

# RISK ASSESSMENT OF WORKERS EXPOSED TO FUMES OF BITUMEN: GENOTOXIC EFFECTS AND URINARY METABOLITES OF NAPHTHALENE, PHENANTHRENE, AND PYRENE



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## INTRODUCTION

### BACKGROUND

- Bitumen is a complex mixture of hydrocarbons consisting of both aliphatic and aromatic compounds, e.g. polycyclic aromatic hydrocarbons (PAH).
- There is inadequate evidence of carcinogenicity of bitumen in humans. Previous studies cannot be interpreted due to major qualitative or quantitative limitations.
- Overall, bitumen is labelled as „high priority substance“ for future evaluation by the International Agency for Research on Cancer.

### OBJECTIVE

- Determination of genotoxic properties of exposure to fumes of bitumen in humans.
- Generation of dose-response relationships which can help to establish health-based threshold limit values for exposed workers.

## METHODS

### STUDY DESIGN

- Cross-sectional and cross-shift study design.

### STUDY SUBJECTS

- 202 bitumen-exposed mastic asphalt workers and 55 construction workers without exposure to bitumen were examined (Table 1).

Table 1: Characteristics of the study groups

	Reference group n = 55	Bitumen-exposed workers n = 202
Age (years; median, range)	37 (19-61)	40 (17-63)
Current smoking (n, %)	23 (41.8)	132 (65.7)
German nationality (n, %)	48 (87.3)	136 (67.7)
Duration of exposure in the current company (years; median, interquartile range)	6.5 (3-10)	8 (3-14)
Exposure to bitumen fumes and aerosols (mg/m <sup>3</sup> ; median, interquartile range)	--	3.7 (1.7-7.1)

### ANALYTICAL PARAMETERS

Exposure was assessed using urinary 1-hydroxypyrene (1-OHP), the sum of 1-, 2+9-, 3-, 4-hydroxyphenanthrene (OHPH) and 1- and 2-naphthol (NOL). Genotoxic effects in white blood cells were determined with non-specific DNA adduct levels of 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxodGuo) and the formation of DNA strand breaks as well as alkali-labile sites.

### STATISTICAL MODEL

- Linear mixed model with log-transformed study variables and control of confounders (SAS Software).
- Implemented in the model are independent fixed factors (time of measurements, smoking status, ethnicity) and a random factor (participants).
- Age is included in the model as a continuous independent variable.
- Results presented are means adjusted for the set of potential confounders and F-tests (P-values) for the exposure effect.

## RESULTS

Personal air monitoring of exposed persons revealed a median shift concentration of fumes of bitumen of 3.7 mg/m<sup>3</sup>. Concentrations of fumes of bitumen showed a moderate association with 1- and 2-naphthol ( $r_s=0.18$ ,  $P<0.01$ ), 1-, 2+9-, 3- and 4-hydroxyphenanthrene ( $r_s=0.36$ ,  $P<0.001$ ) and 1-hydroxypyrene ( $r_s=0.25$ ,  $P<0.001$ ) after shift (Table 2). Significantly increased 8-oxodGuo adduct levels were observed after shift in exposed and non-exposed persons ( $P<0.0001$ , Figure 1). However, decreased DNA strand break frequencies were observed after shift in both groups ( $P<0.05$ , Fig. 1). Significantly higher levels of DNA damage were found in exposed workers compared with reference subjects (Figure 1). DNA strand break frequencies after shift were only moderately associated with NOL ( $r_s = 0.20$ ,  $P = 0.01$ ) and 1-OHP ( $r_s = 0.19$ ,  $P = 0.01$ ) in urine after shift at the group level (Table 2).

Table 2: Spearman rank correlations between exposure to fumes of bitumen, urinary metabolites, 8-oxodGuo and Olive tail moment

Parameter	Independent variables	Preshift			Postshift			Shift difference <sup>a</sup>		
		n	r <sub>s</sub>	P	n	r <sub>s</sub>	P	n	r <sub>s</sub>	P
Exposure to fumes of bitumen (mg/m <sup>3</sup> )	1-OHP [ng/L]				199	0.26	<0.001			
	Sum of five OHPHs [ng/L]				199	0.36	<0.001			
	Sum of two NOLs [μg/L]				190	0.18	0.01			
	8-oxodGuo/10 <sup>6</sup> dGuo				201	-0.02	0.76			
	Olive tail moment (median)				202	0.00	0.97			
1-OHP (ng/L)	Sum of five OHPHs [ng/L]	198	0.73	<0.001	199	0.76	<0.001	195	0.73	<0.001
	Sum of two NOLs [μg/L]	188	0.55	<0.001	188	0.54	<0.001	182	0.51	<0.001
	8-oxodGuo/10 <sup>6</sup> dGuo	196	-0.01	0.94	198	0.05	0.46	192	0.01	0.90
	Olive tail moment (median)	197	-0.05	0.52	199	0.17	0.01	194	0.22	<0.001
Sum of five OHPHs (ng/L)	Sum of two NOLs [μg/L]	188	0.69	<0.001	188	0.67	<0.001	182	0.69	<0.001
	8-oxodGuo/10 <sup>6</sup> dGuo	196	0.05	0.50	198	0.00	0.97	192	0.02	0.81
	Olive tail moment (median)	197	-0.02	0.74	199	0.01	0.84	194	0.12	0.09
	Sum of two NOLs [μg/L]	188	0.06	0.42	189	0.08	0.25	183	-0.02	0.79
8-oxodGuo/10 <sup>6</sup> dGuo	Olive tail moment (median)	189	0.06	0.45	190	0.06	0.40	185	0.20	0.01
	Olive tail moment (median)	199	-0.19	0.01	201	0.08	0.28	198	0.02	0.73

<sup>a</sup> Calculated as postshift minus preshift levels. Abbreviations: 1-OHP, 1-hydroxypyrene; OHPH, hydroxyphenanthrene; NOL, 1- and 2-naphthol

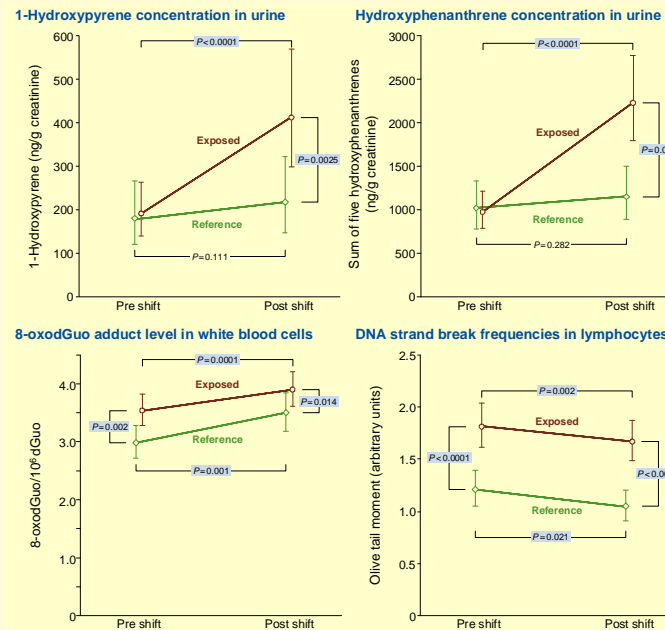


Figure 1: Biomarker levels before and after shift presented as adjusted geometric means and 95% confidence intervals.

- Concentration of 1-hydroxypyrene in urine of workers;
- Sum of five hydroxyphenanthrene concentrations in urine of workers;
- 8-oxodGuo adduct level in white blood cells of workers;
- Olive tail moment in the lymphocyte DNA of workers.

## CONCLUSIONS

- ▶ Exposure to fumes of bitumen results in excretion of 1-OHP, five isomers of OHPH and two isomers of NOL in urine after the shift.
- ▶ The excretion of urinary metabolites was dependent on concentrations of fumes of bitumen. Therefore, 1-OHP, the sum of five OHPH and the sum of NOL are suitable biomarkers to assess exposure to fumes of bitumen.
- ▶ Exposed mastic asphalt workers had higher levels of oxidative DNA damage (8-oxo-dGuo) and higher frequencies of DNA strand breaks in both pre shift and post shift blood samples compared to non-exposed individuals. Consequently these workers show increased biomarkers of genotoxicity.
- ▶ Increases in oxidative DNA damage during the shift were of statistical significance but did not depend on external exposure. Therefore, 8-oxo-dGuo is capable to assess oxidative DNA damage but it is not specific of exposure to fumes of bitumen.
- ▶ Decreases of DNA strand breaks (not significant) were observed after shift in both study groups. Additionally, associations between DNA strand break frequency and PAH metabolites of pyrene and naphthalene in urine after shift were observed.
- ▶ Due to an only weak association between 1-OHP and DNA strand breaks the reasons for increased DNA damage in workers exposed to fumes of bitumen remain unclear.