RESEARCH COMMUNICATION

Cytogenetic Biomonitoring of Road Paving Workers Occupationally Exposed to Polycyclic Aromatic Hydrocarbons

Sudha Sellappa*, Bhuvaneswari Mani, Kripa S Keyan

Abstract

Road pavement workers are exposed to many known carcinogens in their complex occupational environment. The study makes an attempt to investigate exposure to polycyclic aromatic hydrocarbons (PAH) from the bitumen fumes among the road pavement workers engaged in different pavement sites at Coimbatore, Tamil Nadu and to thereby determine the genotoxic effects associated with it. The study included 36 road pavers and 37control subjects with similar mean ages, smoking prevalence and alcohol consumption and was analyzed for DNA damage in blood leucocytes by Micronucleus assay (MN) and the Comet assay. The mean urinary 1-OHP concentration in road pavers (1.68 ± 0.93) was significantly higher than in controls (0.55 ± 0.42). The results of MN test and comet assay showed that the mean micronuclei rate in workers was significantly higher than those in controls (P <0.05). The results of our study indicated that the genetic damage was detectable in road paving workers occupationally exposed to bitumen and also demonstrate the high sensitivity of comet assay to assess early oxidative effects induced by exposure to bitumen fumes at low doses and confirm the suitability of urinary 1-OHP as a biomarker of PAH exposure.

Keywords: Bitumen - micronucleus assay – genotoxicity - comet assay

Asian Pacific J Cancer Prev, **12**, 713-717

Introduction

Millions of workers in a variety of occupational settings have the potential to be exposed to hazardous substances. They can be present in the occupational environment in the form of gases, vapors, fumes and particles (Keshava et al., 1999). Occupational exposure of paving workers is still poorly characterized. This category of workers performs different tasks, including bitumen asphalt preparation and road paving, that chronically expose workers to polycyclic aromatic hydrocarbons (PAHs) both by inhalation and dermal contamination (McClean et al., 2004).

Workers employed in the pavement are potentially exposed to bitumen fumes either during production or laying of asphalt. Bitumen a dark, semisolid material of natural origin or a by-product of oil refining, a common binder used in road construction and its emulsions are often sprayed onto the road surface prior to paving (IARC, 1985).

The road pavers are exposed to asphalt fumes containing relatively low levels of PAHs, but there is a possibility of long-term health effects following chronic exposure by inhalation or skin contamination. The majority of situations involve exposures to low doses for long periods, which in many cases involve the individual's entire lifetime and result in small increments in health risks.

Tar bitumens are increasingly being used as a binder in road works and are applied hot. They consist of a standard product of about 70% bitumen and 25-30% tar. During hot application of bitumen, complex mixtures of aerosols and vapors are emitted, which contain PAHs and their derivatives as well as other compounds many of which are either known or suspected to be human carcinogens (IARC, 1985). The risk of cancer to people inhaling such substances has been established.

Once the substance enters the body, the parental substance or its metabolites are searched for in the urine, blood, feces, other bodily fluids and tissues. Since parental PAH generally present a reduced plasma half-life, hydroxyl metabolites are the most frequent option for investigation. Metabolites excreted in the urine provide more appropriate estimates of total ingestion as compared to exposure assessments based on environmental data. 1-hydroxypyrene (1-OHP) is the most widely used metabolite in PAH exposure, since pyrene is one of the most abundant hydrocarbons in all PAH mixtures (Jacob et al., 2002; Jongeneelen et al., 1997; Sorensen et al., 2003) thus representing a sensitive biomarker of exposure, recommended by various authors as the most relevant parameter in estimates of individual exposure to PAHs (Hansen et al., 2004).

Some of PAH are tumorigenic due to their metabolites and their ability to generate DNA adducts and oxidative DNA damage through the production of reactive oxygen species during metabolism (Jongeneelen et al., 1997).

*Department of Biotechnology, Karpagam University, Coimbatore, Tamilnadu, India *For correspondence: sudhasellappa@yahoo.co.in*

Bitumen may lead to the formation of DNA adducts (Schoket et al., 1988a,b; Genevois et al., 1996). A review indicates that there may be an increased risk of cancer among workers exposed to fumes from bitumen and other components of asphalt (Partanen et al., 1994).

Several epidemiological studies on pavers showed the presence of respiratory diseases and possible association between lung cancer risk and asphalt fume exposure (Partanen et al., 1995; Boffetta et al., 1997; Boffetta et al., 2003), as reported also by IARC (2001), which confirmed an excess of lung cancer in this occupational category. Though some studies have described an excess risk of cancer among asphalt-exposed workers, there is currently insufficient evidence on the genotoxic risk for bitumen exposed workers. To establish a causal relationship between occupational bitumen exposure and cancer risk only little is known regarding the carcinogenic hazard of bitumen exposure, especially taking into account whether emissions of bitumen at the workplace can cause cancer. The principal limitation is the lack of a correct exposure assessment, as workers are not continuously involved in work and also due to the high variability of exposure conditions characterizing the paving activity influenced by environmental factors (wind, heat, etc.) associated with different climatic conditions (Cavallo et al., 2006). Occupational exposure to carcinogens is of great public health concern. Thus, it is of interest to study whether the present occupational exposure of bitumen represents an increased risk of genotoxic damage. To add further knowledge to the genetic risk related to bitumen exposure, we applied the micronucleus (MN) assay and Comet assay to evaluate the biomarkers of early biological effects such as genotoxicity and DNA damage in bitumen exposed workers.

The comet assay has been found to be a very sensitive method for measuring DNA damage (Schmid, 1975). To substantiate our results and to provide a cytogenetic parameter, the MN test was also carried out. This test allows the detection of both clastogenic and aneugenic agents (Salama et al., 1999). The influence of confounding factors like, smoking, alcohol drinking and duration of exposure on the differences in DNA damage was also analyzed.

Materials and Methods

Selection of subjects and collection of specimens

A total of 73 male subjects (36 road pavers and 37controls) were analyzed in this study. Experimental group were employed in road paving works in and around Coimbatore, South India. The road paving workers had varying durations of exposure (5-15 years) and they were in the age group of 26-59 years. All workers were engaged in mixing and paving. The experimental group was further branched as smokers (20) and non-smokers (16). The control group was selected from the general population with no history of occupational exposure to toxic fumes or any known physical or chemical agent in the workplace, but belonged to the same age group and socio-economic status as the road paving workers. The selection criteria for subjects were based on a questionnaire. The questionnaire covered standard demographic questions (age, genetic disorders, number of X-ray diagnoses, vaccinations, medication, smoking, alcohol, etc.) and occupational questions (per day and years of exposure). We ensured that the workers and the controls did not markedly differ from each other except for occupational exposure. We also ensured that all subjects had not been taking any medicines nor had they been exposed to any kind of radiation for 12 months before sampling. The subjects who smoked >5 cigarettes/day at least for 1 year were considered as smokers and those who consumed >120gm of alcohol/day were considered as alcoholics in both groups. All subjects were informed of the objective of the study and gave their consent. The study was conducted in accordance with the principles for human experience as defined by the Helsinki Declaration.

All pavers under study wore only safety shoes, as protective equipment and not gloves or disposable respirators. The exposure assessment was carried out in different paving sites in and around Coimbatore, from June to December 2010. Biological monitoring of the exposure and evaluation of effects in paving workers were performed on samples of urine and blood collected simultaneously in the same working week.

Urinary 1-Hydroxypyrene analysis

Urine samples were collected at the end of the work shift at 5 p.m., and they were kept cold (±4°C) during collection and with 20% glycerol (volume for volume) to minimize cell loss due to lysis after freezing (–20°C). Urine samples were analyzed for 1-hydroxypyrene according to the method developed by Jongeneelen et al (1987). In brief, the urine was acidified and the glucuronic acid and sulfate were enzymatically removed. Creatinine levels were used to estimate urinary dilution using a colometric test, based on the Jaffé reaction between creatinine and sodium picrate, or analyzed according to an enzymatic method described by Mazzachi et al., (2000). Urinary 1-hydroxypyrene was expressed as micromoles per mole of creatinine.

Venous blood (3ml) was collected from all subjects using heparinized syringes. The samples were transported on ice to the laboratory and were processed. Micronucleus assay and Comet assay were performed using the collected blood samples.

Micronucleus Assay: Cytokinesis-block micronucleus (CBMN) assay was conducted according to the method described by Fenech and Morley (1985). Leukocyte cultures were set up by adding 0.5 ml whole blood to 4.5 ml RPMI 1640 medium supplemented with 15% heat-inactivated fetal calf serum, 1% antibiotics (penicillin and streptomycin) and L-glutamine (all obtained from Gibco). Leukocytes were stimulated by 1% phytohaemagglutinin (PHA; Gibco) and incubated for 72 h at 37°C. A final concentration of 6 μg/ml cytochalasin B (Sigma) was added to the cultures 44 h later to arrest cytokinesiss. At 72 h of incubation, the cultures were harvested by centrifugation at 1000 rpm for 8 min and treated with a hypotonic solution (2–3 min in 0.075 M KCl at 4°C). Cells were centrifuged thereafter and a 3:1 (v/v) methanol:

Table 1. Demographic Features of the Study Population and Mean Urinary 1-OHP Concentrations Among Paving Workers and Controls

Characteristics			Sample Size (n=73)	Age (Mean ± SD)	Duration (yrs) (Mean ± SD)	Cigarettes daily (Mean ± SD)	Frequency (EtOHgm/day)	Urinary 1-OHP[1]
Exposed	Total		36	43.8 ± 9.37	11.08 ± 3.31	-	-	1.68 ± 0.93
	Smoking	Yes	20 (55.56%)	43.6 ± 9.67	11.06 ± 3.39	15.2 ± 7.03	-	2.09 ± 1.23*
		No	16 (44.44 %)	44.1 ± 9.31	10.44 ± 3.18	-	-	1.13 ± 0.95*
	Alcohol	Yes	19 (52.78 %)	45.7 ± 8.60	11.16 ± 3.06	-	251.3 ± 91.5	1.63 ± 0.81*
		No	17 (47. 22%)	41.7 ± 9.98	11.00 ± 3.66	-	-	1.47 ± 0.50*
Controls	Total		37	42.9 ± 9.12	-	-	-	0.55 ± 0.42
	Smoking	Yes	19 (51.35%)	44.7 ± 9.62	-	16.6 ± 5.72	-	0.75 ± 0.46
		No	18 (48.65%)	40.9 ± 8.40	-	-	-	0.35 ± 0.33
	Alcohol	Yes	20 (54.05%)	43.1 ± 10.4	-	-	288.4 ± 55.2	0.54 ± 0.73
		No	17 (45.95%)	41.5 ± 8.25	-	-	-	0.50 ± 0.09

[1]μmol/mol creatinine; *P <0.05

acetic acid solution was gently added. This fixation step was repeated twice and the resulting cells were resuspended in a small volume of fixative solution and dropped onto clean slides. The samples were then applied to clean microscope slides. Smears were air dried and fixed in methanol: acetic acid (3:1). Slides were stained with May-Grunewald Giemsa method (Sigma St Louis MO). The MN analysis was done with a light microscope, at x 100 magnification, using coded slides. 1000 cells from each individual were examined.

Comet assay: An aliquot of 40 μl of whole blood was used to quantitate basal DNA damage using Comet assay, which was carried out according to Singh et al (1988). Cell viability was determined by the trypan blue dye exclusion method (Pool Zobel et al., 1994) ranged from 90 to 95% (data not shown). Slides were prepared in duplicate per person. The cell suspension was centrifuged, the pellet obtained was mixed with 0.7% low melting agarose (LMA) and placed on fully frosted roughened slides previously coated with 1% normal melting point agarose. To the solidified agarose, a third layer of 0.1% LMA was applied and were immersed in freshly prepared ice cold lysis solution for 1 h. The slides were then electrophoresed, neutralized, dried and stained with ethidium bromide. A total of 100 randomly captured comets from each slide were examined at 400X magnification using an epifluorescence microscope (Zeiss) connected through to an image analysis system (Comet Assay II; Perceptive Instruments Ltd, UK). An undamaged cell resembles an intact nucleus without a tail and a damaged cell has the appearance of a comet. The length of the DNA migrated in the comet tail, which is an estimate of DNA damage, was measured. The evaluated tail length (length of DNA migration) is related directly to the DNA fragment size and presented in micrometers.

Statistical Analysis

The samples were coded at the time of preparation and scoring. They were decoded before statistical analysis for comparison. Mean and standard deviation (SD) were calculated for each biomarker. The significance of the differences between control and exposed end-point means were analyzed using Student's t-test. Mean values and standard deviations were computed for the scores and the statistical significance (P<0.05) of effects (exposure, smoking and alcohol) was determined.

Results

The demographic characteristics of the study subjects are presented in Table 1. The age, alcohol consumption and smoking status distributions were similar among exposed workers and controls. Among the smokers, the years of smoking and daily cigarette consumption were similar in the two groups.

The road pavers displayed a mean urinary 1-OHP concentration (1.68 ± 0.93 μmol mol-1 of creatinine) which was significantly higher ($p < 0.05$) than the mean (0.55 ± 0.42 μmol mol-1of creatinine) shown by the controls (Table 2). In smokers, there was a significant increase ($p < 0.05$) in the mean urinary 1-OHP levels in workers than controls. Similarly alcoholics also exhibited an increase in mean urinary 1-OHP levels in workers when compared to their controls. Workers who are non smokers and non alcoholics also showed a significant increase ($p < 0.05$) in the mean urinary 1-OHP levels than controls.

The frequency of micronuclei (MN) was studied in 36 pavers and in 37 controls. Pavers revealed a significant induction of MN when compared with controls ($P < 0.05$). Individuals of the exposed as well as control groups with smoking habit and alcohol consumption showed an enhanced frequency of micronuclei when compared to non smokers and non alcoholics (Table 2).

Bitumen exposed workers showed an increased MN frequency with an increase in duration of work (P<0.05). A marginally significant correlation was observed between

Table 2. Micronucleus Frequency and DNA Mean Tail Length (μm) with Respect to Smoking Habit and Alcohol Consumption in Controls and Pavers

Characteristics			N=73	MN (Mean ± SD)	Mean Comet tail (μm)
Controls (n=37)	Smoking Status	Yes	19	4.31 ± 1.29	13.3 ± 3.74
		No	18	3.10 ± 0.76	10.9 ± 2.85
	Alcohol Status	Yes	20	4.15 ± 0.88	11.1 ± 2.92
		No	17	3.05 ± 0.05	9.9 ± 2.83
Workers (n=36)	Smoking Status	Yes	20	5.70 ± 1.08*	19.4 ± 4.99*
		No	16	4.06 ± 0.93*	15.5 ± 4.94*
	Alcohol Status	Yes	19	5.26 ± 0.04*	16.2 ± 2.03*
		No	17	4.94 ± 0.83*	15.1 ± 3.12*
	Years of Exposure	≤10	16	5.39 ± 0.83	17.3 ± 2.70
		>10	20	5.75 ± 1.20	16.8 ± 4.28

*P<0.05

MN induction and duration of exposure in workers (Table 2).

Basal DNA damage (BDD) was studied in a total of 36 subjects using the Comet assay. The results of DNA damage are given in Table 2. In workers a significant increase ($P < 0.05$) in DNA mean tail length indicating BDD was observed when compared with controls. In exposed group, a significant difference was observed between smokers and non-smokers and between alcohol drinkers and never drinkers in relation to DNA migration ($P < 0.05$). DNA damage was significantly higher in subjects with a longer duration of work ($P < 0.05$).

Discussion

Occupational exposure to bitumen fumes emitted during hot application of asphalt carries the risk of exposure to hydrocarbon compounds and also a significant amount of polycyclic aromatic hydrocarbons (PAH) which constitutes a major threat to human health (IARC, 1985).

To predict total internal exposure to PAH we used urinary 1-OHP as internal biomarker of occupational exposure. A higher concentration of 1-OHP was observed in workers and hence our study indicates that bitumen exposed workers have an increased exposure to PAH.

A study among mastic asphalt workers exposed to fumes and aerosols of bitumen and PAH, reveals an increased DNA damage in blood leucocytes. An increased excretion of 1-hydroxypyrene was found in workers exposed to bitumen fumes (Burgaz et al., 1992).

However, there are several sources of PAH, for example, as exhaust from engines, food, and tobacco smoke. Most of the smokers had higher urinary level of 1-OHP than non-smokers (Dor et al., 2000). Our results are in line with the above observation where in the road pavers with smoking habit showed a high level of 1-OHP in urine.

The use of biological markers to determine the extent of prior exposures to a specific chemical and to predict future disease outcome holds great promise. Exploration of correlations between biomarkers will contribute to the development of human biomonitoring to genotoxic exposures and will help to select optimal biomarkers for more efficient monitoring of various human exposures (Erika et al., 2008).

Condensates of bitumen fume can induce DNA damage both in vitro and in vivo (Machado et al., 1993) and are also capable of inducing micronucleus formation and chromosomal aberrations in cultured cells (Qian et al., 1999). In particular an increase in sister chromatid exchange (SCE) and MN formation was reported by Burgaz et al (1998) in lymphocytes of road paving workers. The present study also observed an increase in MN frequency among the bitumen exposed workers. The variation in MN frequency observed within the exposed groups, characterized by the high values of the standard deviation, must be due to the fact that the response to a genotoxic agent is different from person to person.

Concerning the effect of smoking on MN frequency, the data reported in biomonitoring studies are contradictory (Au et al., 1991; Migliore et al., 1991; Tomanin et al., 1991; Norppa et al., 1993; Van Hummelen et al., 1993; Bolognesi et al., 1997; Barale et al., 1998). In our study a significant difference among smokers and non-smokers with regard to MN were found in exposed and non-exposed groups.

Alcohol use can increase the number of micronuclei (Dittberner et al., 1997). Bishop et al. (1997) mentioned that alcohol does not induce mutations in mammalian cells in vitro, whereas in vivo it induces a variety of genetic effects, including sister chromatid exchange and the production of micronuclei. In the present work, an increase in MN frequency was observed between exposed and non exposed groups with and without drinking habit, that may indicate the existence of an influence of alcohol use on the micronuclei formation of exposed individuals.

Toraason et al. (2001, 2002) reported significant DNA strand breaks in leukocytes of bitumen exposed asphalt workers using the comet assay. Similarly a slight increase of DNA strand breaks was reported by Fuchs et al. (1996) in Germany. Our findings are consistent with the above reports.

In conclusion, our findings indicate that road pavers exposed to fumes and aerosols of bitumen contribute to an increase in micronuclei and increased DNA damage. These workers may not be aware of the genotoxic agents they are exposed nor the type and amount of agent to which they have been exposed. Hence, there is a need to educate them about the potential hazard of occupational exposure and the importance of using protective measures. In general, the introduction of comprehensive exposure control measures in road paving is a challenging task but can be attained to an extent by the use of protective equipment such as gloves, safety shoes, and disposable respirators throughout the work shift.

References

Au WW, Walker DM, Ward JB, et al (1991). Factors contributing to chromosome damage in lymphocytes of cigarette smokers. *Mutat Res*, **260**, 137-44.

Barale R, Chelotti L, Davini T, et al (1998). Sister chromatid exchange and micronucleus frequency in human lymphocytes of 1,650 subjects in an Italian population: II. Contribution of sex, age and lifestyle. *Environ Mol Mutagen*, **31**, 228-42.

Bishop JB, Witt KL, Sloane RA (1997). Genetic toxicities of human teratogens. *Mutat Res*, **396**, 9-43.

Bitumens. Polynuclear aromatic compounds, part 4. Bitumens, coaltars and derived products, shale oils and soots. In: IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, (1985) 35. Lyon: IARC, 39 – 81.

Boffetta P, Burstyn I, Partanen T, et al (2003) Cance rmortality among European asphalt workers: an international epidemiological study. Results of the analysis based on job titles. Am J Ind Med, 43: 18–27.

Boffetta P, Jourenkova N, Gustavsson P (1997) Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons. Cancer Causes Control, 8, 444–472.

Bolognesi C, Merlo F, Rabboni R, Valerio F, Abbondandolo A (1997). Cytogenetic biomonitoring in traffic police workers: micronuclei test in peripheral blood lymphocytes. *Environ. Mol Mutagen*, **30**, 396-402.

Burgaz S, Bonn P, Jongeneelen F (1992). Biological monitoring of exposure to bitumen fumes during road paving operations. *Arch Toxicol* Suppl, **15**, 278-81.

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

Cavallo D et al. (2006). Sister chromatid exchange and oxidative DNA damage in paving workers exposed to PAHs. *Ann Occup Hyg*, **50**, 211-8.

Dittberner U, Schmetzer B, Gölzer P, Eisenbrand G, Zankl H (1997). Genotoxic effects of 2-trans-hexenal in human buccal mucosa cells in vivo. *Mut Res*, 390, 161-5.

Dor F, Haguenoer JM, Zmirou D, et al (2000). Urinary 1-hydroxypyrene as a biomarker of polycyclic aromatic hydrocarbons exposure of workers on a contaminated site: influence of exposure conditions. *J Occup Environ Med*, **42**, 391-7.

Erika G, Livia A, Katalin K, Peter R, Bernadette (2008). Correlation between biomarkers of human exposure to genotoxins with focus on carcinogen-DNA adducts. *Mutagenesis*, **23**, 1-18.

Fenech M, Morley AA, (1985) Measurement of micronuclei in lymphocytes. *Mutat Res*, **147**, 29-36.

Fuchs J, Hengstler JG, Boettler G, Oesch F (1996). Primary DNA damage in peripheral mononuclear blood cells of workers exposed to bitumen-based products. *Int Arch Occup Env Hlth*, **68**, 141-6.

Genevois C, Brandt H, Bartsch H, et al (1996). Formation of DNA adducts in skin, lung and lymphocytes after skin painting of rats with undiluted bitumen or coal-tar fume condensates. *Polycyclic Aromatic Compounds*, **8**, 75-92.

Hansen AM, Wallin H, Binderup ML, et al (2004). Urinary 1-hydroxypyrene and mutagenicity in bus drivers and mail carriers exposed to urban air pollution in Denmark. *Mutat Res*, **557**, 7-17.

International Agency for Research on Cancer (1985). Monographs on the evaluation of the carcinogenic risk of chemicals to humans: Polynuclear aromatic compounds, Bitumens, coal tars, derived products, shale-oils, and soots, Lyon: IARC, 35, 39-81.

International Agency for Research on Cancer (2001). IARC epidemiological study of cancer mortality among European asphalt workers. IARC Internal Report No.01/003. Lyon: IARC

Jacob J, Seidel A (2002). Biomonitoring of polycyclic aromatic hydrocarbons in human urine. *J Chromatogr B Analyt Technol Biomed Life Sci*, **778**, 31-47.

Jongeneelen FJ (1997). Methods for routine biological monitoring of carcinogenic PAH-mixtures. *Sci Total Environ*, **199**, 141-9.

Jongeneelen FJ, Anzion RB, Henderson PT (1987). Determination of hydroxylated metabolites of polycyclic aromatic hydrocarbons in urine. *J Chromatogr*, **413**, 227-32.

Keshava N, Ong TM (1999). Occupational exposure to genotoxic agents. *Mutat Res*, **437**, 175-219.

Machado ML, Beatty PW, Fetzer JC, Glickman AH, McGinnis EL (1993). Evaluation of the relationship between PAH content and mutagenic activity of fumes from roofing and paving asphalts and coal tar pitch. *Fundam Appl Toxicol*, **21**, 492-9.

Mazzachi BC, Peake MJ, Ehrhardt V (2000). Reference range and method comparison studies for enzymatic and Jaffe creatinine assays in plasma and serum and early morning urine. *Clin Lab*, **46**, 53-5.

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

Migliore L, Parrini M, Sbrana I, Biagini C, Battaglia A, Loprieno N (1991). Micronucleated lymphocytes in people occupationally exposed to potential environmental contaminants: the age effects. *Mutat Res*, **256**, 13-9.

Norppa H, Luomahaara S, Heikanen H, et al (1993). Micronucleus assay in lymphocytes as a tool to biomonitoring human exposure to aneuploidigens and clastogens. *Environ Health Perspect*, **101**, 139-43.

Partanen TJ, Boffetta P (1994). Cancers risk in asphalt workers and roofers: review and meta-analysis of epidemiologic studies. *Am J Ind Med*, **26**, 721-740.

Partanen TJ, Boffetta P, Heikkila PR et al. (1995) Cancer risk for European asphalt workers. *Scand J Work Environ Health*, **21**, 252-8.

Pool Zobel BL, Lotzmann N, Knoll M, et al (1994). Detection of genotoxic effects in human gastric and nasal mucosa cells isolated from biopsy samples. *Environ Mol Mutagen*, **24**, 23-45.

Qian H, Whong W, Olsen L, Nath J, Ong T (1999). Induction of micronuclei in V79 cells by fractions of roofing asphalt fume condensate. *Mutat Res*, **441**, 163–170.

Salama SA, Serrana M, Au WW (1999). Biomonitoring using accessible human cells for exposure and health risk assessment. *Mutat Res*, **436**, 99-112.

Schmid W (1975). The micronucleus test. *Mutat Res*, **31**, 9-15.

Schoket B, Hewer A, Grover PL, et al (1988a) Formation of DNA adducts in human skin maintained in short-term organ culture and treated with coal-tar, creosote or bitumen. *Int J Cancer*, **42**, 622–626.

Schoket B, Hewer A, Grover PL, et al (1988b). Covalent binding of components of coal-tar, creosote and bitumen to the DNA of the skin and lungs of mice following topical application. *Carcinogenesis*, **9**, 1253-8.

Singh NP, Mc Coy MT, Tice RR, Schneider EL (1988). A simple technique for quantization of low levels of DNA damage in individual cells. *Exp Cell Res*, **175**, 184-91.

Sorensen MAH, Moller P, Hertel O, et al (2003). Linking exposure to environmental pollutants with biological effects. *Mutat Res*, **544**, 255-71.

Tomanin K, Ballarin C, Nardini B, Mastrangelo G, Sarto F (1991). Influence of smoking habits on the frequency of micronuclei in human lymphocytes by the cytokinesis block method. *Mutagenesis*, **6**, 123-6.

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 Env Hlth*, **74**, 396-404.

Toraason M, Hayden C, Marlow D, et al (2002). DNA strand breaks, oxidative damage, and 1-OH pyrene in roofers with coal-tar pitch dust and/or asphalt fume exposure. *Int Arch Occup Env Hlth*, **75**, 279-82.

Van Hummelen P, Gennart JP, Buchet JP, Lauwerys R, Kirsch-Volders M (1993). Biological markers in PHA exposed workers and controls. *Mutat Res*, **300**, 231-9.