Genotoxic Effects in Mastic Asphalt Workers Exposed to Bitumen

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BACKGROUND

➔ Chemically bitumen is a complex mixture of hydrocarbons consisting of both aliphatic and aromatic compounds, e.g., polycyclic aromatic hydrocarbons (PAH).

➔ According to IARC, there is *inadequate evidence* of carcinogenicity of bitumen in humans. Studies cannot be interpreted as showing either the presence or absence of a carcinogenic effect because of major qualitative and quantitative limitation.

➔ Currently an integrated research program is carried out by IARC (epidemiology), Fraunhofer-ITEM (animal experiments) and BGFA (irritative and genotoxic effects in humans) in order to study acute and chronic health effects in humans after exposure to bitumen .

➔ Increased DNA damage in form of DNA strand breaks was determined in white blood cells in a pilot study consisting of 66 mastic asphalt workers exposed to bitumen [Marczynski *et al.* (**2006**), *CEBP* 15: 645].

OBJECTIVE

➔ To extend our investigations on a larger study population thus increasing statistical power and relevance of the obtained results.

➔ To include additional biomarkers of exposure and effect thus employing a „battery approach“ in order to study genotoxic effects in humans.

➔ To exclude confounding factors (*e.g.* smoking habits, coal tar impurities, modifiers and additives, ethnicity, *etc.*).

STUDY DESIGN

➔ Cross-sectional case/control study design including pre- and post-shift biomarker measurements in order to distinguish between acute and chronic effects in humans (the overall study design is outlined in the figure below).

Vorschicht	Während der Schicht	Nachschicht
Medical Examination Lung Function Questionnaire	**(Photo) Documentation** Workplace Description Bitumen Characterization	**Medical Examination** Lung Function
Spot Urine Sampling Metabolites of PAH	**Personal Ambient Monitoring** External Exposure to Fumes of Bitumen	**Spot Urine Sampling** Metabolites of PAH *o*-Cotinine
Blood Sampling Oxidative DNA Exposure DNA-Adduct Formation DNA Strand Breaks Micronuclei Formation Blood Counts	**Stationary Ambient Monitoring** 16-EPA-PAK	**Blood Sampling** Oxidative DNA Exposure DNA-Adduct Formation DNA Strand Breaks Micronuclei Formation SNP Analysis of Genes Blood Counts
Induced Sputum and Nasal Lavage Acute and Chronic Inflammatory Parameters		**Induced Sputum and Nasal Lavage** Acute and Chronic Inflammatory Parameters

STATISTICAL MODEL

➔ Linear mixed model with log-transformed study variables and control of confounders (SAS Software). Results presented are means adjusted for the set of potential confounders and F-tests (*P*-values) for the exposure effect.

➔ The model includes independent fixed factors (time of measurement, smoking status, ethnicity) and a random factor (participants). Age is included in the model as a continuous independent variable

STUDY SUBJECTS

	Controls (n =55)	Exposed (n = 202)
Age [years] median (range)	37 (19 - 61)	40 (17 - 63)
smokers [%]	41.8	65.7
German nationality [%]	87.3	67.7
Duration of exposure [month] median (25 - 75%)		8 (3 - 14)
Fumes of bitumen [mg/m³] median (25 - 75%)		3.7 (1.7 - 7.1)
<10 mg/m³ [n] (%)		172 (85.1)
≥10 mg/m³ [n] (%)		30 (14.9)
Naphthaline [mg/m³] range		0.32 - 1.03
Phenanthrene [mg/m³] range		0.12 - 0.40
Pyrene [mg/m³] range		0.03 - 0.15

ANALYTICAL PARAMETERS

External dose (fumes of bitumen)…

…was determined by personal air sampling using two adjacent filter devices to trap bitumen aerosols (glass fiber filter) and hydrocarbon vapors (XAD-sorbent) at a sampling rate of at least 3L/min. The filters were eluted with CCl_4 and final analysis was carried out by spectroscopy (CH group analysis of hydrocarbons).

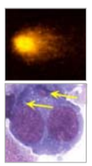
Internal dose (PAH metabolites, creatinine, *o*-cotinine in urine)…

…such as 1- and 2-naphthol (∑OHNaph), 1-hydroxypyrene (1-OHP) as well as 1-, 2+9-, 3-, and 4-hydroxyphenanthrene (∑OH-Ph) are determined by 2D-HPLC and fluorescence detection according to previously published methods [DFG, (**1999**) *Biomonitoring Methods*, Vol. 6; Preuss & Angerer (**2004**), *J Chromatogr B* 801: 307]. Creatinine was determined by a colorimetric method [Taussky (**1954**), *J Biol Chem* 208: 853], while o-cotinine was determined by HPLC/UV.

Effective dose (8-oxo-dGuo and B[*a*]P-DNA-Adduct)…

…were determined in DNA of white blood cells by HPLC/ECD and 2D-HPLC/FLD according to previously published methods [Marczynski *et al.* (**2002**), *Carcinogenesis* 23: 273; Mensing *et al.* (**2005**), *Int J Hyg Environ Health* 208: 173].

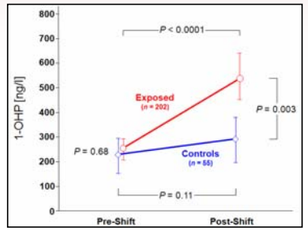
Early biological effects (DNA strand Breaks and micronuclei)…

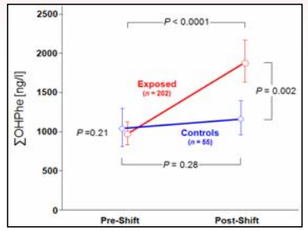


…were determined in lymphocytes by single cell gel electrophoresis (´Comet-Assay´) according to Marczynski *et al.* (**2002**) [*Carcinogenesis* 23: 273] and by microscope scoring according to Fenech (**2000**) [*Mutat Res* 428: 271]. Final parameters were the Olive Tail Moment (OTM) and the number of micronuclei/1000 binucleated cells.

RESULTS AND CONCLUSIONS

INTERNAL DOSE





Parameter	Indep. Variable	Post-Shift		
		n	r_s	*P*
Fumes of Bitumen [mg/m³]	1-OHP [ng/l]	199	**0.26**	**< 0.001**
	∑OHNaph [ng/l]	190	**0.18**	**0,01**
	∑OHPhe [ng/l]	199	**0.36**	**< 0.001**

Parameter	Indep. Variable	Pre-Shift / Post-Shift		
		n	r_s	*P*
1-OHP [ng/l]	∑OHPhe [ng/l]	195	**0.73**	**< 0.001**
	∑OHNaph [ng/l]	182	**0.51**	**< 0.001**
∑OHPhe [ng/l]	∑OHNaph [ng/l]	182	**0.69**	**< 0.001**

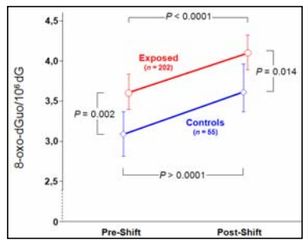
➔ Exposure to bitumen results in an increased excretion of ∑OHNaph, 1-OHP und ∑OHPhe in urine samples after the shift. Therefore, workers are exposed to PAH (in particular naphthalene, pyrene and phenanthrene).

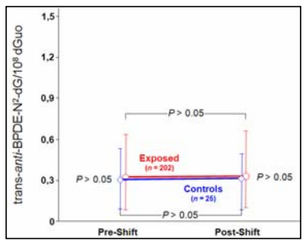
➔ The excretion of all parameters is only *moderate dose-dependent* on external exposure, while biomarkers of internal dose are *highly associated* between each other.

➔ Influence of smoking: ∑OHNaph >> 1-OHP ≥ ∑OHPhe

➔ Determination of the internal dose is clearly superior to the determination of personal air sampling.

EFFECTIVE DOSE





Parameter	Indep. Variable	Post-Shift		
		n	r_s	*P*
Fumes of Bitumen [mg/m³]	8-oxo-dGuo/10⁶ dGuo	201	**-0.02**	**0.76**
	anti-BPDE-N2-dG/10⁹ dG	168	**-0.01**	**0.93**

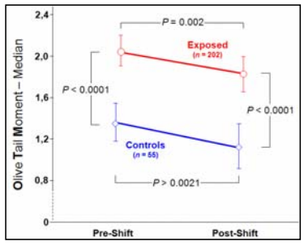
Parameter	Indep. Variable	Post-Shift		
		n	r_s	*P*
1-OHP [ng/l]	8-oxo-dGuo/10⁶ dGuo	198	**0.05**	**0.46**
	anti-BPDE-N2-dG/10⁹ dG	166	**0.02**	**0.84**
∑OHPhe [ng/l]	8-oxo-dGuo/10⁶ dGuo	198	**0.00**	**0.97**
	anti-BPDE-N2-dG/10⁹ dG	166	**-0.03**	**0.71**
∑OHNaph [ng/l]	8-oxo-dGuo/10⁶ dGuo	189	**0.08**	**0.25**
	anti-BPDE-N2-dG/10⁹ dG	164	**0.06**	**0.47**

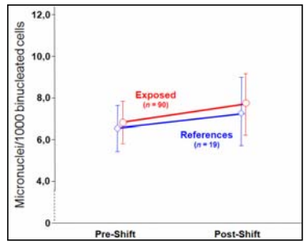
➔ Exposed workers have higher steady-state levels of oxidative DNA exposure (8-oxo-dGuo) (but not B[a]P-DNA adduct levels) in both pre- and post-shift blood samples compared to non-exposed controls.

➔ 8-oxo-dGuo and B[*a*]P-DNA adduct levels are *not associated* to external exposure (fumes of bitumen) nor internal exposure (∑OHNaph, 1-OHP, ∑OHPhe).

➔ The role of oxidative stress (“redox cycling”, here by naphthalene, pyrene and phenanthrene) remains unclear, while exposure to bitumen does not result in increased concentrations of B[*a*]P-DNA adducts.

EARLY BIOLOGICAL EFFECTS





Parameter	Indep. Variable	Post-Shift		
		n	r_s	*P*
Fumes of Bitumen [mg/m³]	Olive Tail Moment	202	**0.00**	**0.97**
	Micronuclei	23	**0.02**	**0.93**

Parameter	Indep. Variable	Post-Shift		
		n	r_s	*P*
1-OHP [ng/l]	Olive Tail Moment	199	**0.17**	**0.01**
	Micronuclei	23	**-0.03**	**0.89**
∑OHPhe [ng/l]	Olive Tail Moment	199	**0.01**	**0.84**
	Micronuclei	23	**-0.04**	**0.85**
∑OHNaph [ng/l]	Olive Tail Moment	190	**0.06**	**0.40**
	Micronuclei	23	**0.04**	**0.86**

➔ Exposed workers have higher steady-state levels of DNA strand breaks (but not micronuclei formation) in both pre- and post shift blood samples compared to non-exposed individuals.

➔ DNA strand breaks and micronuclei are *not associated* to external exposure (fumes of bitumen) nor internal exposure (∑OHNaph, 1-OHP, and ∑OHPhe). The weak association between 1-OHP and DNA strand breaks in post-shift samples can be considered to be a statistical artifact.

➔ A statistical significant decrease during the shift is observed for DNA strand breaks in both exposed and non-exposed workers. The decrease remains unexplained so far.

OVERALL SUMMARY

Mastic asphalt workers show higher levels of oxidative DNA damage and DNA strand break frequencies. However, the observed effects are not dose-dependent on exposure to fumes of bitumen or exposure to naphthalene, phenanthrene, and pyrene in bitumen. In addition, no increased B[*a*]P-DNA adducts and micronuclei frequencies could be observed.

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