 (1)
8-OHd-guanosine (μg/l)	72 (2)	16 (2)	106 (1)	19 (1)

Table 6 Published values of urinary 1-OHPyr (ng/l) in different populations

Authors (year)	Population	Overall levels	Non-smokers	Smokers
Li *et al* (2008)[39]	General population (USA)	50	–	–
Roggi *et al* (1997)[41]	General population (Italy)	286.0*	222.3*	486.5*
Huang *et al* (2004)[40]	General population (USA)	74.8	75.0*	240.0*
Toraason *et al* (2001)[27]	Roofers (USA)	780 (start of week, asphalt only)* 1740 (end of week, asphalt only)* 2220 (start of week, asphalt and coal tar)* 10 650 (end of week, asphalt and coal tar)*	–	–
McClean *et al* (2004)[42]	Asphalt pavers (USA)	186.5 (day 1—pre-shift)* 1554.4 (day 1—post-shift)* 932.6 (day 3—pre-shift)* 1865.3 (day 3—post-shift)*	–	–
McClean *et al* (2007)[43]	Asphalt roofers (USA)	1200 (Monday—pre-shift)* 3000 (rest of week—pre-shift)* 3900 (post-shift)*	–	–
Sobus *et al* (2009)[44]	Asphalt pavers (USA)	375 (baseline) 2110 (post-shift) 1130 (bedtime) 708 (following morning)	–	–
Levin *et al* (1995)[45]	Road pavers (Sweden)	–	210.0 (post-shift)* †	–
Serdar *et al* (2003)[46]	Coke oven workers (China)	25 355.6 (top of oven—post-shift) 11 989.1 (side of oven—post-shift) 1284.0 (clerical workers)	14 585.1 (top of oven) 9393.3 (side of oven) 1188.9 (clerical)	2095.9 (top of oven) 14 013.2 (side of oven) 36 598.2 (clerical)
Onyemauwa *et al* (2009)[34]	Steel workers (Central Europe)	586.0	–	–
Pesch *et al* (2011)[47]	Bitumen-exposed construction workers (Germany)	–	193 (pre-shift)† 419 (post-shift)†	407 (pre-shift)† 793 (post-shift)†
Current study	Roofers (USA)	361.4 (pre-shift) 692.3 (post-shift)	212.7 (pre-shift) 497.7 (post-shift)	692.3 (pre-shift) 1002.2 (post-shift)

*For comparison purposes (assuming a mean urinary creatinine concentration of 13 mmol/l) following conversion factors were used for converting urinary 1-OHPyr levels: 1 μmol/mol creatinine =1.93 μg/g creatinine =3000 ng/l (based on Levin[48]).
†Median levels.

Based on our results, we aim to improve our sampling design to include pre-shift and post-shift samples over three separate days of a workweek.

Despite the increased risks of various cancers, very few studies have quantified occupational exposures among roofers or looked at possible determinants of exposures in this group. Tearing old roofs and potential contact with coal tar have been reported to increase such exposures, particularly via the dermal route.[5 43] In our study, all roofers except one reported tearing old roof containing coal tar during the study day. Thus, it was not clear to what extent removing old roofs contributed to PAH biomarker levels. Higher exposure levels have also been observed in relation to poor workplace ventilation and sheltered areas such as balconies.[58] Asphalt temperature has been linked to PAH composition in asphalt fume, with more carcinogenic PAHs observed at higher temperatures.[11 58] Our results support that dermal absorption may be an important route of PAH exposure among roofers. Skin burn (due to contact with hot asphalt) and use of protective gloves during work may significantly modify levels of PAH biomarkers and of oxidative DNA damage. The prevalence of skin burn in this occupation and its role in increasing the absorption

Table 7 Published values of urinary 8-OHdG (μg/l)

Authors (year)	Method	Population	Overall	Non-smokers	Smokers
Yoshida *et al* (2002)[49]	ELISA	General population (Japan n=72)	11.1	–	–
Chiou *et al* (2003)[50]	ELISA	General population (China, n=24)	53.1*	–	–
		Bladder cancer patients (China, n=15)	103.6*	–	–
Kim *et al* (2004)[51]	ELISA	Boilermakers (USA, n=91)	19.5 (pre-shift)*	21.8*	17.8*
			22.4 (post-shift)*	23.9*	20.9*
Lee *et al* (2010)[52]	ELISA	Diesel emissions inspectors (Taiwan)	–	20.65* (n=20)	23.0* (n=35)
		Controls (Taiwan)	–	9.67* (n=30)	7.31* (n=2)

			Overall			
Authors (year)	Method	Population	Start of week	End of week	Non-smokers	Smokers
Toraason *et al* (2001)[27]	HPLC	Controls (n=12)	4.8*	5.1*	–	–
		Roofers (asphalt only, n=7)	3.8*	3.7*		
		Roofers (coal tar, n=19)	3.7*	4.4*		
Current study	ELISA	Roofers (n=18)	9.30 (pre-shift)		7.85	11.6
			40.4 (post-shift)		28.8	59.1

*For comparison purposes (assuming a mean urinary creatinine concentration of 13 mmol/l, 1.47 g/l).

of carcinogenic compounds need to be further investigated. Based on our results, efforts to reduce cancer risk among roofers should include strategies to prevent skin contact with hot asphalt. Most of the roofers in this pilot study carried hot asphalt to the roof in buckets while climbing a ladder. This practice increases the risk of accidental spill of hot asphalt and should be replaced with safer alternatives (eg, use of transfer pipes to pump hot asphalt from the kettle to the roofs).

A significant number of this small group of roofers reported heavy alcohol consumption and cigarette smoking consistent with the findings of a prior investigation in the US workforce.[18] While these factors did not contribute to urinary 8-OHdG levels in our study, such behavioural risk factors of cancer need to be further investigated and considered in future analyses.

Future direction based on knowledge gained from this pilot study

Our future study is designed to include exposure measurements obtained from personal breathing zone air samples collected repetitively over a workweek. This will enable the comparison of dose–response relationships for each different PAH biomarker. Based on the results of our pilot study, we expect that urinary 1-OHPyr will be the most promising biomarker of PAH exposure among roofers. Urinary 1-OHPyr had also the strongest correlations with the DNA damage marker in post-shift samples. Thus, our future study will include measurements of pyrene in personal air samples. Since pyrene is both in gaseous and in particulate phase, our new study design will cover both phases and include air sampling pumps.

The sample size of this pilot study, which may have underpowered our final analyses, was based on available funds and access to roofing sites during the study period. Sample size calculations of our new study will be based on power calculations to detect minimal difference in DNA damage levels. Furthermore, we will compare biomarkers of exposure and DNA damage at the beginning and the end of the workweek. By comparing personal exposure and biomarker data, repetitively, we will have an improved assessment of the association between PAH exposure and DNA damage over the entire workweek.

Based on our questionnaire data, self-reported smoking status was prevalent among roofers and was an important predictor of urinary PAH metabolites. Quantification of urinary cotinine (biomarker of tobacco smoke exposure) will provide more reliable measures in addition to the questionnaire data. Results of this pilot study support the importance of dermal exposure and possible modifying effects of skin burn due to contact with hot asphalt. Based on these, we plan to improve the assessment of dermal exposure by including skin wipe samples and an improved questionnaire design.

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