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ORIGINAL ARTICLE

Mark Toraason · Charles Hayden · Dave Marlow
Richard Rinehart · Patty Mathias · Dwight Werren
D. Gayle DeBord · Thomas M. Reid

DNA strand breaks, oxidative damage, and 1-OH pyrene in roofers with coal-tar pitch dust and/or asphalt fume exposure

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Abstract ***Objective***: To determine the potential for asphalt fume exposure to increase DNA damage, we conducted a cross-sectional study of roofers involved in the application of roofing asphalt. ***Methods***: DNA strand breaks and the ratio of 8-hydroxydeoxyguanosine (8-OHdG) to 2-deoxyguanosine (dG) were measured in peripheral blood leukocytes of roofers. In addition, urinary excretion of 8-OHdG and 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-PGF) was also measured. The study population consisted of 26 roofers exposed to roofing asphalt and 15 construction workers not exposed to asphalt during the past 5 years. A subset of asphalt roofers ($n = 19$) was exposed to coal-tar pitch dust (coal tar) during removal of existing roofs prior to applying hot asphalt. Personal air monitoring was performed for one work-week to measure exposure to total particulates, benzene-soluble fraction of total particulates, and polycyclic aromatic compounds (PACs). Urinary 1-OH-pyrene levels were measured as an internal biomarker of PAC exposure. ***Results***: Full-shift breathing zone measurements for total particulates, benzene-solubles and PACs were significantly higher for coal-tar exposed workers than for roofers not exposed to coal tar. Similarly, urinary 1-OH-pyrene levels were higher in coal-tar exposed roofers than roofers not exposed to coal tar. Total particulates or benzene-soluble fractions were not associated with urinary 1-OH-pyrene, but PAC exposure was highly correlated with urinary 1-OH-pyrene. When stratified by 1-OH-pyrene excretion, DNA strand breaks increased in a dose-dependent manner, and leukocyte 8-OHdG/dG decreased in a dose-dependent manner. Significant changes in DNA damage appeared to be linked to PACs from coal-tar exposure, although asphalt fume alone was associated with a small but significant increase in urinary 1-OH-pyrene and DNA strand breaks. ***Conclusions***: Results are consistent with previous reports that asphalt or coal-tar exposure can cause DNA damage. Urinary 8-epi-PGF remained relatively constant during the week for virtually all subjects, regardless of exposure indicating that neither asphalt nor coal-tar exposure induces an overt oxidative stress. A small, but statistically significant increase in 8OHdG was evident in end-of-week urine samples compared with start-of-week urine samples in roofers exposed to coal-tar. The increase in urinary 8OHdG coupled with the decrease in leukocyte 8-OHdG/dG, suggests that coal-tar exposure induces protective or repair mechanisms that result in reduced levels of steady-state oxidative-DNA damage.

Key words DNA strand breaks · Oxidative DNA damage · 1-OH Pyrene · Asphalt fume · Coal-tar pitch · 8-Hydroxydeoxyguanosine · 8-Epi-prostaglandin$_{2\alpha}$

M. Toraason (✉) · C. Hayden · D. Marlow · P. Mathias
D. Werren · D. G. DeBord · T. M. Reid
National Institute for Occupational Safety and Health,
4676 Columbia Parkway, Cincinnati, OH 45226, USA

R. Rinehart
Harvard School of Public Health, Boston,
MA, USA

Introduction

Asphalt is used extensively in the construction and highway maintenance industries. It is a useful commodity due to its adhesive properties, flexibility, durability, and water and acid resistance. As of 1999, an estimated 50,000 workers were exposed to roofing asphalt in the United States (Asphalt Roofing Environmental Council (AREC) 1999, unpublished report). However, serious research gaps exist regarding the human health effects of asphalt in general, and roofing asphalt in particular. Both experimental and epidemiological studies have suggested cause for concern. While an early study failed to demonstrate a carcinogenic potential for petroleum-derived roofing asphalt (Emmett et al. 1981), specific fractions of laboratory-generated roofing asphalt-fume condensates have been shown to

be carcinogenic in mice (Sivak et al. 1997). A summary of human epidemiological studies has noted increases in the risk of lung, stomach, and bladder cancer in asphalt workers (Partanen and Boffetta 1994), although the specific contribution of asphalt could not always be assessed.

Among asphalt-exposed workers, roofers appear to have higher risks of cancer than highway maintenance workers (Chiazze et al. 1991; Partanen and Boffetta 1994). In 1988, the National Institute for Occupational Safety and Health (NIOSH) recommended that asphalt fumes be considered a potential occupational carcinogen (NIOSH 1988) based on the findings of Niemeier et al. (1988). However, a definitive link has not been established between asphalt exposure alone and cancer in worker populations (IARC 1985). In contrast, coal-tar pitch is a well-established carcinogen (Emmet et al. 1981; IARC, 1985) and a component of many new and existing roofs. Coal-tar pitch has a higher content of polycyclic aromatic compounds (PACs) than asphalt, and has a higher concentration of complex PACs thought to be more carcinogenic than those found in asphalt. Indeed, the in vitro mutagenic activity of coal-tar-pitch fumes has been shown to be far greater than that of asphalt fumes (Machado et al. 1993). It has been noted, but not assessed, that during removal of old roofs, roofers are exposed to coal-tar-pitch dust (referred to hereafter as coal-tar) which, because of its high PAC content, could contribute to DNA damage (Fuchs et al. 1996).

Assessing occupational exposure to a mixture, such as asphalt fume, is especially problematic as there may be no specific component identified as an indicator of exposure. However, as asphalt contains numerous PACs, 1-OH-pyrene, a commonly used biomarker of PAC exposure, can be utilized to estimate asphalt fume exposure (Jongeneelen et al. 1986). In addition, increased genetic damage in the form of DNA strand breaks, DNA adducts, and sister chromatid exchanges (SCE) have been reported in workers exposed to asphalt (Fuchs et al. 1996; Burgaz et al. 1998). Therefore, assessment of genotoxicity in workers exposed to roofing asphalt constitutes an effective means of assessing health risks of exposure. The present study was undertaken to assess DNA damage in roofers while monitoring their exposure to total particulates, the benzene-soluble fraction of total particulates, and PACs.

Urinary 1-OH-pyrene was measured as in internal biomarker of PAC exposure. In addition, because elevated PAC exposure has been associated with oxidative DNA damage (Autrup et al. 1999), roofers were also assessed for increased oxidative DNA damage. The most thoroughly studied and frequently used biomarker of oxidative DNA damage is 8-hydroxy-2′-deoxyguanosine (8-OHdG) (Toraason 1999). Urinary 8-OHdG was used as an estimate of repaired oxidative DNA damage, and 8-OHdG in DNA from peripheral blood leukocytes was used as an estimate of steady-state oxidative DNA damage. Excretion of the isoprostane, 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-PGF), was measured as an index of lipid peroxidation (Morrow and Roberts 1997). The single-cell gel electrophoresis assay (Singh et al. 1988), commonly referred to as the comet assay, was used to detect DNA strand breaks.

Materials and methods

Subjects

This study was approved by the NIOSH Human Subjects Review Board. The study population is shown in Table 1. Roofing contractors were contacted by phone and requested to participate in a study assessing the association between asphalt fume exposure and DNA damage. All participants gave informed consent and completed a questionnaire. Only smoking status, age, and history of asphalt exposure were considered in the present evaluation. All workers classified as smokers smoked cigars daily or smoked more than ten cigarettes per day. The five asphalt roofing crews that participated in the study were comparable in their make up and work practices. Each crew was monitored for one work-week. Crews were typically paid weekly at the end-of-shift on

Table 1 Study population and exposures (*PAC* polycyclic aromatic compounds, *TWA* time-weighted average, *8-epi-PGF* 8-epi-prostaglandin $F_{2\alpha}$, *8-OHdG* 8-hydroxydeoxyguanosine, *Y* yes, *N* no, *NA* not available)

Crew[a]	*n*	Age	Smoker[b]		Asphalt[c]	Coal-tar tear-off[d]	Total particulates[e]	Benzene-soluble[e]	PAC[f] 254/360	PAC[f] 254/400
			Y	N						
1	8	35 ± 11	7	1	Y	Y	0.88 ± 0.82	0.52 ± 0.60	94 ± 74	1.5 ± 0.9
2	5	49 ± 11	1	4	Y	Y	1.19 ± 0.40	0.73 ± 0.38	166 ± 39	32.2 ± 11.9
3	6	37 ± 10	4	2	Y	Y	0.87 ± 0.45	0.19 ± 0.10	86 ± 21	12.6 ± 7.0
4	6	39 ± 4	3	3	Y	N	0.24 ± 0.10	0.08 ± 0.02	15 ± 5	1.4 ± 0.4
5	1	49	1	0	Y	N	0.31	0.18	30	3.9
6	12	43 ± 10	3	8	N	N	NA	NA	NA	NA
7	3	36 ± 14	0	3	N	N	NA	NA	NA	NA

[a] Crews 1–5 are roofers monitored for all variables during one week of asphalt application. Crew 6, construction workers not using asphalt, monitored only for 8-epi-PGF, 8-OHdG, DNA strand breaks with comet assay. Crew 7, construction workers not using asphalt, monitored only for 1-OH-Pyrene. Ages and TWAs are mean ± SD

[b] Workers self-reported smoker or non-smoker of tobacco

[c] Workers applied hot asphalt during each day of study

[d] Workers spent part of each study-day tearing off old roof containing coal-tar

[e] TWA for period monitored, values are mg/m^3

[f] TWA for period monitored, values are $\mu g/m^3$

Thursdays, and Thursday was defined as end-of-week for this study. Because rain was forecast, crew number 3 did not anticipate working on the Thursday, and end-of-week for this crew was the Wednesday.

Exposures

This study evaluated workers in the commercial roofing industry. The two major roof-replacement processes involved were removal (tear-off) of the existing roof and the laying down of a new roof that included the application of hot asphalt and potential exposure to asphalt fume. The tear-off process exposed roofers to dusts that may contain coal-tar, depending on the composition of the existing roof. A subset (crews 4 and 5) of roofers in this study did not have significant exposure to coal-tar for at least 3 months prior to the study, either due to working with existing rubber-based roofs or working on new construction sites. Determination that old roofs being torn off contained coal-tar was made at the work site and was based on the judgment of the crew foreman and the investigating industrial hygienist. Crew members typically covered exposed skin during tear-off procedures to avoid the accompanying irritation. Materials used for cover-up and the extent of covering varied among individuals. Typical attire worn during tear-off consisted of hats, long-sleeved shirts, gloves, and scarves over mouth and nose. This protection was not worn by roofers during removal of the rubber roof or during application of hot asphalt. One or two complete cycles of tear-off and asphalt application took place each workday. Crew number 4 removed a rubber roof prior to applying an asphalt roof. Crew number 5, which had only one roofer who agreed to participate, applied asphalt to a new structure.

The asphalt used by the crews was either Trumbull, type III steep ASTM D312-95A (Owens Corning, Toledo, Ohio, USA) or Type IV, PA-100, 210/225 extra-steep roofing asphalt ASTM D312–84 (ERT = 400/450 °C) (Sprilast, Arkadelphia, Ark., USA). For all crews, hot asphalt was pumped to a wheeled cart on the roof and transported to the asphalt application site. Application was by spreader and/or mopping. With the exception of kettlemen, all crew members were essentially involved in all work practices, and were considered to have the same exposures. Kettlemen appeared to have considerable exposure to asphalt fume, but were less likely to participate in tear-off activities. However, this varied by crew, by day, and by time of day. Therefore, no attempt was made to analyze data by job category. Ethnicity was not given consideration as only two participants were non-white (African-American). All were male.

Controls

The control participants were recruited individually through newspaper advertisements and had labor practices similar to roofers but without exposure to asphalt fume or the tear-off process. Controls were roofers who applied only shingles, installers of aluminum window and siding, carpenters working on small outdoor structures, and site suppliers of building materials.

Total particulates, benzene-soluble fraction, and polycyclic aromatic compounds

Twenty-three of the roofers agreed to wear personal air monitors that collected samples from their breathing zone. Personal breathing zone samples were analyzed for total particulates and the benzene-soluble fraction of total particulates using method 5042 of the 4th edition of the NIOSH *Manual of Analytical Methods* (NMAM) (NIOSH 1994) (http://www.cdc.gov/niosh/nmam/nmampub). PACs were measured using NMAM 5800. Low molecular weight PACs (2- and 3-member ring compounds) were measured at spectrofluorometer settings of 254 nm excitation and 360 nm emission. High molecular-weight PACs (4- and 5-member ring compounds including pyrene) were measured at spectrofluorometer settings of 254 nm excitation and 400 nm emission. Workers wore the air monitors during the entire work shift. The time-weighted average (TWA) concentration (mg/m^3) was calculated for each day of the entire air monitoring period, and the daily values were used to calculate an average weekly TWA for each roofer.

Blood and urine samples

Venous blood and urine were collected prior to work on the first day of the work-week and at the end-of-shift on the Wednesday or Thursday of the same week. Blood samples were fractionated into various components the same day they were collected. For comet analysis, 60-µl aliquots were added to 1 ml of Hank's balanced salt solution plus 20 m*M* EDTA and 10% dimethylsulfoxide. These samples were flash-frozen in liquid nitrogen and stored at −80 °C until used. Start-of-week urine and end-of-week urine were collected at the same time as blood samples. Additional urine samples were collected during the work-week but are not part of the present analysis, with one exception. For three roofers, urine available at end-of-week shift was inadequate for 1-OH-pyrene analysis to be performed. Alternate urine samples were used to estimate end-of-week urinary 1-OH-pyrene. This estimate was used only to stratify workers into low, medium or high urinary 1-OH-pyrene categories (Figs. 2–4). Urine samples were frozen on the same day as they were collected and stored at −80 °C.

Urinary 1-OH-pyrene

A modification of the HPLC method of Jongeneelen (Tolos et al. 1990) was used to assay for 1-OH-pyrene in all urine samples from 22 roofers. Urinary 1-OH-pyrene is presented as µmol/mol creatinine. Creatinine was determined by Stanibo Creatinine Procedure No. 400 (Stanibo Laboratories, San Antonio Tex., USA).

Urinary 8-epi-prostaglandin $F_{2\alpha}$

Enzyme immunoassay (EIA) kits from Cayman Chemical (Ann Arbor, Mich. USA) were used to measure 8-epi-PGF. Prior to 8-epi-PGF immunoassay analysis, urine samples were extracted using an automated solid phase extraction (SPE) procedure recommended by Cayman. The eluates were dried under a stream of nitrogen and stored at −20 °C under nitrogen until required for analysis. All samples were assayed in triplicate. Urinary 8-epi-PGF is expressed as ng/g creatinine.

8-hydroxydeoxyguanosine in leukocyte DNA and urine

Peripheral blood leukocyte DNA was extracted from 2 ml whole blood on the day it was drawn using a WAKO DNA extractor WB kit (WAKO Chemicals USA, Richmond Va., USA). DNA extracted from leukocytes was hydrolyzed by incubation with nuclease P1 and alkaline phosphatase. 8-OHdG in leukocyte DNA and urine was measured using HPLC and electrochemical detection by ESA Laboratories, Chelmsford, Mass., USA. DNA extracted from leukocytes is expressed as the ratio of 8-OHdG to deoxyguanosine (dG) (8-OHdG/dG, nmol/mmol). Urinary 8-OHdG is expressed as µg/g creatinine.

Estimation of DNA strand breaks by the comet assay

Prior to use, blood samples were thawed on ice and centrifuged at 1800 *g* for 5 min at 4 °C. Six hundred microliters of supernatant were removed and the remaining sample mixed briefly to resuspend the cells. Ten microliters of each sample were added to 200 µl of 0.5% low-melting agarose at 37 °C and 50 µl of this mixture was added to each of two wells of a CometSlide (Trevigen; Gaithersburg, Md., USA). The agarose was allowed to harden for 15 min at

4 °C. Slides were then placed in lysis buffer (10 m*M* Tris, 2.5 *M* NaCl, 0.1 *M* EDTA, 10% DMSO, 1% Triton X-100, pH 10) for 90 min at 4 °C. Slides were placed in a 22 × 32 cm electrophoresis chamber filled with 1,200 ml of 0.3 *M* NaOH, 1 m*M* EDTA for 30 min. Electrophoresis was performed at a constant voltage of 300 mA (22–24 V) for 30 min in the same NaOH/EDTA solution. Slides were washed three times with 0.4 *M* Tris (pH 7.4) followed by incubation in 100% methanol for 5 min and 100% ethanol for an additional 5 min. Slides were allowed to dry for 30 min in a fume hood and stored at room temperature. To score slides, 30 µl of ethidium bromide (2 µg/ml) was added to each well of the slide and covered with a cover slip. The percentage of DNA in the comet tail (%Tail DNA) was assessed with an epi-fluorescence microscope paired with a computerized image analysis system (Komet 4.0; Kinetic Imaging). A minimum of 100 cells was analyzed for each individual.

Statistical analysis

All statistical procedures were performed using Statgraphics statistical package (STSC, Rockville, Md., USA). Data were compared using ANOVA, Student's *t*-test, paired *t*-test, or linear regression. $P < 0.05$ was considered statistically significant.

Results

Total particulates, benzene-soluble fraction, and polycyclic aromatic compounds

Personal breathing zone air concentrations for total particulates, benzene-soluble fraction, or PACs varied considerably among the crews (Table 1). Grouping roofers as to whether they participated in tear-off of roofs containing coal tar, or only in the application of hot asphalt, revealed that coal-tar tear-off contributed significantly to exposures (Fig. 1). Linear regression analysis was also performed between these environmental exposure indices and the biomarkers of biological effect, but no statistically significant correlations were evident (data not shown).

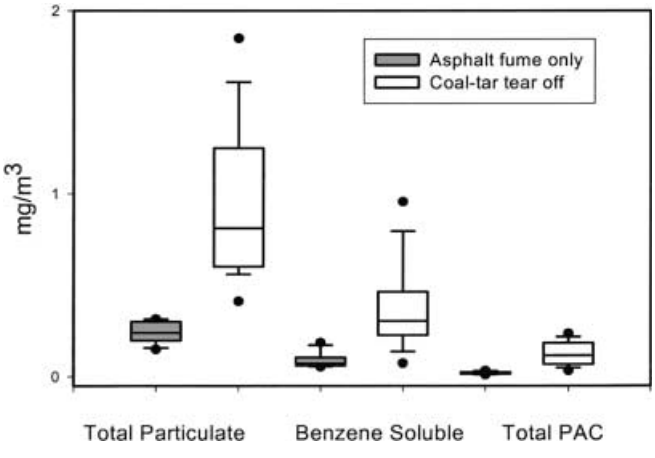

Fig. 1 Box and whisker plots (with 5th/95th percentiles ●) of exposure levels of total particulates, benzene-soluble compounds, and total polycyclic aromatic compounds (*PACs*) (PAC 254/360 plus PAC 254/400) in personal breathing zone air samples of roofers. Asphalt-fume-only roofers applied hot asphalt but were not involved in tear-off of coal-tar roofs. Roofers involved in tear-off of coal-tar roofs were also exposed to hot asphalt. In all cases, exposure levels were significantly higher for roofers involved in coal-tar tear-off (Student's *t*-test)

1-OH-Pyrene

Regression analysis demonstrates the absence of a significant association between urinary 1-OH-pyrene and exposure to particulates or benzene-soluble compounds (Table 2). In contrast, a significant association occurred between 1-OH-pyrene and PAC exposure, and the greatest statistical significance was evident for 4- to 5-ring PACs (254/400 nm). Separation of roofers into asphalt only and asphalt plus coal-tar tear-off groups revealed that elevated levels of 1-OH-pyrene in roofers was associated with tear-off. Linear regression analysis was also performed between 1-OH-pyrene and the biomarkers of biological effect, but no statistically significant correlations were evident (data not shown). However, when workers were categorized on the basis of urinary 1-OH-pyrene levels, statistically significant associations were evident. Figure 2 illustrates the division of the roofer population into three urinary pyrene categories; low < 1 ($n = 8$), medium $> 1 < 4$ ($n = 7$) and high > 4 ($n = 8$). The control values ($n = 3$) were obtained from three non-roofers who were also nonsmokers and for whom 1-OH-pyrene was the only biomarker measured. The assumption was made that urinary 1-OH-pyrene values from these three workers were representative of the urinary 1-OH-pyrene levels in the 12 controls for whom urinary 1-OH-pyrene was not measured. The low 1-OH-pyrene category included nearly all of the roofers not involved in coal-tar tear-off. Interestingly, two roofers in the low 1-OH-pyrene category who were part of coal-tar tear-off crews were kettlemen. Typically, kettlemen prepared the hot asphalt while the rest of the crew tore off the old roof. As a consequence, one would expect their exposure to coal-tar to be lower, which is supported by the low urinary 1-OH-pyrene. All but one roofer in the medium and high 1-OH-pyrene categories were involved in coal-tar tear-off.

Table 2 Correlation of urinary 1-OH-pyrene with exposure indices. Regression analysis was end-of-week urinary 1-OH-pyrene versus mean of daily time-weighted averages (TWAs) for the environmental exposures. *PAC* polycyclic aromatic compounds

Air sampling measures	R	*P*
Total particulate (mg/m^3)	0.231	0.328
Benzene-soluble fraction (mg/m^3)	0.289	0.215
PAC (254/360) (µg/m^3)	0.504	0.023
PAC (254/400) (µg/m^3)	0.592	0.006
PAC (total) (µg/m^3)	0.629	0.013

Urinary 8-epi-prostaglandin F_{2a} and 8-hydroxydeoxyguanosine

8-Epi-PGF was used as a biomarker of lipid peroxidation, and 8-OHdG was used as a biomarker of oxidative DNA damage repair. Together, 8-epi-PGF and 8-OHdG can be considered to be an index of oxidative stress. No significant difference between start-of-week and end-of-week values for 8-epi-PGF and 8-OHdG were evident in controls or asphalt roofers (Table 3); nor were there significant differences between controls and asphalt roofers. Although 8-epi-PGF and 8-OHdG were not significantly different between controls and coal-tar exposed roofers, end-of-week 8-OHdG in coal-tar exposed workers was significantly elevated over start-of-week values. Regardless of exposure category, there were no significant differences between smokers and non-smokers for either urinary 8-epi-PGF or urinary 8-OHdG (data not shown).

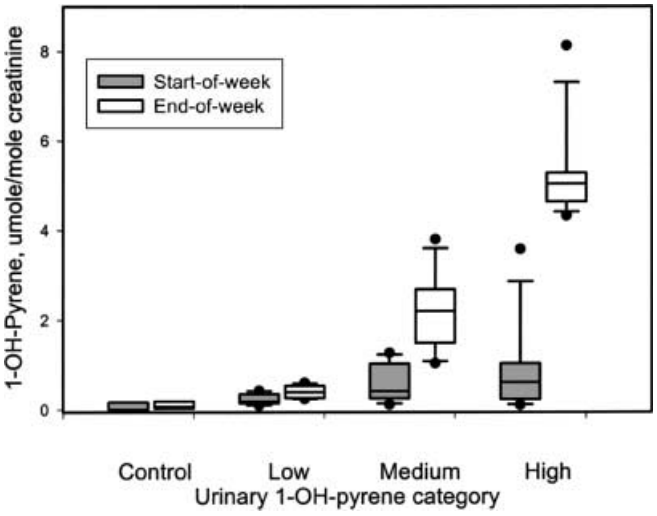

Fig. 2 Box and whisker plots (with 5th/95th percentiles ●) of the four categories of end-of-week urinary 1-OH-pyrene levels. Controls are non-roofers without exposure to asphalt or coal-tar tear-off. 1-OH-pyrene categories for roofers exposed to asphalt and, in some cases, coal tar are low <1 ($n = 8$), medium >1 and <4 ($n = 7$), and high >4 ($n = 8$) μmol/mol creatinine. Start-of-week values were from urine collected on the Monday morning before work. End-of-week values are from urine collected at the end of the shift on the Wednesday or Thursday. End-of-week values are significantly greater than start-of-week values for medium and high categories (paired *t*-test)

Leukocyte 8-hydroxydeoxyguanosine/2-deoxyguanosine

Steady-state oxidative DNA damage, as assessed by leukocyte DNA 8OHdG/dG, was significantly less than controls in asphalt roofers with coal-tar exposure, but not in roofers with only asphalt fume exposure (Table 3). Regardless of exposure category, there were no significant differences between smokers and non-smokers for 8-OHdG/dG (data not shown).

Leukocyte 8-OHdG/dG was significantly decreased in the low, medium and high urinary 1-OH-pyrene group relative to the control group (Fig. 3). In addition, end-of-week 8OHdG/dG values were significantly decreased compared with start-of-week values for the medium 1-OH-pyrene category.

Leukocyte DNA strand breaks

Estimates of DNA strand breaks as determined by the comet assay were significantly greater in asphalt roofers than controls (Table 3). Also, in asphalt roofers, but not controls, end-of-week estimates of strand breaks were higher than start-of-week values (Table 3). This was true for roofers with only asphalt fume exposure and for roofers with asphalt fume and coal-tar exposure.

Table 3 Biomarkers of exposure and effect for controls and roofers applying hot asphalt. Values are means ± SD. *8-epi-PGF* 8-epiprostaglandin $F_{2\alpha}$, *8-OHdG* 8-hydroxydeoxyguanosine, *dG* 2-deoxyguanosine

Biomarker	Controls		Asphalt-fume-only exposure		Coal-tar tear-off exposure	
	Start of week[a]	End of week[b]	Start of week	End of week	Start of week	End of week
Urinary 1-OH-pyrene μmol/mol Creatinine	0.08 ± 0.12 (3[c])[d]	0.12 ± 0.12 (3)	0.26 ± 0.13 6	0.58 ± 0.29* 5	0.74 ± 0.86 16	3.55 ± 2.17*† 15
Urinary 8-epi-PGF ng/g Creatinine	355 ± 214 12	256 ± 152 12	486 ± 224 7	278 ± 184 7	431 ± 286 19	390 ± 249 19
Urinary 8-OHdG μg/g Creatinine	3.3 ± 1.9 12	3.5 ± 1.7 12	2.6 ± 0.8 7	2.5 ± 0.8 7	2.5 ± 1.7 19	3.0 ± 1.7† 19
Leukocyte 8-OHdG/dG μmol/mol Creatine	19.6 ± 8.3 12	19.6 ± 8.3 7	12.4 ± 7 6	15.7 ± 16.5 4	10.3 ± 7.1* 18	5.6 ± 2.4*† 18
Leukocyte % Tail DNA (Comet)	13.9 ± 4.0 11	13.9 ± 3.3 11	13.6 ± 1.9 7	16.7 ± 1.4† 7	15.6 ± 3.1 18	16.9 ± 3.3*† 18

* Significantly different from corresponding control (*t*-test); † significantly different from corresponding start-of-week value (paired *t*-test)

[a] Urine and blood sample collected the Monday morning prior to shift

[b] Urine and blood sample collected the Wednesday or Thursday at end-of-shift

[c] Number of assay values included in mean

[d] Population for (control) 1-OH-pyrene values independent of other controls; see Fig. 1

Separation of roofers into low, medium and high urinary 1-OH-pyrene categories revealed significant differences between start-of-week values and end-of-week values for strand breaks in medium and high urinary 1-OH-pyrene categories, but not in low 1-OH-pyrene or control categories (Fig. 4). No significant differences for estimates of strand breaks were observed between smokers and non-smokers (data not shown).

Discussion

The population of asphalt roofers evaluated in the present study had three exposures previously associated with DNA damage. All had exposure to asphalt, approximately one-half were tobacco smokers, and 19 of 26 had apparent exposure to coal-tar during the tear-off of old roofs. The determination of coal-tar in the old roofs was made at each site on the first day of assessment. Exposure to coal-tar was associated with significant increases in urinary 1-OH-pyrene and DNA strand breaks. Paradoxically, the coal-tar exposure was also associated with a significant reduction in oxidative DNA damage. Asphalt fume exposure alone did not result in a significant increase in detectable oxidative stress, but did result in a significant increase in end-of-week DNA strand breaks relative to start-of-week values. Smoking status was without a significant effect on the biological endpoints examined.

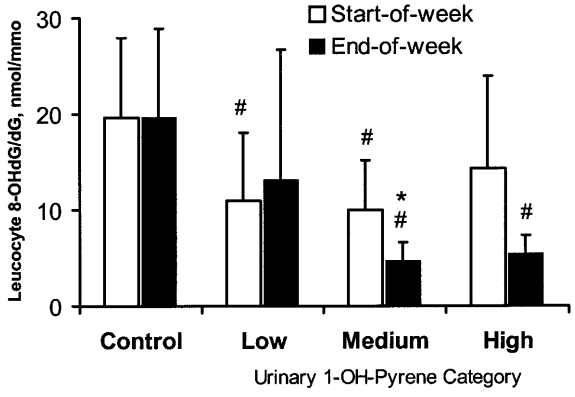

Fig. 3 Leukocyte DNA 8-hydroxydeoxyguanosine/2-deoxyguanosine (*8-OHdG/dG*) (nmol/mmol) in controls and low-, medium-, and high-1-OH-pyrene categories. *Bars* are mean ± SD Start-of-week values are from blood collected on the Monday morning before work. End-of-week values are from blood collected at the end of the work shift on the Wednesday or Thursday. # Indicates that values are significantly different from corresponding control value (ANOVA). * Indicates that end-of-week value is significantly different from corresponding start-of-week values (paired *t*-test)

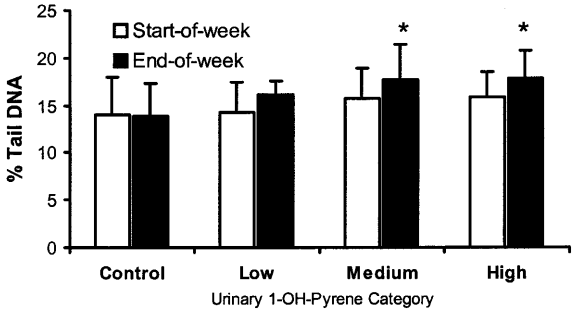

Fig. 4 DNA strand breaks in controls and low-, medium-, and high-1-OH-pyrene categories. DNA strand breaks were estimated by comet assay as increase in % tail DNA. *Bars* are mean ± SD. Start-of-week values are from urine collected on the Monday morning before work. End-of-week values are from urine collected at the end of the work shift on the Wednesday or Thursday. * Indicates that end-of-week values are significantly different from corresponding start-of-week values (paired *t*-test)

Urinary 1-OH-pyrene as a biomarker of polycyclic aromatic compound exposure

Because air monitoring of PACs may not adequately predict bioavailability, use of an internal biomarker such as 1-OH-pyrene can assist in assessing a worker's exposure. Bouchard and Viau (1999) recently reviewed workplace studies examining the relationship between PAC exposure and 1-OH-pyrene excretion, and their overview was used in the evaluation of the present data. They report that during a 5-day exposure week the relationship between PAC exposure and 1-OH-pyrene excretion is complex, and that both dermal and inhalation routes of exposure are important. They conclude that the difference between start-of-week and end-of-week urinary 1-OH-pyrene values are an indication of the average PAC exposure over the work-week. They also note that urinary 1-OH-pyrene levels tend to level off after 3–4 days of exposure to elevated PACs In the present study, end-of-week 1-OH-pyrene was highly correlated ($r = 0.934$, $P < 0.00001$) with the difference between start-of-week and end-of-week 1-OH-pyrene. Therefore, end-of-week 1-OH-pyrene served as an equivalent of average weekly 1-OH-pyrene. This avoided the use of negative values in analysis as, for some participants, urinary 1-OH-declined slightly during the week. The observed significant association between PAC exposure and urinary 1-OH-pyrene confirmed previous reports of the utility of 1-OH-pyrene as a biomarker of PAC exposure (Bouchard and Viau 1999). The 1-OH-pyrene levels in present controls are comparable or lower (0.01–0.22 μmol/mol creatinine) than those reported in other non-occupationally exposed individuals (0.03–0.68 μmol/mol creatinine) (Bouchard and Viau 1999). The wide range of 1-OH-pyrene values in exposed groups allowed stratification of asphalt roofers to be made, into low (< 1 μmol/mol, $n = 8$), medium (> 1 and < 4 μmol/mol, $n = 7$), and high (> 4 μmol/mol, $n = 8$) 1-OH-pyrene categories. The 1-OH-pyrene values reported here are higher than reported for asphalt pavers, higher than, or comparable with those reported for engine mechanics and aluminum workers, and lower than those reported for coke-oven workers (Bouchard and Viau 1999; Pan et al. 1998; Granella and Clonfero 1993).

Assessment of 1-OH-pyrene in the present study indicated that exposure to asphalt fume may be a factor in elevated urinary 1-OH-pyrene, but that PAC exposures during tear-off of roofs containing coal-tar was the primary contributor. 1-OH-pyrene levels in roofers with only asphalt exposure were less than 1 μmol/mol creatinine; levels that are comparable to those reported in pavers (Burgaz et al. 1998). Although PAC exposures were significantly elevated in roofers performing tear-off, TWA values in Table 1 are probably an underestimate of exposures. Fluorescence analysis was performed on hexane-soluble material, and the old roof material was not completely soluble in hexane.

Tobacco smoking

Many of the biomarkers used in the present study have been reported to be associated with smoking. The three primary exposures examined in the present study were tobacco smoking, asphalt fume, and coal-tar. While the asphalt roofer population consisted of nearly equal numbers of smokers and non-smokers, smokers were under-represented in the control population and over-represented in the coal-tar-plus-asphalt exposure group (Table 1). In well-controlled studies, levels of 8-epi-PGF have been consistently elevated in smokers relative to non-smokers (Reilly 1996; Morrow et al. 1995; Morrow and Roberts 1997). Although no statistically significant effect was noted here, 8-epi-PGF tended to be higher in smokers than in non-smokers (data not shown). The absence of a significant effect can be attributed to the fact that smokers and non-smokers were not matched by exposure, age, or other lifestyle factors; and smokers, regardless of number of cigarettes smoked per day or years of smoking, were placed in a single category due to the small population size.

While no significant changes were noted, urinary 8-OHdG tended to be lower in smokers than in non-smokers. Although several studies have demonstrated a direct association between smoking and 8-OHdG (Loft and Poulsen 1996), occupational studies rarely demonstrate a positive association (Toraason 1999). Recently, van Zeeland et al. (1999) reported significantly decreased leukocyte DNA 8-OHdG in smokers relative to non-smokers, and attributed the effect to enhanced rapid repair. The absence of an effect of smoking on DNA strand breaks has also been reported elsewhere (Fuchs et al. 1996).

Coal-tar

The carcinogenic potential of coal-tar pitch and coal-tar pitch dust is well-recognized (Emmett et al. 1981; IARC 1985). The mutagenic potential of coal-tar and asphalt fumes is generally attributed to PACs, which can be several orders of magnitude higher in coal-tar fume than in asphalt fume (Machado et al. 1993). While the presence of coal-tar in tear-off material was not determined analytically, the conclusions of the roofers and subsequent air sample analysis for PAC are consistent with the supposition that old roofs contain coal-tar. The increased PAC exposure associated with tear-off of roofs was manifested as increased urinary 1-OH-pyrene. The elevated PAC exposure resulted in significantly increased DNA strand breaks. The increased strand breaks are consistent with several studies demonstrating increased genetic damage in roofers and pavers (Fuchs et al. 1996; Burgaz et al. 1998).

Elevated PAC exposure was not associated with increased oxidative damage. While Carstensen et al. (1999) did not see an increase in urinary 8-OHdG in potroom workers with high PAC exposure, Autrup et al. (1999) found a positive association between PAC-albumin adducts and elevated urinary 8-OHdG in bus drivers and postal workers. In the present study, the urinary biomarker of oxidative damage to cell membranes, 8-epi-PGF, was unchanged, while urinary 8-OHdG was increased in coal-tar exposed workers. Typically, elevated urinary 8-OHdG is considered an indication of increased oxidative DNA damage. However, this increase occurred in the face of a significant decrease in leukocyte DNA 8-OHdG/dG. No change during the work-week was evident in controls, but in roofers exposed to asphalt fume and coal-tar dust there was a 1-OH-pyrene concentration-dependent change in the 8OHdG/dG. This result demonstrates a possible biological effect of PACs despite the fact that the effect would not be considered adverse. That is, reduced oxidative damage would generally be considered beneficial. As the present results demonstrate, little or no indication of increased oxidative stress associated either with asphalt fume or coal-tar exposure, the decrease in oxidative DNA damage observed, appears to result from increased antioxidant capacity or increased repair of endogenous damage that is associated with increased PAC exposure. Hydroxylated guanine is repaired by specific base and nucleotide excision repair pathways (Bessho et al. 1993). If increased repair were responsible for a decrease in leukocyte 8-OHdG/dG, a corresponding increase in urinary 8-OHdG would be expected. This is the case, but the effect appears modest in light of the marked decrease in leukocyte 8-OHdG/dG. This suggests that decreased 8-OHdG/dG may be the result of increased antioxidant capacity. PACs have been linked to induction of xenobiotic response element (XRE) and antioxidant response element (ARE) (Ng et al. 1998; Burczynski and Penning, 2000). Therefore, repeated PAC exposure of roofers may lead to enhanced capacity to prevent endogenous oxidative stress.

Asphalt

Coal-tar is clearly a factor in increased DNA damage in roofers. What is less clear is the contribution of asphalt fume alone. Several studies have examined the genotoxic

potential of asphalt fume either from road paving or roofing application (Fuchs et al. 1996; Burgaz et al. 1998). Recently, Burgaz et al. (1998) demonstrated an association between increased urinary 1-OH-pyrene and elevated frequencies of SCE and micronuclei in road paving workers exposed to asphalt fume. While this study controlled for smoking, it did not include dosimetry, nor was there any exposure metric other than the urinary biomarker of exposure 1-OH-pyrene. In the present study, the sub-population of roofers with only asphalt fume exposure did exhibit statistically significant increases in DNA damage. Increased DNA strand breaks in asphalt roofers was also reported by Fuchs et al. (1996). They found a non-significant increase in DNA strand breaks in asphalt pavers and a decrease in DNA strand breaks in asphalt painters. They noted that roofers had a potential for coal-tar exposure during tear-off of old roofs, but did not account for it in their analysis. Present results demonstrate that coal-tar exposure during tear-off can contribute significantly to DNA damage in roofers applying hot asphalt. The present study is also consistent with previous reports that asphalt fume exposure can be genotoxic, and suggests that examination of a larger cohort may establish the genotoxicity of asphalt fumes.

Conclusions

Several studies attempting to assess the human genotoxicity of asphalt fume have not accounted for potential confounders other than smoking (Fuchs et al. 1996; Hatjian et al. 1997; Burgaz et al. 1998). The present study demonstrates that substantial PAC exposure can occur as the result of old-roof tear-off, which can be an inescapable component of asphalt roofers' regular work. The cohort of asphalt roofers exhibited significant increases in DNA strand breaks relative to controls. The study also demonstrated that increased DNA strand breaks were closely associated with urinary 1-OH-pyrene, which was closely associated with PAC exposure. Elevated PAC exposure occurred primarily in roofers who applied hot asphalt and were involved daily in the tear-off of old roofs believed to contain coal-tar. Urinary 1-OH-pyrene exposure was slightly elevated in roofers without tear-off exposure, relative to controls, and there was a corresponding increase in end-of-week DNA strand breaks. The present study also examined the potential for asphalt fume exposure to increase oxidative stress and oxidative DNA damage. The results provide no real indication that asphalt fume or PAC exposure increases oxidative damage. A small increase in urinary 8-OHdG was observed, but this appeared to be the result of increased DNA repair. PAC exposure is highly associated with a reduction in oxidative DNA damage. While this observation is perplexing, it could easily reflect increased repair or induction of a stress response and increased antioxidant capacity. Regardless, it does represent a biological response, and in that respect serves as a biomarker of a biologically effective dose. Taken together with the increased DNA strand breaks, there is clearly a biological response in the roofers of our study. While present results indicate that the role of asphalt fume is minimal, it is clear that the work practices surrounding tear-off of roofs containing coal-tar may be a health hazard. NIOSH considers coal-tar products to be potential carcinogens, and recommends an exposure limit (REL, 10-h TWA) of 0.1 mg/m^3 (cyclohexane extractable) (NIOSH 1977). The US Occupational Safety and Health Administration's permissible exposure limit (OSHA PEL, 8-h TWA) is 0.2 mg/m^3 (benzene-soluble fraction) (29CFR 1910.1000, p.678). Although no crew in our study had coal-tar-only exposure, the contribution of coal-tar to the benzene-soluble fraction of total particulates can be approximated from data in Table 1. Crews 1–3, with asphalt fume plus coal-tar exposure, had an average benzene-soluble fraction TWA exposure of 0.48 mg/m^3. Crews 4 and 5, with only asphalt fume exposure, had an average benzene-soluble fraction TWA exposure of 0.13 mg/m^3. The difference between the two average values is 0.35 mg/m^3. As the procedure for application of hot asphalt was similar among all crews, the 0.35 mg/m^3 TWA serves as an approximate average exposure to the benzene-soluble fraction of total particulates from coal-tar. Although an estimate of coal-tar exposure from dust, this value exceeds the OSHA PEL for coal-tar exposure from fume. One interpretation of the present results is that a consequence of exceeding this limit is increased DNA damage.

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