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DNA Adducts among Asphalt Paving Workers

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Objective: Asphalt is used extensively in the highway construction industry and contains a complex mixture of polycyclic aromatic hydrocarbons, some of which are known or suspected to be human carcinogens. Though numerous epidemiologic studies have described an excess cancer risk among asphalt workers, a causal relationship has not been established. Accordingly, the primary objective of this study was to use DNA adducts as a biomarker of biologically effective dose and determine whether DNA damage resulted from occupational exposure to asphalt among paving workers. Methods: Over a 12 month period, four peripheral blood samples (spring, summer, fall and winter) were obtained from 49 asphalt paving workers (169 samples) and 36 non-paving construction workers (103 samples). The spring, summer and fall samples were collected during the work-season, whereas the winter samples were collected during the off-season (due to the seasonality of paving work). Mononuclear white blood cells were isolated and analyzed for DNA adducts via the ^{32}P-postlabeling assay and generalized linear models were used to evaluate the DNA adduct data. Results: Among paving workers during the work-season, DNA adducts increased during each day of the workweek such that mean adduct levels were lowest on Mondays (3 adducts per 10^{10} nucleotides) and highest on Fridays (46 adducts per 10^{10} nucleotides). Additionally, a 3-fold difference in adduct burden was observed by paving task such that mean adduct levels were lowest among roller operators (7 adducts per 10^{10} nucleotides) and highest among screedmen (23 adducts per 10^{10} nucleotides). Using adducts as a measure of biologically effective dose, these findings (weekday trend and task-based differences) were consistent with a previous evaluation of absorbed dose in the same population. Adduct levels were not, however, higher among paving workers than among non-pavers. Adducts were also highest during the winter months, suggestive of a seasonal effect that has been observed in previous studies. Conclusion: These findings indicate that adduct burden increased throughout the workweek among paving workers, suggesting that DNA damage may be associated with occupational exposure to hot-mix asphalt. However, the lack of contrast with non-paving workers, as well as the seasonal variation warrants additional investigation.

Keywords: asphalt; biomarkers; DNA adducts; polycyclic aromatic hydrocarbons; ^{32}P-postlabeling assay

INTRODUCTION

Hot-mix asphalt is used internationally as an industrial material, such that annual asphalt production amounts to ~267 million tons in Western Europe and 440 million tons in the United States (Partanen and Boffetta, 1994). Road paving is one of the most common applications, with the United States road paving industry accounting for 87% of domestic asphalt production and employing ~300 000 workers (NIOSH, 2000).

Asphalt contains a complex mixture of polycyclic aromatic hydrocarbons (PAHs), many of which are either known or suspected to be human carcinogens (IARC, 1985). Accordingly, numerous epidemiologic studies have described an excess risk of cancer among asphalt-exposed workers (Partanen and Boffetta, 1994; Boffetta *et al*., 1997; Boffetta *et al*., 2003), though possible confounding by smoking and/or coal tar exposure has limited the ability to establish a causal relationship between asphalt and cancer risk (Chiazze *et al*., 1991; NIOSH, 2000). Additionally, existing studies have been criticized for lacking quantitative measurements of exposure to asphalt or its constituents (Chiazze *et al*., 1991; NIOSH, 2000).

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Recent studies of asphalt workers have evaluated DNA damage in the form of sister-chromatid exchange, micronuclei and strand breaks, reporting significantly higher levels of genetic damage among exposed workers versus controls (Burgaz *et al*., 1998; Toraason *et al*., 2001). However, previous investigations of DNA adducts among asphalt workers are limited (Fuchs *et al*., 1996). The formation of DNA adducts occurs when reactive metabolites bind to sites within the DNA molecule, providing a useful measure of DNA damage that has been found to be associated with both PAH exposure and cancer risk (Tang *et al*., 1995; Wiencke *et al*., 1995). Accordingly, DNA adducts have been proposed as biomarkers of 'biologically effective dose'.

This study of DNA adducts is the third phase of a project evaluating a population of asphalt paving workers. In the first phase, inhalation and dermal exposures to polycyclic aromatic compounds (PACs) were found to be higher among paving workers than among highway construction workers who are not regularly exposed to asphalt fumes (McClean *et al*., 2004a). Also, asphalt containing a high percentage of recycled asphalt product (RAP) resulted in higher inhalation and dermal exposures than when workers were exposed to low-RAP asphalt (McClean *et al*., 2004a). The differences in PAC exposure by job category and RAP content suggested that the inhalation and dermal PAC exposures experienced by the paving workers were asphalt-related.

In the second phase, the pyrene metabolite 1-hydroxypyrene (1-OHP) was measured in urine from the same workers (McClean *et al*., 2004b). The main objective was to evaluate urinary 1-OHP as a biomarker of total absorbed dose that results from inhalation and dermal exposure to pyrene. Among paving workers, the urinary 1-OHP levels were found to increase throughout the workweek such that the average pre-shift level on Thursday (1.4 $\mu g\ g^{-1}$ creatinine) was 3.5 times higher than the average pre-shift results on Monday (0.4 $\mu g\ g^{-1}$ creatinine). The urinary 1-OHP results were consistent with the analysis of environmental measurements, indicating that urinary 1-OHP levels were higher among paving workers than among non-paving construction workers (McClean *et al*., 2004b).

The current study was designed to evaluate DNA adducts as a biomarker of biologically effective dose of potentially carcinogenic PACs in asphalt paving workers. Since PAH-DNA adducts are also formed as a result of non-occupational exposures, it is crucial to control for potential confounders such as cigarette smoking. While controlling for the effects of smoking and body mass index (BMI), the objectives of this study were to: (i) determine whether DNA adduct levels among paving workers increased throughout the workweek; (ii) determine whether DNA adduct levels were significantly different by paving task; and (iii) determine whether DNA adduct levels were higher among paving workers than among non-paving workers.

MATERIALS AND METHODS

Study population

The study population included 85 highway construction workers: 49 pavers, 16 millers and 20 roadside construction workers. All participants were male, worked for the same company and lived in the Greater Boston Area (Eastern Massachusetts). Written and informed consent was obtained from each study participant prior to sampling, and all sampling was conducted in accordance with a standardized human subjects protocol that was approved by the Institutional Review Board at the Harvard School of Public Health.

The paving, milling and roadside construction workers have been described previously (McClean *et al*., 2004a). Briefly, the paving workers applied hot-mix asphalt while resurfacing roads and included workers from each of four task categories (paver operators, screedmen, rakers and roller operators). The milling workers used grinding equipment to remove layers of existing roads in preparation for the paving crews, and the roadside construction workers repaired curbs and guardrails. Since the millers and roadside construction workers did not regularly work with hot-mix asphalt, the millers and construction workers were combined here and referred to as the non-paving workers (36 workers).

Study design

Blood samples were collected from participants on four occasions during the 12 month study period such that a maximum of four samples were collected from each worker. Round 1 samples were collected from May to July (1999), Round 2 from August to October (1999), Round 3 from October to December (1999) and Round 4 from February to early May (2000). Questionnaire data regarding personal characteristics (e.g. age, height, weight), job-related information (e.g. job title, task characteristics) and non-occupational PAC exposure (e.g. cigarette smoking) were also obtained. The peripheral blood samples were collected for the purpose of measuring DNA adducts in mononuclear white blood cells (MNCs).

The first three rounds of blood samples represent the adduct burden at different points during the work-season and were collected during early morning visits to work sites. Given the seasonal nature of Massachusetts highway work, paving workers do not work with hot-mix asphalt during the winter months; therefore, the Round 4 blood samples represent the adduct burden following 1–2 months of no asphalt exposure and were primarily collected

during morning visits to the participants' local communities. Though some Round 4 samples were obtained from paving workers who had sporadically worked with asphalt during the preceding month, all Round 4 samples were retained since analysis with and without these workers yielded very similar results.

Blood samples were only collected during the workweek (Monday through Friday) and each worker was never sampled more than once per round. Accordingly, 'weekday' was simply a characteristic of each blood sample that indicated at what point in the workweek each sample was collected; however, while an attempt was made to sample each worker once during each round (season), it was not feasible to sample each worker during each day of the week.

Blood samples and DNA adduct analysis

The blood samples were obtained by a trained phlebotomist using the Vacutainer blood collection system with heparin-treated tubes. Following sample collection, the blood samples were immediately transported to the laboratory and applied to Ficoll–Hypaque density gradients to separate MNCs from erythrocytes and granulocytes. The isolated MNCs were brought to a final volume of 1 ml and stored at −20°C. The frozen MNC samples were coded, packed in dry ice and shipped to the University of California in San Francisco (UCSF) for DNA adduct analysis.

The laboratory analysts at UCSF were blind to the identity and exposure status of the study participants. The MNCs were homogenized in 0.1 M Tris–HC1 (pH 8.0), 0.1 M NaCl, 50 mM EDTA and 1% sodium dodecyl sulfate on ice and then extracted twice with equal volumes of chloroform/isoamyl alcohol, 24:1 (vol/vol). The aqueous supernatant was incubated with ribonuclease (RNase) A and RNase T1 (250 μg ml^{-1}; Sigma Chemical Co., St Louis, MO, USA) at 37°C for 60 min followed by digestion with proteinase K (10 μg ml^{-1}; 37°C for 60 min; Life Technologies Inc., Gaithersburg, MD, USA). The digest was extracted twice with chloroform/isoamy alcohol, after which sodium acetate (0.4 M, final concentration) was added to the aqueous supernatant. The DNA was then precipitated with ethanol at −20°C and dissolved in 0.1× standard saline citrate. The quantity of DNA was determined by a fluorometric method (Hoechst 33258; Hoeffer Scientific, San Francisco, CA, USA).

The DNA adduct analysis was conducted using the ^{32}P-postlabeling assay with nuclease P_1 enhancement, the details of which have been previously described (Reddy and Randerath, 1986; Wiencke *et al*., 1995). For each experiment, a positive control sample of DNA containing benzo[*a*]pyrene diol-epoxide-labeled deoxyguanosine was diluted to a level that would be seen in human tissues in vivo. Repeated analyses of the positive control yielded a coefficient of variation of 50%. The detection limit was 1 adduct per 10^{10} nucleotides.

Statistical analysis

The DNA adduct data were analyzed using descriptive statistics and generalized linear models. Units for DNA adduct levels are reported as the number of adducts per 10^{10} nucleotides. For samples that were analyzed more than once, the adduct levels were averaged to obtain one adduct measurement per sample. All statistical analyses were conducted using SAS statistical software (SAS Institute, Cary, NC, USA).

Adduct count data have commonly been analyzed using regression models that assume a Poisson distribution, though recently negative binomial models have also been used (Lawless, 1987; Wiencke *et al*., 1999). Although the Poisson distribution is useful for analyzing count data with a variance that is approximately equal to the mean, the negative binomial distribution is more appropriate for overdispersed data (variance larger than mean). Given the overdispersion of the adduct data and the fact that multiple samples were collected from each worker, a repeated measures generalized linear model framework (generalized estimating equation) and log link function were used to evaluate the DNA adduct data, while the error term was assumed to have a negative binomial distribution. Regression models were applied to the DNA adduct data using the GENMOD procedure with an exchangeable correlation matrix to obtain generalized estimating equation estimates of the parameters (SAS Institute, Cary, NC, USA).

Using weekday as a surrogate for dose, the DNA adduct data were first analyzed to determine whether the DNA adducts increased throughout the workweek. Accordingly, the DNA adducts collected during the work-season were analyzed to evaluate the fixed effects of weekday, paving task, smoking and BMI, and had the following form:

$$Y_{ijml} = \ln(X_{ijml}) = \alpha + \beta_{lm}\mathrm{TASK}_{ijm} + \beta_{21}\mathrm{WEEKDAY}_{ijl} + \beta_3\mathrm{SMOKING}_i + \beta_4\mathrm{BMI}_i + \varepsilon_{ijml}$$

where X_{ijml} represents the exposure level of the *i*-th worker during the *j*-th round, Y_{ijml} is the natural logarithm of measurement X_{ijml}, and the β's represent the fixed effects for each of the covariates where m = {Roller Operator, Laborer, Paver Operator, Screedman} and l = {Monday, Tuesday, Wednesday, Thursday, Friday}. For comparison, this model was evaluated separately for paving workers during the work-season, paving workers during the off-season and non-paving workers during the work-season. An additional model was constructed to determine whether significant differences existed by worker group (pavers versus non-pavers) or round (1, 2, 3, 4)

while controlling for the effect of smoking and BMI. In all models, cigarette smoking was evaluated as a continuous variable (number of cigarettes), a three-category variable (current, former, never) and a two-category variable (current smoker, current non-smoker) to determine the optimal method of controlling for smoking in the final models.

The DNA adduct dataset was unbalanced due to missing values. Data were missing for the following reasons: the participant changed jobs or retired, limitations of the sampling schedule or laboratory error. None of the participants chose to discontinue their involvement with the study. The data are assumed to be missing at random since it is unlikely that the reasons for missing values were related to either the observed or to the missing DNA adduct data.

RESULTS

Table 1 presents the summary statistics for the DNA adduct data by worker group. DNA adducts were detected in 60% of the 169 samples (median = 11 adducts per 10^{10} nucleotides) collected from paving workers, and in 61% of the 103 samples (median = 12 adducts per 10^{10} nucleotides) collected from non-paving workers. Though the median adduct levels were similar for both worker groups, the mean adduct level for paving workers (23 adducts per 10^{10} nucleotides) was lower than the mean adduct level for non-pavers (29 adducts per 10^{10} nucleotides) due to a small number of extreme values observed for non-pavers (non-smokers). The summary statistics are stratified by worker group, round, smoking status, age and BMI.

Approximately 39% of the data were found to be less than the detection limit of 1 adduct per 10^{10} nucleotides. Owing to the overdispersion of the adduct data, all results that follow are based on repeated measures generalized linear models that assume a negative binomial distribution. All regression models initially controlled for the effect of smoking, BMI and age; however, age was not found to be significant in any model and was therefore excluded from the final models. Cigarette smoking was evaluated in the models as a continuous variable (number of cigarettes smoked), a three-category variable (current, former, never) and a two-category variable (current smoker, current non-smoker). A positive association was observed between 'number of cigarettes smoked' and DNA adduct levels, but the parameter estimate was not significant in any of the models. In fact, the two-category variable evaluating current smoking status was the only method that approached statistical significance. Since there was evidence that smoking and BMI had a significant effect on DNA adduct burden, current smoking status and BMI were retained in all models regardless of the statistical significance.

Table 2 presents the results for models evaluating DNA adducts by weekday and paving task. The parameter estimates, standard errors and *P*-values are presented for each variable. Since the presented

Table 1. Summary statistics for DNA adducts by worker group

	Paving workers					Non-paving workers				
	Number of workers[a]	Number of samples[a]	Mean[b]	Median[b]	Range[b]	Number of workers[a]	Number of samples[a]	Mean[b]	Median[b]	Range[b]
Round										
1 (Spring)	42	42	26	11	0–105	13	13	48	8	0–215
2 (Summer)	45	45	6	0	0–116	34	34	28	0	0–210
3 (Fall)	40	40	24	8	0–165	30	30	6	0	0–37
4 (Winter)	42	42	37	36	0–78	26	26	47	43	23–91
Smoking status										
Smoker	11	39	29	15	0–156	13	33	21	16	0–91
Non–smoker	38	130	21	10	0–165	23	70	33	11	0–215
Age (years)										
<35	17	57	24	8	0–160	15	38	22	10	0–179
35–43	12	41	24	16	0–156	13	44	36	11	0–215
>43	20	71	22	9	0–165	8	21	27	17	0–202
BMI (kg m^{-2})										
<26.0	12	47	21	7	0–165	17	45	20	12	0–141
26.0–28.5	18	67	25	12	0–160	8	24	26	13	0–202
>28.5	18	55	22	14	0–105	11	34	44	20	0–215
Total	49	169	23	11	0–165	36	103	29	12	0–215

[a]Numbers of workers or samples may not sum to total due to missing values.
[b]Mean, median and range in units of DNA adducts per 10^{10} nucleotides.

Table 2. Models evaluating DNA adduct data[a] by weekday and paving task

Parameters	Pavers during work-season		Pavers during off-season		Non-pavers during work-season	
	Estimates (SE)	*P*-values	Estimates (SE)	*P*-values	Estimates (SE)	*P*-values
Intercept	2.3 (1.2)		1.7 (1.0)		−3.3 (1.6)	
Weekday		0.02		0.04		0.07
Monday	−2.7 (0.7)		0.05 (0.5)		−0.3 (0.6)	
Tuesday	−1.8 (0.4)		0.5 (0.3)		−0.9 (0.4)	
Wednesday	−1.1 (0.6)		0.6 (0.3)		−1.7 (0.5)	
Thursday	−0.6 (0.5)		−0.9 (0.5)		0.7 (0.4)	
Friday	0.0 (Ref.)		0.0 (Ref.)		0.0 (Ref.)	
Smoking		0.09		0.1		0.5
Smoker	0.6 (0.2)		0.4 (0.3)		0.4 (0.4)	
Non-smoker	0.0 (Ref.)		0.0 (Ref.)		0.0 (Ref.)	
BMI		0.3		0.2		0.09
(kg m^{-2})	0.06 (0.04)		0.05 (0.04)		0.2 (0.06)	
Paving task		0.1		0.7		
Roller operators	−1.2 (0.4)		0.3 (0.3)		·	
Rakers	−0.7 (0.3)		0.1 (0.3)		·	
Paver operators	−0.4 (0.4)		0.4 (0.4)		·	
Screedman	0.0 (Ref.)		0.0 (Ref.)		·	

[a]Analyzed as ln (adducts per 10^{10} nucleotides).

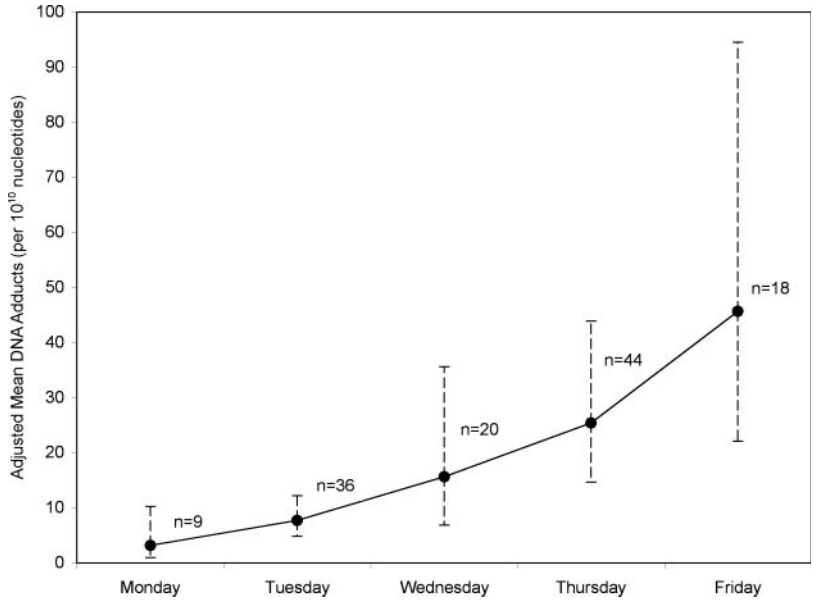

Fig. 1. DNA adducts among paving workers by weekday (during work-season only).

parameter estimates represent differences in log-transformed adduct levels, the following text translates the results into predicted mean adduct levels to assist with interpretation. Among paving workers during the work-season, adducts increased throughout the workweek and were different by paving task. The parameter estimates by weekday show that adduct levels were lowest on Mondays (3 adducts per 10^{10} nucleotides), highest on Fridays (46 adducts per 10^{10} nucleotides) and increased during each day of the week, while the parameter estimates by paving task show that adducts were lowest for roller operators (7 adducts per 10^{10} nucleotides) and highest for screedmen (23 adducts per 10^{10} nucleotides). Figure 1 presents the adjusted mean adduct levels by weekday for paving workers during the work-season.

In contrast, when the same model was evaluated for paving workers during the off-season, there was

Table 3. Model evaluating DNA adduct data[a] by job and round

Parameters	Estimates (SE)	*P*-values
Intercept	2.3 (0.7)	
Job		0.3
Pavers	−0.3 (0.2)	
Non-pavers	0.0 (Ref.)	
Round		0.0001
1 (Spring)	−0.3 (0.2)	
2 (Summer)	−1.2 (0.3)	
3 (Fall)	−0.9 (0.3)	
4 (Winter)	0.0 (Ref.)	
Smoking status		0.6
Smoker	0.2 (0.3)	
Non-smoker	0.0 (Ref.)	
BMI		0.08
(kg m^{-2})	0.06 (0.03)	

[a]Analyzed as ln (adducts per 10^{10} nucleotides).

no increasing trend throughout the workweek and no difference by paving task. In fact, the weekday parameter estimates indicate that the adduct levels were actually higher on Monday, Tuesday and Wednesday than on Thursday or Friday. As an additional comparison, when the same model was evaluated for non-paving workers during the work-season, there was no increasing trend from Monday to Friday. Accordingly, the weekday trend and differences by paving task were only observed among paving workers during the work-season.

Table 3 presents the results of a regression model indicating that adduct levels did not vary significantly by worker group ($P = 0.3$) or by smoking status ($P = 0.6$); however, the DNA adduct levels did vary significantly by season ($P = 0.0001$). The results of the model indicated that mean adduct levels were highest in winter (45 adducts per 10^{10} nucleotides) and lowest in summer (13 adducts per 10^{10} nucleotides). Additionally, the effect of BMI ($P = 0.08$) was marginally significant with DNA adducts increasing with increasing BMI.

DISCUSSION

Occupational exposure to hot-mix asphalt may increase the risk of cancer (lung, stomach, bladder, leukemia and non-melanoma skin cancer) among asphalt workers (Partanen and Boffetta, 1994; Boffetta *et al*., 1997; Boffetta *et al*., 2003). In the present study, we have evaluated DNA adducts in a population of paving workers to assess the role of asphalt exposure on DNA damage. Since the formation of DNA adducts has been recognized as an important initiating event that can lead to mutation, an association between asphalt-related exposure and DNA adduct burden in this population would support previous findings that occupational exposure to asphalt may increase cancer risk. A longitudinal study design was selected to evaluate changes in DNA adduct burden during the workweek and work-season while assessing interindividual and intraindividual differences.

The results of this study indicate that DNA adducts collected from paving workers during the work-season increased during each day of the workweek, such that the mean DNA adduct level on Friday morning was ∼14 times higher than the mean DNA adduct level on Monday morning. The results also revealed that DNA adduct levels were different among the four paving tasks, such that mean adduct levels were lowest for roller operators and highest for screedmen (3-fold difference).

The differences in DNA adducts by weekday suggests that the pattern of DNA damage observed among the paving workers may have resulted from occupational asphalt exposure. First, the increasing trend by weekday and the task-based differences were observed for pavers during the *work-season* (Round 1, Round 2 and Round 3) but were not observed for pavers during the *off-season* (Round 4). Similarly, the increasing trend by weekday was observed during the work-season for *pavers* but was not observed during the work-season for *non-pavers*. In both comparisons, the weekday trend and/or the task-based differences were observed for the asphalt-exposed group but not for the unexposed reference group.

The weekday trend and task-based differences observed for DNA adducts are consistent with a previous evaluation of urinary 1-OHP in a subset of the same population (20 pavers and 6 non-pavers). Urinary 1-OHP measurements were obtained at pre-shift, post-shift and bedtime during consecutive workdays and evaluated as a biomarker of total absorbed dose (McClean *et al*., 2004b). Among pavers, the mean pre-shift urinary 1-OHP concentrations were found to increase throughout the workweek, such that the mean pre-shift result on Thursday morning was 3.5 times higher than the mean pre-shift result on Monday morning. Similarly, the urinary 1-OHP concentrations were found to vary significantly by paving task, with results indicating that mean concentrations were lowest for roller operators and highest for screedmen (3-fold difference).

While excretion of urinary 1-OHP occurs with an approximate half-life of 20 h (6–35 h), the half-life of DNA adducts is considerably less clear (Dor *et al*., 1999). Since the majority of leukocytes (∼70%) are short-lived granulocytes, we targeted MNCs (monocytes and lymphocytes) in an effort to focus on longer-living subsets (Wiencke *et al*., 1995); however, even in these longer living cells, previous studies have demonstrated a 3- to 10-fold variation in the ability of cultured MNCs to form DNA adducts

after treatment with benzo(a)pyrene (Rothman *et al*., 1990).

Urinary 1-OHP is a biomarker of total absorbed dose that results from exposure to pyrene, whereas the DNA adducts are a biomarker of biologically effective dose that results from exposure to PACs. Pyrene and PACs were found to be strongly correlated in inhalation ($r = 0.87$, $P < 0.001$) and dermal samples ($r = 0.65$, $P = 0.002$) collected from the same paving workers (McClean *et al*., 2004a). Among paving workers, the increasing weekday trend and differences by paving task were observed for both urinary 1-OHP (total absorbed dose) and DNA adducts (biologically effective dose), such that both types of biomarkers were found to be lowest for roller operators and highest for screedmen (3-fold difference in each case). However, among non-paving workers, the weekday trend was not observed for either biomarker (McClean *et al*., 2004b).

In light of the weekday trend and task-based differences that were observed for DNA adducts among paving workers, it was somewhat surprising that the average DNA adduct level for pavers was slightly lower than for non-paving workers (milling and construction workers). There are several possible explanations for this finding. First, these results could also be the result of temporal differences since pavers and non-pavers were never measured on the same day (for logistical reasons). In fact, workers sampled at the beginning of a round were sampled ~2 months earlier than workers sampled at the end of the same round. Second, we had a limited ability to control for the effect of diet which has been shown to have a significant effect on DNA adduct levels and could have varied by exposure group (Kang *et al*., 1995). Finally, the milling and roadside construction workers were not truly unexposed populations. Milling workers had exposure to asphalt dust while grinding existing roads, while the roadside construction workers occasionally worked as rakers on asphalt crews during particularly busy periods; thus, these 'non-paving' construction workers may not have been an ideal comparison group for the pavers in this study.

In general, the levels of DNA adducts observed in the asphalt paving workers were low compared to those observed in other studies of PAC-exposed workers; however, the workers in those studies (i.e. foundry, coke oven, aluminum) primarily worked indoors and typically had PAC exposures that were much higher than the exposures experienced by the paving workers in this study (Dor *et al*., 1999).

Other types of DNA damage have been evaluated in previous studies of asphalt workers, finding significantly higher levels of sister-chromatid exchange (Burgaz *et al*., 1998), micronuclei (Burgaz *et al*., 1998) and strand breaks (Toraason *et al*., 2001) among exposed workers versus controls. Previous investigations of DNA adducts among asphalt workers are limited. Fuchs *et al*. (1996) measured DNA adducts in peripheral MNCs from 12 asphalt paving workers and 2 bitumen painters and reported adduct levels that were considerably lower than the DNA adduct levels observed among the paving workers in this study. In addition to measuring DNA adducts, Fuchs *et al*. (1996) evaluated strand breaks from 18 paving workers, collecting samples on Monday and Friday. Twelve of the eighteen workers showed higher strand breaks on Friday than on Monday, which is consistent with our finding that DNA adducts increased throughout the workweek.

Residual confounding by smoking is unlikely to explain the observed pattern of DNA adducts among paving workers during the work-season. Continuous smoking data were collected during each round of sampling, though the measurement error associated with asking a worker about typical cigarette consumption may have prevented us from observing a significant linear relationship between 'number of cigarettes smoked' and DNA adduct levels; however, the effect of current smoking status was found to be marginally significantly associated with DNA adduct levels, with results indicating that DNA adducts were higher among current smokers than among current non-smokers. Smoking is unlikely to explain the increasing trend by weekday since this pattern of adduct burden was observed for pavers but not for non-pavers. Additionally, the weekend represents a break from occupational asphalt exposure but does represent a break from cigarette consumption. Similarly, smoking is unlikely to explain the observed task-based differences since these differences were observed during the work-season but not during the off-season. The observed effect of smoking in our data is consistent with numerous studies that were summarized in a recent review (Wiencke, 2002).

The DNA adduct measurements in this study were obtained via the ^{32}P-postlabeling assay with nuclease P_1 enhancement, which has the strength of being an extremely sensitive method and the limitation of being a non-specific method. Though the assay was standardized for benzo(*a*)pyrene diol-epoxide DNA adducts, the DNA adducts could not be specifically identified.

Our results also show that DNA adduct levels were higher during the winter than during the summer for both pavers and non-pavers, which is generally consistent with the seasonal variability of DNA adducts that has been previously observed (Wiencke, 2002). Though the explanation for this seasonal variation remains unclear, previous studies have suggested a possible link to PAC concentrations in ambient air (Moller *et al*., 1996; Topinka *et al*., 2000). However, since the pavers in our study have occupational exposure to PACs during all seasons *except* the winter,

changes in PAC exposure seem unlikely to explain the seasonal variation observed in this study.

Another consideration is the seasonal variation of white blood cells, which accumulate PACs over the life of the cell and were used for our DNA adduct analysis. Maes *et al*. (1994) analyzed peripheral blood MNCs in 26 normal volunteers and found statistically significant annual variations with seasonal rhythms in the number of lymphocytes and monocytes. The counts of other leukeocyte subsets were also found to exhibit similar seasonal patterns (Maes *et al*., 1994). Since different subsets of leukocytes also have different lifespans, the change in cell counts by season may explain some of variability in DNA adduct measurements by season. As another possible explanation, the seasonal differences in DNA adduct levels may actually be attributable to endogenous variation (i.e. biological rhythms). For instance, since highly lipophilic PACs such as benzo(a)pyrene partition into the lipid components of cells and tissues, DNA adducts may be affected by the mobilization of fat and release of sequestered PACs during the winter months. Further research is necessary to fully understand the biological basis of seasonal variation in DNA adduct burden.

Among this group of paving workers, the difference in DNA adducts by weekday and paving task suggests that the pattern of DNA damage may have resulted from occupational asphalt exposure. Additionally, the weekday trend and task-based differences observed in this evaluation of DNA adducts (biomarker of biologically effective dose) were consistent with a previous evaluation of urinary 1-OHP (biomarker of total absorbed dose) conducted in a subset of the same workers. However, given the weekday trend and task-based differences that were observed among paving workers, the fact that the mean DNA adduct level among the paving workers was not different from the mean DNA adduct level among the non-pavers was somewhat surprising and requires additional investigation. Finally, our results demonstrate the importance of seasonal variation in longitudinal studies of DNA adducts.

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REFERENCES

Boffetta P, Jourenkova N, Gustavsson P. (1997) Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. Cancer Causes Control; 8: 444–72.

Boffetta P, Burstyn I, Partanen T *et al.* (2003) Cancer mortality among European asphalt workers: an international epidemiological study. II. Exposure to bitumen fume and other agents. Am J Ind Med; 43: 28–39.

Burgaz S, Erdem O, Karahalil B *et al.* (1998) Cytogenic biomonitoring of workers exposed to bitumen fumes. Mutat Res; 419: 123–30.

Chiazze L, Watkins DK, Amsel J. (1991) Asphalt and risk of cancer in man. Br J Ind Med; 48: 538–42.

Dor F, Dab W, Empereur-Bissonnet P *et al.* (1999) Validity of biomarkers in environmental health studies: the case of PAHs and benzene. Crit Rev Toxicol; 29: 129–68.

Fuchs J, Hengstler JG, Boettler G *et al.* (1996) Primary DNA damage in peripheral mononuclear blood cells of workers exposed to bitumen-based products. Int Arch Occup Environ Health; 68: 141–6.

IARC. (1985) Polynuclear aromatic compounds, Part 4, Bitumens, coal-tars and derived products, shale-oils and soots. IARC Monogr Eval Carcinog Risk Chem Hum; 35: 1–247.

Kang DH, Rothman N, Poirier MC *et al.* (1995) Interindividual differences in the concentration of 1-hydroxypyrene-glucuronide in urine and polycyclic aromatic hydrocarbon-DNA adducts in peripheral white blood cells after charbroiled beef consumption. Carcinogenesis; 16: 1079–85.

Lawless JF. (1987) Negative binomial and mixed Poisson regression. Can J Stat; 15: 209–25.

Maes M, Stevens W, Scharpe S *et al.* (1994) Seasonal variation in peripheral blood leukocyte subsets and in serum interleukin-6, and soluble interleukin-2 and -6 receptor concentrations in normal volunteers. Experientia; 50: 821–9.

McClean MD, Rinehart RD, Ngo L *et al.* (2004a) Inhalation and dermal exposure among asphalt paving workers. Ann Occup Hyg; 48: 663–71.

McClean MD, Rinehart RD, Ngo L *et al.* (2004b) Urinary 1-hydroxypyrene and polycyclic aromatic hydrocarbon exposure among asphalt paving workers. Ann Occup Hyg; 48: 565–78.

Moller L, Grzybowska E, Zeisig M *et al.* (1996) Seasonal variation of DNA adduct pattern in human lymphocytes analyzed by 32P-HPLC. Carcinogenesis; 17: 61–6.

NIOSH. (2000) Health effects of occupational exposure to asphalt. Cincinnati, OH: US Department of Health and Human Services. Report No.: 2001–110.

Partanen T, Boffetta P. (1994) Cancer risk in asphalt workers and roofers: review and meta-analysis of epidemiologic studies. Am J Ind Med; 26: 721–40.

Reddy MV, Randerath K. (1986) Nuclease P1-mediated enhancement of sensitivity of ^{32}P -postlabeling test for structurally diverse DNA adducts. Carcinogenesis; 7: 1543–51.

Rothman N, Poirier MC, Baser ME *et al.* (1990) Formation of polycyclic aromatic hydrocarbon-DNA adducts in peripheral white blood cells during consumption of charcoal-broiled beef. Carcinogenesis; 11: 1241–3.

Tang D, Santella RM, Blackwood AM *et al.* (1995) A molecular epidemiological case-control study of lung cancer. Cancer Epidemiol Biomarkers Prev; 4: 341–6.

Topinka J, Schwarz L, Wiebel F *et al.* (2000) Genotoxicity of urban air pollutants in the Czech Republic. Part II. DNA adduct formation in mammalian cell by extractable organic matter. Mutat Res; 469: 83–93.

Toraason M, Hayden C, Marlow D *et al.* (2001) DNA strand breaks, oxidative damage, and 1-OH pyrene in roofers with coal-tar pitch dust and/or asphalt fume exposure. Int Arch Occup Environ Health; 74: 396–404.

Wiencke JK. (2002) DNA adduct burden and tobacco carcinogenesis. Oncogene; 21: 7376–91.

Wiencke JK, Kelsey KT, Varkonyi A *et al.* (1995) Correlation of DNA adducts in blood mononuclear cells with tobacco carcinogen-induced damage in human lung. Cancer Res; 55: 4910–4.

Wiencke JK, Thurston SW, Kelsey KT *et al.* (1999) Early age at smoking initiation and tobacco carcinogen DNA damage in the lung. J Natl Cancer Inst; 91: 614–9.