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Exposure to polycyclic aromatic hydrocarbons and genotoxic effects on nonsmoking Swedish road pavement workers

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Objectives The objective of this study was to investigate exposure to polycyclic aromatic hydrocarbons (PAH) from asphalt fumes among Swedish road pavement workers and determine whether any effects could be detected with genotoxic tests.

Methods The study included 28 nonsmoking road pavers and 30 nonsmoking referents. The concentration of PAH was determined in the breathing zone of the road pavers. 1-Hydroxypyrene was analyzed before and after shifts of asphalt work and during the afternoon for referents. Sister chromatid exchanges (SCE) and micronuclei (MN) were determined in peripheral lymphocytes.

Results Several 3- or 4-ring PAH were found, and the analysis indicated that they occurred in bitumen fumes rather than in traffic fumes. The average total concentration of PAH was 2.3 (range 0.2—23.8) $\mu\text{g}/\text{m}^3$. The concentration of 1-hydroxypyrene in urine was higher for the road pavers than for the referents, but there was no significant difference between the pre- and postshift values of the road pavers. The road pavers had no significant increase in SCE or MN.

Conclusions The study showed that Swedish road pavers have an increased exposure to PAH from bitumen fumes, but no genotoxic effects could be detected by SCE or MN tests.

Key terms asphalt, bitumen, epidemiology, micronuclei, occupation, sister chromatid exchanges.

A recent review indicates that there may be an increased risk of cancer among workers exposed to fumes from bitumen and other components of asphalt (1). In Sweden there are about 3500 workers exposed to bitumen fumes from road paving or asphalt mixing.

Bitumen is a complex mixture of heavy hydrocarbons derived from processing crude petroleum oil. It contains a relatively high proportion of hydrocarbons with carbon numbers greater than C_{25} , but also some polycyclic aromatic hydrocarbons (PAH) and trace amounts of such metals as nickel, iron, and vanadium. At room temperature bitumen is almost nonvolatile, and the concentration

of PAH is typically in the range below 10 mg/kg (ppm) (2, 3). During paving bitumen is heated to 150—200°C, and the concentration of PAH in the condensed fumes may be 10—100 times higher (2—4). Coal tar is mixed with bitumen in some applications, but the use of coal tar in road paving has decreased. In Sweden, the use of coal tar in road paving has been abandoned on a voluntary basis since the early 1970s. Today coal tar is rarely used, and then only for very special applications (eg, sealing of asphalt on some airfields).

There are a few studies of the exposure to bitumen fumes among road pavement workers. Pyrene is a

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constituent of all PAH mixtures, and urinary 1-hydroxypyrene has been suggested and used as a biomarker for exposure to PAH (5). Jongeneelen et al (6) found no increased excretion of 1-hydroxypyrene in the urine of workers exposed to bitumen fumes, while asphalt workers exposed to coal tar had an increased excretion. The thioether excretion was not increased in 44 workers exposed to bitumen fumes during asphalt mixing or road paving (7). In a later study an increased excretion of 1-hydroxypyrene was found in workers exposed to bitumen fumes (8). A study by Pasquini et al (9) found an increased excretion of mutagens in the urine of workers exposed to bitumen fumes, while there was no increase in the excretion of thioethers or D-glucaric acid.

The objective of this study was to investigate the possibility of exposure to fumes from bitumen and other components in asphalt being detected with genotoxic tests. The concentration of some PAH was used as a marker of exposure to bitumen fumes. Since smoking is an important source for exposure to PAH, the study included nonsmokers only. The investigation was a cross-sectional study which was carried out in 1990 and in which the concentration of PAH in the breathing zone was measured along with the excretion of 1-hydroxypyrene in urine. In order to study possible genotoxic effects, sister chromatid exchanges (SCE) and micronuclei (MN) in lymphocytes were investigated. Ordinary road paving operations were selected to avoid special asphalt mixtures (eg, mastic asphalt) or repaving. The temperature of the asphalt varied between 140 and 185°C.

Subjects and methods

Subjects

Asphalt paving is done almost exclusively by men in Sweden. Furthermore, it is seasonal work due to the coldness of the winters. The plan was to study as many as 30 male asphalt workers during the road paving season. All the men participating in the study had to be lifelong nonsmokers or ex-smokers who had stopped smoking at least 3 years before the examination. A similar number of male carpenters in the construction industry acted as referents. All the subjects were asked about smoking habits and their medical history. Men that had undergone previous treatment with cytostatic drugs or radiation were excluded.

Altogether 28 exposed workers and 30 referents participated. Twenty-one of each group were life long nonsmokers, and 7 and 9, respectively, reported that they had stopped smoking at least 3 years earlier. Their average ages were 41.1 (range 23–63) and 38.4 (range 21–61) years, respectively. Fifty-five air samples were taken,

consisting of 2 samples for all persons except 1 exposed worker.

Asphalt-paving operations were performed in teams that consisted of 4–7 members with different tasks. All nonsmoking members in each team offered to participate, and the teams were selected so that the travel distance would be as short as possible for the investigators. The study was approved by the ethics committee of the University of Göteborg.

Air sampling

Air samples were taken in the breathing zone by personal sampling during 2 full shifts. Air was drawn through a 37-mm glass fiber filter (type A/E, Gelman Sciences Inc, Ann Arbor, MI, USA) in series with an adsorbent tube (Amberlite XAD-2, 40/80 mg, SKC Inc, Eighty-four, PA, USA). The filter collected the particles and the adsorbent the vapors. A flow of about 1 l/min was used, and the sampling time was around 8 hours. The flows of the pumps were calibrated prior to the sampling and checked after the sampling.

Analysis of polycyclic aromatic hydrocarbons in air samples

The chemical analysis was performed according to a method developed by Östman et al (10). An internal standard (2,2'-binaphthyl) was added to the filter and to the adsorbent when placed in a Soxhlet extractor. The filter and adsorbent were extracted with 15 ml of acetone:dichloromethane (1:1) during 24 hours in 6-minute cycles. As a keeper 100 µl of dodecane was added to the extracts, which were dried and dissolved in 200 µl of cyclohexane and sonicated for 30 seconds. The extracts were filtered on a column (6 × 0.5 mm) packed with silica gel (0.063–0.20 mm, Kieselgel 60, Merck, Darmstadt, Germany). The gel had previously been heated to 450°C for 24 hours and was subsequently deactivated with 10 weight percent of distilled water. A fraction containing aliphatics, monoaromatics, diaromatics, and also PAH was eluted from the column by the use of 8 ml of cyclohexane. Polar components and possible particles were retained on the column.

The eluate was reduced to 1500 µl, of which 100 µl was injected into an on-line coupled liquid chromatographic-gas chromatographic (LC-GC) system. The PAH fraction was isolated by the use of normal phase liquid chromatography on an aminopropyl column (µ-Bondapak-NH₂, 300 × 3.9 mm Waters Associates, Milford, MA, USA, operated using a model 9001 HPLC chromatograph, Varian, Walnut Creek, CA, USA). Pentane was used as the liquid phase with a flow of 1.0 ml/min. Aliphatics and mono- and diaromatics were eluted, after which the mobile phase flow through the column was reversed and the PAH fraction was eluted as a single peak. By means of a loop-type interface the contents of

this peak were transferred to a gas chromatograph (Model 3700, Varian, Walnut Creek, CA, USA). Separation was subsequently performed using a capillary column (DB-5, 28 m $\times$ 0.32 mm, 25 μ m, J&W Scientific, Folsom, CA USA) and utilizing a flame ionization detector. The temperature program was as follows: 54°C for 2 minutes followed by a linear temperature increase of 10°C/min to 300°C, a temperature that was kept for 10 minutes.

Quantification of the PAH components was performed by calculation using the added internal standard and a standard solution of 20 PAH, including the internal standard. A linear response was demonstrated by the LC-GC system in the range 1–1000 ng of the injected amount of single PAH components, and thus single point calibration was used. Control of the coupled chromatographic LC-GC system, registration of the detector signals, and the calculations were performed by a computerized system (ELDS 900, Chromatography Data Systems AB, Kungshög, Sweden).

The PAH components were identified by gas chromatography-mass spectrometry (GC-MS) (Incus 50, Finnigan, San Jose, CA, USA). A pooled air sample which was 50% of 10 selected sample extracts was cleaned up off-line using the high pressure-liquid chromatographic method already described. The cleaned-up sample was dissolved in 100 μ l cyclohexane:acetone (1:1), of which 1 μ l was injected into the GC-MS system. Individual PAH components were identified by use of the relative retention and mass spectra of the components in a comparison with a standard solution of 20 PAH.

Urinary and blood samples

Urinary samples were taken before and after 2 shifts for the exposed workers. Urine (20–50 ml) was collected in plastic bottles, chilled, and then frozen before transport to the laboratory. One urinary sample was taken from the referents in the afternoon. Venous blood samples were taken early in the morning for both the exposed workers and the referents and were transported to the laboratory the same day.

Analysis of urinary 1-hydroxypyrene

A method modified from Jongeneelen et al (11) was used (12) to determine urinary 1-hydroxypyrene. Urine (15 ml) was adjusted to pH 5.0 with 1 M hydrochloric acid, 6.0 ml of 0.1 M sodium acetate buffer, and 3.0 ml of β -glucuronidase (500 units/ml, Sigma Chemical Co) was added. The sample was hydrolyzed in a water bath at 37°C for 15 hours. The sample was put on an adsorption column (Waters SEP-PAK C₁₈), pretreated with 5 ml of methanol, followed by 10 ml of water. The column was washed with 5 ml of water and eluted with 3 $\times$ 3 ml of methanol. Ten microliters of the eluate was injected in a HPLC with two pumps (Waters Mod 6000 A), a gradi-

ent former (Waters Mod 680), an autoinjector (Waters Mod 710 B), a fluorescence detector (Perkin Elmer LS-4), and an integrator (Spectra Physics Chrom Jet). For quantification an external standard of 1-hydroxypyrene was used (Janssen Chimica). The standard curve was linear between 0.01 and 0.4 μ g/ml. The samples were analyzed on a C₁₈ column (Chromosphere PAH, 150 $\times$ 4.6 mm, Chromopak), with a flow of 0.9 ml/min and a gradient consisting of water and acetonitrile. 1-Hydroxypyrene was detected at an excitation wavelength of 242 nm and an emission wavelength of 388 nm.

Analysis of sister chromatid exchanges in peripheral lymphocytes

The lymphocytes were cultivated for 72 hours with phytohemagglutinin and bromodeoxyuridine. Bromodeoxyuridine was added to a final concentration of 0.000064 g/ml. The suspension of cells was dripped onto an object glass and incubated with Hoechst 33258 for 20 minutes. After 60 minutes of ultraviolet radiation the samples were stained with Giemsa. The number of SCE per cell was determined for 25 cells, and the average was used in the further analysis (13).

Micronuclei in peripheral lymphocytes

MN were determined according to Högstedt (14) and Högstedt et al (15). Lymphocytes from the buffy coat were added to RPMI 1640 and 15% fetal calf serum. Either phytohemagglutinin or pokeweed was used as the mitogen, and the cells were cultivated for 72 hours. One thousand activated lymphocytes from each mitogen were analyzed for each person.

Data analysis

The differences in the geometric means were calculated by the t-test. To calculate the geometric mean of samples taken before or after 2 shifts, the mean of pre- or postshift values was first calculated for each person. In the analysis of the difference in the urinary concentration of 1-hydroxypyrene between Mondays and other days, the difference between the other day and the Monday value was first calculated for each person. All the P-values were 2-tailed.

Results

Polycyclic aromatic hydrocarbons in the workers' breathing zone

Several PAH with 3–4 rings that were methylated derivatives dominated over unsubstituted compounds. PAH compounds with more than 4 rings were detected, but the concentrations were too low to perform a mass spectrometric analysis for identification. The gas phase con-

Table 1. Average concentration of polycyclic aromatic hydrocarbons (PAH) in the breathing zones of 28 workers during paving. The figures cover 55 full-shift measurements. PAH 10 is the sum of the identified PAH. (See the text.)

	Vapor phase ($\mu\text{g}/\text{m}^3$)		Particle phase ($\mu\text{g}/\text{m}^3$)		Total ($\mu\text{g}/\text{m}^3$)	
	Geometric mean	Range	Geometric mean	Range	Geometric mean	Range
Total PAH	1.1	0.2–13.0	1.3	0.02–10.8	2.3	0.2–23.8
PAH 10	0.47	0.02–6.4	0.12	0.02–1.2	0.59	0.02–7.6
Pyrene	0.018	0.004–0.12	0.014	0.004–0.09	0.032	0.004–0.20

tained PAH with 3–4 rings, while the particle-associated phase contained PAH with 3–5 rings. The PAH were calculated as (i) the total amount of PAH detected, (ii) the sum of the identified PAH (PAH10) [phenanthrene, 3-methylphenanthrene, 2-methylphenanthrene, the sum of 4- and 9-methylphenanthrene (coeluting on GC), 1-methylphenanthrene, 3 different dimethylphenanthrenes, fluoranthene and pyrene], and (iii) pyrene in the vapor and particle phase (table 1).

1-Hydroxypyrene in urine

The geometric means of the urinary 1-hydroxypyrene were 0.96 (range 0.04–3.8) $\mu\text{mol}/\text{l}$ preshift and 0.96 (range 0.23–4.0) $\mu\text{mol}/\text{l}$ postshift compared with 0.60 (range 0.14–2.2) $\mu\text{mol}/\text{l}$ (afternoon values) for the referents ($P < 0.01$, t-test, comparing pre- or postshift values with the referents' values). There was no significant increase in 1-hydroxypyrene in the urine between the post- and preshift values [mean 0.00 (SE 0.09) $\mu\text{mol}/\text{l}$]. For the exposed workers the preshift concentration was lower on Monday morning than on the mornings of the other days [mean of difference 0.64 (SE 0.18) $\mu\text{mol}/\text{l}$, $N = 23$, $P < 0.01$].

Sister chromatid exchanges and micronuclei in lymphocytes

There were no significant increases in the SCE or MN between the workers exposed to bitumen fumes and the referents (table 2). There was no significant association between SCE, MN, and employment time in asphalt work in a linear regression model including age as a possible confounder.

Table 2. Sister chromatid exchanges (per cell) and micronuclei per 1000 cells. (PHA = phytohemagglutinin mitogen, PW = pokeweed mitogen)

	Asphalt workers (N=28)		Referents (N=30)	
	Geometric mean	SD	Geometric mean	SD
SCE	8.9	1.3	9.6	1.3
Micronuclei (PHA)	4.1	2.0 ^a	4.5	1.7
Micronuclei (PW)	5.8	1.4 ^a	6.4	1.5

^a Due to technical reasons the micronuclei test failed for one person, $N = 27$.

Discussion

This study indicates that asphalt workers have an increased exposure to PAH. However, there are several sources of PAH, for example, as exhaust from engines, food, and tobacco smoke. Tobacco smoke seems highly improbable in that all the examined asphalt workers were nonsmokers and environmental tobacco smoke from other workers is improbable as the workers were outdoors. The exposure to pyrene was between 4 and 200 (mean 46) ng/m^3 for the asphalt workers; this value is higher than the concentration on streets in Stockholm with heavy traffic, where concentrations between 10 and 30 ng/m^3 have been measured (16). Furthermore, common components of PAH in car exhaust, such as benzo[ghi]perylene and coronene, could not be identified in the air samples taken from the breathing zone of the asphalt workers. Therefore, exhaust from traffic does not seem to be a major source of the PAH exposure in this investigation. The road pavers showed a low 1-hydroxypyrene excretion, as a result of the low exposure. However, they had significantly higher concentrations than the referents. There was no significant difference between the road pavers' post- and preshift samples. Monday morning urine, however, had significantly lower 1-hydroxypyrene concentrations than other weekday mornings, and therefore indicated occupational PAH exposure.

There was a correlation between pyrene in air and 1-hydroxypyrene in urine ($r = 0.4$ for postshift urine samples and $r = 0.6$ for urine samples taken the morning after the air samples). Thus the increased excretion of 1-hydroxypyrene in asphalt workers was probably due to inhaled pyrene rather than to pyrene ingested by food.

The inhaled PAH may come from the asphalt fumes of other sources. The pattern of PAH indicated that exhaust from traffic was not the major source of PAH. All the workers were outdoor nonsmokers, and therefore tobacco smoke could be excluded as a major cause of their PAH exposure. Thus asphalt fumes are the most probable source of their PAH exposure. Bitumen is a major component in asphalt, and it contains PAH. There was no coal tar in the asphalt. Thus it is probable that the asphalt workers' increased exposure to PAH was mainly from bitumen fumes.

The measurements were not selected by random, but instead according to the period of study and the region in which the study was performed. Thus the levels may not be totally representative of exposure to bitumen fumes in the entire country. There was no previous knowledge of the exposures influencing the selection of the workplaces. The temperature of the asphalt strongly influences exposure. In Sweden there is one major supplier of asphalt; therefore the instructions for asphalt temperature are similar and differences in asphalt type form a rather improbable cause of regional differences. Thus we believe that these measurements represent an estimate of the exposure during ordinary road paving in Sweden around 1990.

The exposure to PAH measured as total PAH was between 0.2 and 23.8 $\mu\text{g}/\text{m}^3$, which is 1 to 100 times higher than the average concentration on the streets in Stockholm with heavy traffic (16). The average concentration of 1-hydroxypyrene in urine was twice the concentration of the referents. A similar ratio was found by Burgaz et al (8) in nonsmokers exposed to bitumen fumes. Workers exposed to coal tars have considerably higher concentrations of 1-hydroxypyrene in urine (17).

There was no difference in the SCE or MN tests between the workers exposed to bitumen fumes and the referents; yet the former had a higher excretion of 1-hydroxypyrene in urine. However, these tests may have too low a sensitivity to detect a difference at the exposure levels to which these asphalt workers were exposed. Furthermore, 1-hydroxypyrene is a metabolite from a nongenotoxic component of PAH and the concentration and uptake of other PAH may not follow those of pyrene. In vitro studies have shown that condensates from fumes of roofing asphalt may cause the induction of micronuclei (18). Our results are in accordance with those of a study of German road pavement workers exposed to bitumen fumes (19). There was no increase in DNA (deoxyribonucleic acid) strand breaks in peripheral mononuclear blood cells among the road pavers.

The risk of an increased inhalation of PAH from bitumen is difficult to estimate. Some studies indicate an increased risk of cancer for workers exposed to asphalt fumes, but it is probable that the exposure to PAH was much higher when coal tar was a common component in asphalt (1). The concentrations of PAH from coal tar pitch are some orders of magnitude higher than those from bitumen (20). There is no obvious correlation between the total concentration of PAH in bitumen fumes from paving asphalt and mutagenicity; yet, as several PAH cause cancer, exposure to PAH should be as low as feasible. The size of the cancer risk can be estimated from epidemiologic studies, and a collaborative international study is in progress in Europe.

In conclusion, this study shows that road pavers in Sweden have an increased exposure to PAH due to ex-

posure to bitumen fumes, but the sensitivity of 2 genotoxic tests, SCE and MN, seem to be too low to detect any effects in exposed nonsmokers.

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