Assessment of DNA Damage in WBCs of Workers Occupationally Exposed to Fumes and Aerosols of Bitumen

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Abstract

We conducted a cross-shift study with 66 bitumen-exposed mastic asphalt workers and 49 construction workers without exposure to bitumen. Exposure was assessed using personal monitoring of airborne bitumen exposure, urinary 1-hydroxypyrene (1-OHP), and the sum of 1-, 2 + 9–,3-,4-hydroxyphenanthrene (OHPH). Genotoxic effects in WBC were determined with nonspecific DNA adduct levels of 8-oxo-7, 8-dihydro-2′-deoxyguanosine (8-oxodGuo) and the formation of DNA strand breaks and alkali-labile sites. Concentration of fumes and aerosols of bitumen correlated significantly with the concentrations of 1-OHP and OHPH after shift (r_s = 0.27; P = 0.03 and r_s = 0.55; P < 0.0001, respectively). Bitumen-exposed workers had more DNA strand breaks than the reference group (P < 0.0001) at both time points and a significant correlation with 1-OHP and OHPH in the postshift urines (r_s = 0.32; P = 0.001 and r_s = 0.27; P = 0.004, respectively). Paradoxically, we measured higher levels of DNA strand breaks, although not significant, in both study groups before shift. 8-OxodGuo adduct levels did not correlate with DNA strand breaks. Further, 8-oxodGuo levels were associated neither with personal exposure to bitumen nor with urinary metabolite concentrations. Significantly more DNA adducts were observed after shift not only in bitumen-exposed workers but also in the reference group. Only low-exposed workers had significantly elevated 8-oxodGuo adduct levels before as well as after shift (P = 0.0002 and P = 0.02, respectively). Our results show that exposure to fumes and aerosols of bitumen may contribute to an increased DNA damage assessed with strand breaks. (Cancer Epidemiol Biomarkers Prev 2006;15(4):645–51)

Introduction

There is specific concern about the potential carcinogenicity of occupational exposure to bitumen fumes and aerosols. Bitumen is mainly used in roofing and in a mixture with stone in asphalt for road paving. During hot application of bitumen, complex mixtures of aerosols and vapors are emitted, which contain polycyclic aromatic hydrocarbons (PAH) and their derivatives as well as other compounds (1). However, little is known regarding the carcinogenic hazard of asphalt fumes to exposed workers. To evaluate the carcinogenicity of bitumen, the IARC conducted a retrospective cohort study in asphalt workers in eight European countries (2) but confounding by former exposure to coal tar was a main uncertainty. Therefore, cross-sectional studies on bitumen-related DNA damage in tar-free settings are a supplemental study design to assess genotoxicity by markers of DNA damage. Although data are still limited, the results point to an increased DNA damage in asphalt workers (3-6).

The potential carcinogenicity of bitumen has been attributed to the presence of PAH (1). However, some data suggest that PAH are not the sole genotoxic compounds in bitumen fume condensates (7). Nitrogen-, sulfur- and/or oxygen-containing PAH or their alkyl-substituted analogues may also contribute to genotoxic effects (7, 8). The urinary metabolite of pyrene, 1-hydroxypyrene (1-OHP), showed a moderate association with exposure to asphalt fumes (9-12) and thus may serve as a marker of internal exposure not only to PAH but also to bitumen.

Condensates of bitumen fume can induce DNA damage both *in vitro* and *in vivo* but the mutagenic effect is less strong than for condensates from coal-tar fumes (13). They are weakly mutagenic to bacteria and are capable of inducing micronucleus formation and chromosomal aberrations in cultured cells (14). In experimental animals, exposure to asphalt fume was genotoxic to alveolar macrophages, as revealed by DNA migration in the Comet assay, but no micronuclei formation was detected (15). Further, bitumen condensate binds covalently to DNA in lung cells but not in WBCs of rats (16).

Carcinogens exert probably their biological effect not only through direct DNA damage, but also through the generation of reactive oxygen species. Bioactivation of PAH and other compounds requires oxidative metabolism by phase I enzymes and in particular the cytochrome P-450 system (17). These result in the formation of radical cations, anti–diol-epoxides, and reactive and redox-active *o*-quinones. Reactive oxygen species may lead to the formation of oxidative DNA damage and have been shown to participate in all stages of the carcinogenesis process (18). The interacting pathways for prevention and repair of oxidative DNA damage have been recently reviewed (19). The spectrum of oxidative DNA damage includes strand breaks, apurinic/apyrimidinic sites, and oxidized bases. A commonly measured marker for oxidized bases is 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodGuo), a major product with a highly mutagenic potential generated by reactive oxygen species (20, 21). Previously published results found evidence for DNA damage in the form of 8-oxodGuo

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and DNA strand breaks in workers exposed to PAH (22, 23) and to bitumen fume condensates in particular (4, 6).

Information on genotoxic risk for bitumen workers is limited. To evaluate the genotoxic and irritative effects of bitumen fumes and aerosols, we conducted a cross-shift study in German mastic asphalt workers and construction workers without exposure to bitumen. We measured personal exposure to fumes and aerosols of bitumen and metabolites of pyrene and phenanthrene, 1-OHP and the sum of 1-, 2 + 9−, 3- and, 4-hydroxyphenanthrene (OHPH), respectively. Pyrene and phenanthrene are frequently measured variables of external PAH exposure. Their metabolites 1-OHP and OHPH serve as biomarkers for internal PAH exposure assessment in humans and have been used as suitable variables for the biological monitoring of exposure to PAH in various studies. Here, we report on the genotoxic effects of bitumen assessed with 8-oxodGuo as biomarker of steady-state oxidative DNA damage and the formation of DNA strand breaks and alkali-labile sites in WBC of workers before and after shift. The irritative effects on the lung and upper airways will be presented elsewhere.

Materials and Methods

Study Groups. We conducted a cross-shift study with 115 workers insured by the German Employer's Liability Insurance Association for Construction (Tiefbau-Berufsgenossenschaft). The study group consisted of 66 mastic asphalt workers who were exposed to fumes and aerosols of bitumen released under high processing temperature and 49 construction workers at outdoor construction sites without exposure to bitumen as reference group. The mastic asphalt workers, not exposed to coal-tar pitch since at least 2 years, were exposed to bitumen fumes and aerosols during paving in basement garages. All workers were examined twice a day immediately before and after shift. At both time points, spot urine and blood samples were collected. A structured questionnaire was applied in a face-to-face interview to assess detailed information on the workplace, occupational history, potential confounders, health complaints, and other factors. We asked the workers for their nationality. Three different nationalities (German, Polish, and Turkey) are included in the study. All of them were Caucasians. We adjusted the effects and mean values for German nationality (yes/no). All study subjects provided written informed consent before examination. The study was approved by the Ethics Committee of the Ruhr-University Bochum and was conducted in accordance with the principles for human experience as defined by the Helsinki Declaration.

Ambient Monitoring. Personal air sampling in the workers' breathing zone was carried out to determine exposure to bitumen fumes and aerosols. For measuring both particle and vapor form, a closed-face Gesamtstaub/Gas-Probenahmekopf sampler (24) was used. The Gesamtstaub/Gas-Probenahmekopf system is a true inhalable aerosol sampler as specified in Methods for the Determination of Hazardous Substances 14/3 (24) and corresponds to European norm 481 (25). The aerosol phase was collected on a 37-mm glass fiber filter and the gaseous phase was adsorbed in a cartridge containing 3 g XAD-2 (size 0.5-0.9 mm) with a flow rate of 3.5 L/min. Filter and adsorbent were extracted with tetrachloroethene in an ultrasonic bath. For the quantification, Fourier transform IR spectroscopy (2,800-3,000 cm^{-1}) was applied using nonaromatic mineral oil for spectroscopy as a standard. The limit of quantification was 0.5 mg/m^3 for a sampling volume of 420 liters.

Urine and Blood Sampling. All workers (exposed workers as well as reference group) were examined in the midweek (from Tuesday to Thursday). No sampling was done on Monday and Friday to exclude week start and weekend effects. Urine samples were immediately frozen and transported in a box containing dry ice. Whole blood samples were collected in EDTA-treated tubes for 8-oxodGuo measurement (9 mL) and in heparin-treated tubes for lymphocyte preparation in Comet assay (5 mL). During the study, there were great efforts to avoid any influence of storage and transport conditions on DNA damage and to avoid differences in the sampling procedure for preshift or postshift samples. Therefore, after collecting the blood, the samples were transported to our laboratory immediately. The time processing of samples was in the exact same manner. Samples for Comet assay were transported at 5°C to 8°C (during the transport, we monitored the temperature), whereas the samples for the measurement of 8-oxodGuo adducts were transported in a box containing dry ice. Usually, sample preparation started within 12 to 15 hours after blood collection for both preshift and postshift samples. Preliminary results showed that this time has no influence on the DNA damage.

PAH Metabolites in Urine. The determination of 1-OHP and OHPH in preshift and postshift spot urine samples of the workers was carried out using a modified high-performance liquid chromatography method developed by Lintelmann and Angerer (26) as reported previously (23).

Creatinine in Urine. Urinary creatinine was determined photometrically as picrate, according to the Jaffe method (27).

8-OxodGuo Adducts in WBC DNA. DNA from WBCs was isolated within 2 days and frozen at −80°C. DNA extraction and 8-oxodGuo adduct isolation were carried out using the procedure from Marczynski et al. (23). For the analysis of nucleosides in WBC DNA, a Shimadzu high-performance liquid chromatography/UV apparatus, connected to a Coulochem II (model 5200) electrochemical detector (ESA, Chelmosford, MA), was used.

DNA Strand Breaks by the Comet Assay (Alkaline Single-Cell Gel Electrophoresis). Lymphocytes were isolated by a standard method of centrifugation on a Ficoll density gradient. A modified protocol of the original description by Östling and Johanson (28) and Singh et al. (29) was used for the Comet assay (22). The altered migration of DNA toward the anode can be quantified in several ways. Using the image-analyzing program, the total area of each tail, its absolute average intensity, and its distance to the center position of the head were determined. These data enabled to calculate several indicators of DNA damage, from which we have selected the Olive tail moment [OTM: (tail mean − head mean) × (%tail DNA / 100)] to quantify DNA damage (30, 31). This variable was used to estimate the DNA strand break frequency, as it comprised both the length of DNA migration and the percentage of migrated DNA, as one value. Thus, the Comet tail moment reflects the net result of DNA damage. Standard scanning criteria were followed. Median OTM was calculated from 51 cells per slide, using two different slides prepared from each subject.

Statistical Analysis. Based on the individual exposure levels, a classification of the mastic asphalt workers into low-exposed (n = 38) and high-exposed (n = 28) workers had been carried out with 10 mg/m^3 as cutoff (current German threshold limit value for exposure to fumes and aerosols of bitumen). A mixed linear model was done on the different outcomes with exposure group [nonexposed, exposed (low-exposed, high-exposed)], time of measurement (preshift, postshift), current smoking (yes, no), German nationality (yes, no), and age (<30, 30 to <50, ≥50 years) as fixed factors and subjects as random factor. Due to the skewed distributions, continuous variables were log-transformed for the model calculations. If adjusted means were presented, the variables were retransformed to

their original scale. The relative changes by exposure and during shift were expressed as factors for log-transformed variables. All tests were conducted two-sided with a significance level of 5% using the statistical software package SAS 8.2 (SAS Institute, Inc., Cary, NC). Spearman rank correlation coefficients (r_s) were calculated to describe the correlations between the different variables.

Results

Characteristics of the Study Population. Table 1 depicts the characteristics of the study population. All bitumen-exposed workers were males, with a median age of 40 years (range, 17-63 years). The reference group was also male workers but with a median age of 36 years (range, 19-61 years). Of the bitumen-exposed workers, 66.7% were current smokers compared with only 40.8% in the reference group. Whereas almost 90% of the workers in the reference group were of German nationality, this was the case for only 57.6% of those with bitumen exposure. Median duration of occupational exposure to bitumen fumes and aerosols was 84 months (interquartile range, 24-168). Personal monitoring devices were used to evaluate the exposure of the workers to bitumen fumes and aerosols during shift. Median concentration of the bitumen fumes and aerosols during the shift was 5.3 mg/m^3 (interquartile range, 2.5-16.2). We included age, smoking, and nationality in the statistical models for the potential confounder.

Effects of Bitumen Exposure on Urinary PAH Metabolites. The concentrations of 1-OHP and OHPH in urine were measured to assess internal exposure to bitumen fumes and aerosols. Concentration of fumes and aerosols of bitumen at the workplace was strongly correlated with concentrations of 1-OHP and OHPH in the urine of the workers after shift ($r_s = 0.27$, $P = 0.03$ for 1-OHP and $r_s = 0.55$, $P < 0.0001$ for OHPH; Table 2). The urinary concentrations of both 1-OHP and OHPH in exposed workers increased significantly ($P < 0.0001$ for both variables) during shift (Fig. 1A and B; Table 3). However, no significant increase of these variables was found in the reference group ($P = 0.20$ for 1-OHP, $P = 0.33$ for OHPH; Fig. 1A and B; Table 3). The urinary concentrations of 1-OHP and OHPH of exposed and nonexposed workers were similar before shift ($P = 0.62$ and $P = 0.91$, respectively; Table 3) but different after shift ($P = 0.005$ and $P < 0.0001$, respectively; Fig. 1A and B; Table 3), with higher levels in bitumen-exposed workers when adjusted for age, smoking, and nationality. Only current smoking, but not age and nationality, had an effect on the concentrations of OHPH ($P = 0.002$) and 1-OHP ($P < 0.0001$), showing higher levels in current smokers (data not shown). Additionally, a strong association was observed between changes during shift of urinary concentrations of 1-OHP and OHPH ($r_s = 0.78$, $P < 0.0001$; Table 2).

Urinary PAH Metabolites Compared with 8-OxodGuo Adducts and DNA Strand Breaks. Steady-state oxidative DNA damage, as assessed by 8-oxodGuo, was higher before shift in all bitumen-exposed workers compared with the reference group ($P = 0.01$, Fig. 1C) as well as after shift. However, DNA adduct rate was significantly increased after shift ($P = 0.02$) only in low-exposed workers (Table 3) but not in high exposed workers. Additionally, significantly higher levels of 8-oxodGuo adducts were found after shift not only in asphalt workers but also in the reference group ($P = 0.01$ and $P = 0.001$, respectively; Fig. 1C; Table 3). Age, smoking, and nationality had no significant effect on 8-oxodGuo.

At both time points, the number of DNA strand breaks determined by OTM was significantly higher in asphalt workers than in the reference group ($P < 0.0001$ for preshift and postshift; Fig. 1D; Table 3). In both groups, OTM decreased slightly but not significantly during shift ($P = 0.23$ for asphalt workers and $P = 0.13$ for the reference group; Fig. 1D; Table 3). No different trend was found between the exposure groups for both markers of DNA damage (Fig. 1C and D). Age, smoking, and nationality had no significant influence on DNA strand breaks (data not shown). Spearman rank correlation between the changes during shift (postshift minus preshift) of 8-oxodGuo and OTM showed no association ($P = 0.22$; Table 2).

Changes of the formation of 8-oxodGuo adducts during shift showed no association with exposure to bitumen nor with internal exposure assessed with PAH metabolites (1-OHP and OHPH; Table 2). Bitumen-exposed workers showed significantly more DNA strand breaks with 1-OPH and OHPH in the postshift urines ($r_s = 0.32$; $P = 0.001$ and $r_s = 0.27$, $P = 0.004$, respectively). No correlation was found between changes during shift for DNA strand break levels and OHPH ($r_s = 0.09$, $P = 0.37$, Table 2), but a statistically significant association with internal exposure assessed with 1-OHP ($r_s = 0.24$, $P = 0.01$, Table 2) as well as with external exposure concentrations of bitumen fumes and aerosols ($r_s = -0.27$, $P = 0.03$, Table 2) was observed.

Discussion

This cross-sectional study in mastic asphalt workers was conducted to evaluate the genotoxic and irritative effects of fumes and aerosols of bitumen under tar-free exposure conditions. In the present analysis, we focused on the genotoxic effects of bitumen exposure. Overall, we found evidence for genotoxic effects in form of DNA strand breaks and alkali-labile sites but not for 8-oxodGuo. Further, we found no correlation between these two markers. There was an association of DNA strand breaks with airborne bitumen exposure during the shift and with internal exposure to pyrene and phenanthrene metabolites. We confirmed the bitumen influence on internal exposure to PAH metabolites as reported by Vaananen et al. (32) in mastic asphalt workers. Airborne monitoring of bitumen may not adequately predict total internal exposure due to the additional dermal route of exposure (33, 34). To account for this, we used urinary 1-OHP and OHPH as internal biomarkers of occupational exposure to PAH, although other compounds of the bitumen may also contribute to the health effects. Although the levels of 1-OHP and OHPH of both exposed and nonexposed workers before shift are higher than the median of the general population in Germany, they are still lower than the 90th percentile (35). During shift, there was an increase of 1-OHP

Table 1. Characteristics of the study groups

	Reference group ($n = 49$)	Bitumen-exposed workers ($n = 66$)
Age (y), median (range)	36 (19-61)	40 (17-63)
Current smoking, n (%)	20 (40.8)	44 (66.7)
German nationality, n (%)	44 (89.8)	38 (57.6)
Duration of exposure (mo), median (interquartile range)	0	84 (24-168)
Exposure to bitumen fumes and aerosols (mg/m^3), median (interquartile range)	—	5.3 (2.5-16.2)

Table 2. Spearman rank correlations between exposure to fumes and aerosols of bitumen, urinary metabolites, 8-oxodGuo, and OTM

Variable	Independent variables	Preshift			Postshift			Shift difference*		
		n	r_s	*P*	*n*	r_s	*P*	*n*	r_s	*P*
Exposure to fumes and aerosols of bitumen (mg/m^3)	1-OHP (ng/L)				65	0.27	0.03	65	0.25	0.05
	1-OHP (ng/g crn)				65	0.23	0.07	65	0.32	0.01
	Sum of five OHPHs (ng/L)				65	0.55	<0.0001	65	0.55	<0.0001
	Sum of five OHPHs (ng/g crn)				65	0.51	<0.0001	65	0.68	<0.0001
	8-OxodGuo/10^6 dGuo				66	−0.31	0.01	66	−0.10	0.44
	OTM (median)				66	−0.18	0.14	66	−0.27	0.03
1-OHP (ng/L)	Sum of five OHPHs (ng/L)	114	0.82	<0.0001	114	0.72	<0.0001	113	0.78	<0.0001
	8-OxodGuo/10^6 dGuo	113	−0.03	0.72	113	0.01	0.96	111	−0.11	0.27
	OTM (median)	113	0.07	0.47	114	0.32	0.001	112	0.24	0.01
1-OHP (ng/g crn)	Sum of five OHPHs (ng/g crn)	114	0.61	<0.0001	114	0.64	<0.0001	113	0.68	<0.0001
	8-OxodGuo/10^6 dGuo	113	−0.02	0.84	113	0.02	0.81	111	−0.11	0.25
	OTM (median)	113	0.05	0.57	114	0.32	0.0004	112	0.18	0.06
Sum of five OHPHs (ng/L)	8-OxodGuo/10^6 dGuo	113	−0.01	0.91	113	0.00	0.97	111	−0.05	0.58
	OTM (median)	113	0.20	0.03	114	0.27	0.004	112	0.09	0.37
Sum of five OHPHs (ng/g crn)	8-OxodGuo/10^6 dGuo	113	0.02	0.83	113	0.00	0.97	111	−0.07	0.49
	OTM (median)	113	0.22	0.02	114	0.26	0.01	112	0.06	0.52
8-OxodGuo/10^6 dGuo	OTM (median)	113	−0.02	0.80	114	0.13	0.18	112	−0.12	0.22

Abbreviation: crn, creatinine.
*Calculated as postshift minus preshift value.

but we found only a weak association with personal exposure to bitumen even when taking into account that mastic asphalt workers have higher exposure levels than paving workers (3, 6, 9). After shift, the concentrations of 1-OHP asphalt workers were much lower than in occupational settings with high PAH exposure, such as in the manufacture of graphite electrodes and in coke-oven and fireproof material plants (22, 23). We found an even stronger effect for OHPH, which confirms another study where OHPH turned out superior to 1-OHP in occupational settings with high PAH exposure (36).

To our knowledge, this is the first study on workers exposed to fumes and aerosols of bitumen that uses the cross-shift design to assess DNA damage in asphalt workers. The cross-shift design with preshift and postshift sampling offers the possibility of measurements of acute effects and has the advantage that test subjects poses as their own controls because an appropriate reference group is crucial to establish. Potential ''carry-over effects'' have to be considered regarding higher levels of DNA strand breaks before shift in the high-exposed group. Further, a possible healthy worker effect was found for the low-exposed group that turned out healthier in terms of lung function and irritative effects compared with both the reference group and the high-exposed workers (data not shown).[5] The higher adduct levels in the low-exposed group were associated with a better lung function. Thus, adduct levels might be less informative for exposure to bitumen fumes and aerosols. There was no association between DNA adducts and strand breaks with irritative variables (data not shown). Therefore, these data were not included in statistical models to evaluate possible associations between genotoxic and inflammatory effects.

Urinary 1-OHP and OHPH reflects recent exposure. In contrast, DNA damage in WBCs may not only reflect the exposure during a 1-day shift but might have accumulated subchronic effects, which seems to be supported by elevated preshift values of OTM. 8-OxodGuo adduct levels were associated neither with personal exposure to fumes and aerosols of bitumen nor with internal exposure assessed with 1-OPH and OHPH. Further, only the low-exposed group had higher 8-oxodGuo levels than the reference group before and after shift. All groups showed an increase during shift that was significant for the reference group and the high-exposed group only. Presumably, the low-exposed group showed a ''healthy-worker effect,'' which was shown by better lung function variables and less irritative effects (data not shown). Thus, 8-oxodGuo might reflect not only bitumen effect but also other effects because this DNA adduct is not a specific biomarker for PAH exposure but a general marker for oxidative stress. There are a variety of possible explanations for these effects. First, WBCs might not be a suitable target cell for bitumen effects as shown in experimental animals where bitumen condensate binds covalently to DNA in lung cells but not in WBCs of rats (16). Second, these unspecific DNA adducts might represent other influences than occupational exposure, such as physical activity, during work. The increase in DNA adduct levels during work shift in both groups may support such an effect. In particular, the ''healthy'' low-exposed workers may exert more physical activities and different job tasks than the highly qualified high-exposed workers. Smoking was not associated with adduct levels in our study as well as in a large population-based cohort study (37), which also points toward other influences than PAH, e.g., bitumen exposure. The presence of 8-oxodGuo reveals a lower fidelity in the replication process and enhances the probability of adenine incorporation into the complementary strand, causing G to T transversions (38, 39). Hydroxylated guanine on DNA and removal by DNA repair system is a complex mechanism and depends on many various factors leading to substantial intraindividual and interindividual variations (39). Third, PAH exposure level in this bitumen study was considerably lower than in previous studies among PAH-exposed workers where 8-oxodGuo levels were up to 3-fold higher in several settings, such as in the manufacture of graphite electrodes (22, 23). Fourth, although the range of the adduct levels is within the recommended background level (3.0-4.2 8-oxodGuo/10^6 dGuo), there are still critical steps in sample processing to rule out adventitious oxidation (20). However, we chose a method for preparation of nucleosides, which, to our knowledge, is less prone to the artifact of additional oxidation. According to the European Standards Committee on Oxidative DNA Damage, we used a high-performance liquid chromatography/UV/electrochemical detector method that is capable of measuring 8-oxodGuo with high accuracy.

[5] Submitted for publication.

Detailed analysis of the frequency of DNA strand breaks in lymphocytes of the workers revealed significantly more DNA strand breaks with increasing internal exposure assessed by urinary pyrene and phenanthrene metabolites. OTM correlated better during shift with 1-OHP compared with OHPH. Moreover, elevated DNA damage was shown when compared with the reference group not only after shift but also before. OTM showed higher levels in both exposure groups but no dose-response relationship after shift. Additionally, we measured decrease in the frequency of DNA strand breaks (not significant) during shift in all groups. The reason for this is difficult to explain. Confounding by age, current smoking and nationality was adjusted for, although we cannot exclude other unmeasured sources of confounding between the groups. DNA repair might have an effect for example, but no data are available on this variable. Care was taken to transport samples both before and after shift in the exact same manner to minimize the effect of transportation. The results might indicate carry-over effects from previous occupational exposure and thus potential (sub)chronic effects that are supported by similar carry-over effects in the mediators of irritations found in induced sputum and nasal lavage (data not shown).

Similar studies, however, with different time points of sampling were carried out by Fuchs et al. (4) and Toraason et al. (6). Fuchs et al. (4) sampled blood from workers occupationally exposed to bitumen in different settings on Mondays and Fridays. Genotoxic damage assessed with DNA strand breaks and alkali-labile sites was detected in roofers but not in road pavers. Paradoxically, bitumen painters exhibited a relatively high level of alkaline DNA strand breaks on Mondays after a weekend free of exposure and a decreased mean level of DNA strand breaks on Fridays (4). Fuchs et al. (4) hypothesized that a decrease in the level of alkaline DNA strand breaks from Monday to Friday in bitumen workers can possibly be explained by the formation of DNA cross-links, which reduce the elution velocity of the DNA. During the weekend or overnight, these cross-links may be partly repaired by excision, leading to a higher level of DNA strand breaks on Mondays or in the morning. Also, Toraason et al. (6) determined DNA strand breaks and 8-oxodGuo in WBCs of roofers sampled on Mondays and Thursdays. In this study, asphalt fume exposure was also associated with significant increases in DNA strand breaks but without significant changes in 8-oxodGuo. DNA strand breaks and DNA cross-links have a counteracting effect due to a masking of the effect of DNA strand breaks by simultaneously existent DNA cross-links. Particularly, changes in DNA repair capacity, cell turnover, and apoptosis may influence level of DNA damage measured at different time points. Our results and previous reports (4, 6) are consistent and show changes in DNA strand break frequency (refs. 4, 6 and our study) and in the level of 8-oxodGuo adducts (ref. 6 and our study) in workers occupationally exposed to bitumen-based products.

In the alkaline Comet assay version, DNA lesions, such as DNA double- and single-strand breaks, and alkali-labile sites lead to increased DNA migration. Besides these effects, DNA strand break formations during nucleotide excision repair can also increase DNA migration in the Comet assay. We found no association between 8-oxodGuo adducts and DNA strand breaks. The results based on alkaline single-cell gel electrophoresis include DNA strand breaks, but also base modifications (40), as the oxidized purine bases (8-oxodGuo and others) and pyrimidine bases could be converted into additional DNA single-strand breaks (41). On a steady-state level, the contribution of repair through enzyme-mediated DNA cleavage at the site of oxidized bases is negligible with respect to the overall formation of DNA strand breaks (42-45). The level of hydroxyl radical–induced base damage stays in the same

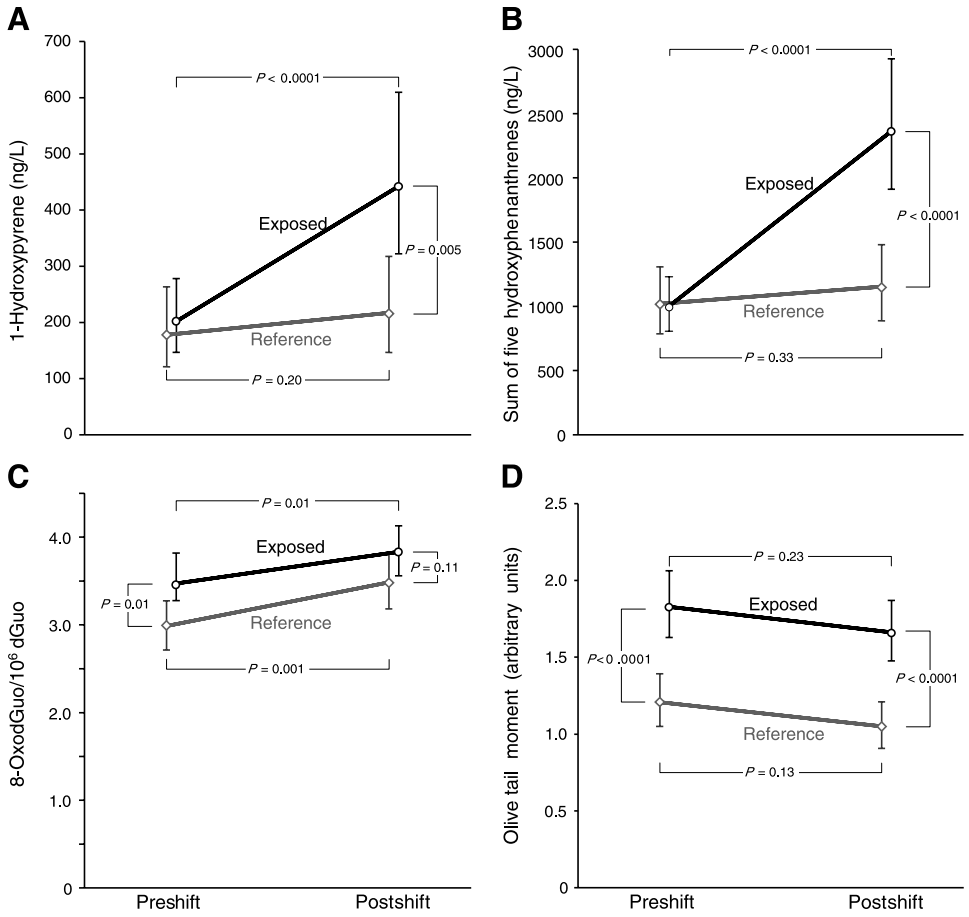

Figure 1. Biomarker levels before and after shift presented as adjusted geometric means and 95% CIs. **A.** Concentration of 1-OHP in urine of workers (group comparison preshift, $P = 0.62$; postshift, $P = 0.005$). **B.** Sum of five OHPH concentrations in urine of workers (group comparison preshift, $P = 0.91$; postshift, $P < 0.0001$). **C.** 8-OxodGuo adduct level in WBCs of workers (group comparison preshift, $P = 0.01$; postshift, $P = 0.11$). **D.** OTM in the lymphocyte DNA of workers (group comparison preshift, $P < 0.0001$; postshift, $P < 0.0001$).

Table 3. Biomarkers of internal exposure and of effect in workers exposed to fumes and aerosols of bitumen (n = 66, low n = 38, high n = 28) and in the reference group (n = 49)

Variable	Group	Preshift			Postshift			During shift	
		Adjusted mean*	Exposure effect†	P	Adjusted mean*	Exposure effect†	P	Shift effect†	P
1-OHP (ng/L)	Reference	178	1	—	216	1	—	1.21	0.20
	Exposed	202	1.13	0.62	444	2.05	0.005	2.19	<0.0001
	Low	155	0.87	0.62	296	1.37	0.26	1.90	0.0002
	High	263	1.48	0.21	666	3.08	0.0004	2.53	<0.0001
1-OHP (ng/g crn)	Reference	148	1	—	179	1	—	1.21	0.11
	Exposed	165	1.12	0.59	296	1.66	0.02	1.79	<0.0001
	Low	145	0.98	0.94	218	1.22	0.38	1.51	0.003
	High	189	1.28	0.34	402	2.25	0.002	2.13	<0.0001
Sum of five OHPHs (ng/L)	Reference	1,015	1	—	1,150	1	—	1.13	0.33
	Exposed	997	0.98	0.91	2,372	2.06	<0.0001	2.38	<0.0001
	Low	933	0.92	0.65	1,645	1.43	0.05	1.76	0.0002
	High	1,064	1.05	0.82	3,421	2.98	<0.0001	3.22	<0.0001
Sum of five OHPHs (ng/g crn)	Reference	894	1	—	945	1	—	1.06	0.47
	Exposed	850	0.95	0.67	1,556	1.65	<0.0001	1.83	<0.0001
	Low	876	0.98	0.88	1,190	1.26	0.08	1.36	0.0007
	High	825	0.92	0.58	2,034	2.15	<0.0001	2.47	<0.0001
8-OxodGuo/10^6 dGuo	Reference	2.99	1	—	3.50	1	—	1.17	0.001
	Exposed	3.47	1.16	0.01	3.85	1.10	0.11	1.11	0.01
	Low	3.85	1.29	0.0002	4.09	1.17	0.02	1.06	0.24
	High	3.14	1.05	0.51	3.61	1.03	0.66	1.15	0.03
OTM (median)	Reference	1.21	1	—	1.05	1	—	0.87	0.13
	Exposed	1.83	1.52	<0.0001	1.66	1.59	<0.0001	0.91	0.23
	Low	1.72	1.42	0.001	1.71	1.63	<0.0001	1.00	0.98
	High	1.96	1.62	<0.0001	1.61	1.54	0.0002	0.82	0.12

*Adjusted for age (<30, 30 to <50, ≥50 years), smoking, and German nationality.
†Relative changes by exposure and during shift were expressed as factors for log-transformed variables.

range as the extent of radical reactions leading to DNA strand cleavage (44, 46, 47). However, the origin of the direct DNA strand breaks and alkali-labile sites that may include modified sugar and base residues is difficult to establish using the alkaline Comet assay and, obviously, this strongly depends on the DNA-modifying agent.

In conclusion, our findings indicate that occupational settings, such as mastic asphalt works that expose workers to fumes and aerosols of bitumen and PAH, contribute to an increased DNA damage in WBC. It is possible that factors other than bitumen or PAH have contributed to the observed DNA damage, such as elevated temperature at the workplaces of mastic asphalt workers. Although confounding cannot be ruled out, the results of this study indicate that PAH-containing fumes and aerosols of bitumen might exert DNA damage assessed as strand breaks in a dose-dependent manner, which could become subchronic. The results are consistent with previous reports attempting to assess the human genotoxicity of bitumen but are yet limited. However, efforts are under way to expand the study size and thus the statistical power to allow a more precise estimation of the bitumen-related health effects.

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