

Occupational exposure to polycyclic aromatic hydrocarbons and DNA damage by industry: a nationwide study in Germany

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Abstract Exposure to polycyclic aromatic hydrocarbons (PAH) and DNA damage were analyzed in coke oven ($n = 37$), refractory ($n = 96$), graphite electrode ($n = 26$), and converter workers ($n = 12$), whereas construction workers ($n = 48$) served as referents. PAH exposure was assessed by personal air sampling during shift and biological monitoring in urine post shift (1-hydroxypyrene, 1-OHP and 1-, 2 + 9-, 3-, 4-hydroxyphenanthrenes, Σ OHPHE). DNA damage was measured by 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodGuo) and DNA strand breaks in blood post shift. Median 1-OHP and Σ OHPHE were highest in converter workers (13.5 and 37.2 $\mu\text{g/g}$ crea). The industrial setting contributed to the metabolite concentrations rather than the air-borne concentration alone. Other

routes of uptake, probably dermal, influenced associations between air-borne concentrations and levels of PAH metabolites in urine making biomonitoring results preferred parameters to assess exposure to PAH. DNA damage in terms of 8-oxo-dGuo and DNA strand breaks was higher in exposed workers compared to referents ranking highest for graphite-electrode production. The type of industry contributed to genotoxic DNA damage and DNA damage was not unequivocally associated to PAH on the individual level most likely due to potential contributions of co-exposures.

Keywords Polycyclic aromatic hydrocarbons · Occupational exposure · DNA damage · Biological monitoring · PAH industries

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Introduction

Polycyclic aromatic hydrocarbons (PAH) are produced by incomplete combustion or pyrolysis of organic materials and are important occupational and environmental pollutants. Epidemiological studies have shown an increased incidence of lung cancer in various industrial settings with high exposure to PAH (IARC 1984; Boffetta et al. 1997). Exposures during coal gasification, coke production, coal tar distillation, as a chimney sweep, during paving and roofing with coal tar pitch and during aluminum production were categorized by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (Group 1), whereas exposures during graphite-electrode manufacture were categorized as probably carcinogenic to humans (Group 2A) (IARC 2009).

PAH are a complex mixture of more than 100 compounds (Boström et al. 2002). Sixteen PAH compounds (Σ PAH) are recommended for monitoring of air-borne exposure by the US Environmental Protection Agency. Σ PAH comprises of seven carcinogenic PAH (c-PAH) recently categorized by IARC in group 1 (benzo[*a*]pyrene, B[*a*]P), group 2A (dibenz[*a,h*]anthracene) and group 2B (possibly carcinogenic in humans: benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, chrysene, and indeno[1,2,3-*cd*]pyrene) (Straif et al. 2005). PAH exposure is also assessed by biological monitoring of the internal dose taking into account all routes of uptake rather than inhalation only. The most prominent biomarker is 1-hydroxypyrene (1-OHP) in urine (Jongeneelen 1997). In addition, metabolites of other PAH including phenanthrene such as isomers of hydroxylated phenanthrenes (1-, 2-, 3-, 4-, and 9-hydroxyphenanthrenes, Σ OHPHE) in urine have also been suggested as biomarkers of exposure (Seidel et al. 2008; Hecht 2002).

PAH require metabolic activation in order to induce carcinogenic effects. A major pathway includes the activation of PAH to diol epoxides by CYP450 and epoxide hydrolase. Diol epoxides serve as ultimate carcinogens with tumor-initiating properties by directly forming covalently bound DNA adducts with N2 of purines (Dipple et al. 1999). A second pathway involves the induction of oxidative stress including the formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxo-dGuo) as a by-product of redox cycling between PAH-dihydrodiols and PAH-o-chinons (Burczynski et al. 1999). DNA adducts formed by both the direct and the indirect pathway can lead to DNA damage such as DNA strand breaks and mutations and are associated with subsequent cancer (Luch 2005; Klaunig et al. 1998).

The purpose of the study presented here was to analyze biomarkers of PAH exposure and DNA damage by type of industry and at various workplaces with high PAH expo-

sure. The data presented here are part of a nationwide cross-sectional study in Germany. Results on statistical approaches for dose–response modeling and the influence of polymorphic genes on the excretion of PAH metabolites in urine already have been published earlier (Pesch et al. 2007; Rihs et al. 2005).

Materials and methods

Study subjects

A cross-sectional study was conducted in male workers from different PAH industries in Germany. Face-to-face interviews were performed with all workers using a structured questionnaire on characteristics of the workplace, smoking habits, medical history, and use of personal protection devices during work. Air-borne PAH exposure was measured during shift with personal devices, whereas urine and blood samples were collected on the same day post shift. A complete set of data consisting of personal monitoring of air-borne PAH, biological monitoring of PAH metabolites, level of DNA damage, and questionnaire information were available for 171 employees. Forty-eight construction workers without occupational exposure to PAH served as referents. Exposed workers were enrolled from coke ($n = 37$), refractory ($n = 96$), and graphite-electrode production ($n = 26$). In addition, converter workers ($n = 12$) employed in steel industry and performing brickwork lining and repair of converters were studied. Coke-oven workers included top-oven workers ($n = 11$), side-oven workers ($n = 15$), and persons working close to the coke-oven batteries in the side-product area ($n = 11$). Employees from the manufacture of refractory materials were producing clay and non-clay refractories in terms of formed objects and unformed granulated compositions. Workers from the production of graphite electrodes were recruited from all workplaces including the production of green electrodes, impregnation, graphitization, and finishing. All persons gave their informed consent. The study was conducted under the provisions of the World Medical Association declaration of Helsinki.

Exposure assessment

Personal air sampling of Σ PAH in the workers' breathing zone was carried out during shift. Samples were analyzed according to NIOSH method 5506 (NIOSH 1994). Depending on the compound the limit of detection (LOD) varied between 0.01 and 0.05 $\mu\text{g}/\text{m}^3$. 1-OHP and Σ OHPHE were analyzed in spot urine samples post shift according to a method developed by Lintelmann and Angerer (1999). LODs were between 0.02 and 0.09 $\mu\text{g}/\text{L}$ depending on the

respective metabolite. Volume-related concentrations of PAH metabolites in urine were adjusted to creatinine (crea). Creatinine was determined according to a colorimetric method (Jaffé reaction) published by Taussky (1954). Urinary cotinine was determined in post-shift samples according to a method published by Scherer et al. (2001) with a LOD of 1.0 µg/L. All methods were fully validated prior to use using external quality control material of the German External Quality Assessment Scheme (G-EQUAS, Schaller et al. 2002).

DNA damage

Blood samples were collected at the end of the shift. White blood cells (WBC) were isolated for the analysis of 8-oxo-dGuo according to a procedure previously published by Marczynski et al. (2005). Nucleosides were analyzed according to the methods proposed by Floyd et al. (1986) and Pouget et al. (1999) with a LOD of 0.1 adduct/10⁶ nucleotides. The method has been recommended by the European Standards Committee on Oxidative DNA Damage (ESCODD 2003). DNA strand breaks in lymphocytes were determined with the Comet assay using a modified protocol of the original description by Östling and Johanson (1984) and Singh et al. (1988). All quality control measures in accordance with the guidelines of the In Vivo Comet Assay Workgroup of the International Conference on Harmonization (ICH) were taken into account (Burlinson et al. 2007). Clean up steps were performed under red light to prevent additional DNA damage and artifact formation. Final analysis was performed by fluorescence microscopy and automated image analysis. The Olive tail moment (OTM) was selected to quantify DNA damage of each cell.

Statistical analyses

Statistical analyses were performed with SAS v. 9.1 (SAS Institute Inc., Cary, NC, USA). c-PAH were defined as sum of the concentrations of B[a]P, dibenz[a,h]anthracene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, and indeno[1,2,3,-cd]pyrene. Values below LOD were set half of the LOD. The distributions of the values are presented by type of industry with median and inter-quartile range. Spearman rank correlation coefficients (r_s) were calculated to describe the associations between the various variables. In order to determine the variation by type of industry and to estimate the influence of air-borne PAH on biomarkers, potential confounders such as smoking were included in the statistical model. Due to highly skewed distributions, air-borne PAH and biomarker concentrations were log-transformed in order to apply a linear model. PAH exposure was implemented as continuous and independent variable. Type of workers

(coke, refractory material, graphite electrodes, and converter workers), current smoking (yes, no), German nationality (yes, no), and age (<30, 30–49, ≥50 years) were modeled as categorical variables. PAH concentrations were centered for exposed workers by the corresponding median concentration. Occupational PAH exposure of the referents was set to zero for statistical analyses. Quantitative PAH effects were presented as $\exp(\beta)$, which corresponds to the factor of alteration of a biomarker if air-borne PAH concentrations doubles. All tests were conducted two-sided with a significance level of 5%.

Results

Characteristics of the study populations

Table 1 depicts the socio-demographic characteristics of the various study groups. Median age of exposed workers was 39 years (range 19–62 years) and 36 years in the reference subjects (range 19–61 years). Self-reported smoking status was assessed by questionnaire for all persons. The percentage of current smokers differed between exposed workers (67.8%) and referents (41.7%) but not between workers in the various PAH industries ($P = 0.65$). Self-reported smoking status was used for statistical analyses. For 114 out of 171 exposed workers, urinary cotinine was available for correction. In these workers, current smoking was re-categorized based on cotinine in urine using a cut-off level of 100 µg/l. The comparison between self-reported smoking status and measured urinary cotinine revealed a high concordance of 93%.

Air-borne PAH exposure

Table 2 shows the distribution of air-borne PAH exposure by type of industry. Median Σ PAH was 30.2 µg/m³ for all exposed workers. The highest median concentrations were observed for converter workers (576 µg/m³), followed by workers in the production of refractory materials (30.0 µg/m³), coke (22.5 µg/m³), and graphite electrodes (13.5 µg/m³). Similar results were obtained for pyrene (PYR) and phenanthrene (PHE). Concentrations of Σ PAH were strongly associated to PYR ($r_s = 0.80$; $P < 0.0001$) and PHE ($r_s = 0.88$; $P < 0.0001$) (Table 3). The association between c-PAH and Σ PAH was significant but weaker ($r_s = 0.51$; $P < 0.0001$). With particular focus on c-PAH including B[a]P, the highest median concentrations were observed for converter workers (12.7 and 2.4 µg/m³). c-PAH and B[a]P concentrations were about 2–3 times higher than those measured in coke production (4.7 and 1.0 µg/m³). The lowest concentrations of c-PAH and B[a]P were found in the production of refractory materials (0.9 and

Table 1 Characteristics of the study population

	Reference group	Exposed group	Coke production	Refractory materials	Carbon electrodes	Converter workers
Number (<i>n</i>)	48	171	37	96	26	12
Age (years)						
Median	36	39	35	40	41	34
Range	19–61	19–62	22–51	19–62	21–58	26–52
Current smokers <i>n</i> (%)	20 (41.7)	116 (67.8)	27 (73.0)	65 (67.7)	16 (61.5)	8 (66.7)
German nationality <i>n</i> (%)	43 (89.6)	130 (76.0)	23 (62.2)	80 (83.3)	19 (73.1)	8 (66.7)

Table 2 Concentrations of air-borne PAH during work shift and of biomarkers post shift by industry presented with median and inter-quartile range

	Reference group	Exposed group	Coke production	Refractory materials	Carbon electrodes	Converter workers
Number (<i>n</i>)	48	171	37	96	26	12
ΣPAH (μg/m ³)	–	30.2 (15.2–98.0)	22.5 (15.9–91.0)	30.0 (17.8–59.7)	13.5 (5.2–175)	576 (182–881)
c-PAH (μg/m ³)	–	1.5 (0.6–6.5)	4.7 (2.2–13.2)	0.9 (0.4–3.9)	1.0 (0.1–3.7)	12.7 (6.0–99.5)
B[a]P (μg/m ³)	–	0.2 (0.1–1.0)	1.0 (0.4–2.5)	0.1 (0.0–0.4)	0.1 (0.0–0.5)	2.4 (1.0–24.1)
Pyrene (μg/m ³)	–	1.4 (0.5–3.7)	1.5 (0.4–3.6)	1.2 (0.4–2.7)	0.79 (0.3–3.2)	9.3 (5.1–13.4)
Phenanthrene (μg/m ³)	–	6.9 (2.0–17.1)	2.9 (1.0–7.4)	9.6 (3.0–18.1)	3.2 (1.4–37.2)	36.2 (13.4–57.1)
1-OH-Pyrene (μg/g crea)	0.18 (0.1–0.3)	7.8 (3.2–17.1)	3.8 (2.2–6.9)	8.4 (3.8–17.8)	9.7 (5.6–17.2)	13.5 (7.5–31.3)
ΣOH-Phenanthrenes (μg/g crea)	1.0 (0.7–1.5)	12.4 (5.6–30.4)	6.8 (4.1–14.4)	13.0 (6.8–30.4)	15.8 (4.5–31.3)	37.2 (30.0–61.9)
8-oxo-dGuo/10 ⁶ dGuo	3.5 (2.8–4.5)	5.7 (4.3–7.1)	4.9 (2.8–6.5)	5.4 (4.4–6.5)	11.3 (9.0–12.2)	4.4 (3.9–6.1)
DNA strand breaks (Olive tail moment)	1.3 (1.2–1.8)	2.3 (1.7–3.0)	2.2 (1.7–3.0)	2.1 (1.7–2.7)	3.0 (2.3–4.1)	1.4 (1.3–2.6)

Table 3 Spearman correlation coefficients between concentrations of air-borne PAH and biomarkers post shift in 171 workers from the production of steel, coke, refractory material, and carbon electrodes (*P* values in brackets)

	c-PAH (μg/m ³)	Pyrene (μg/m ³)	Phenanthrene (μg/m ³)	1-OH-Pyrene (μg/g crea)	ΣOH-Phenanthrenes (μg/g crea)
ΣPAH (μg/m ³)	0.51 (<0.0001)	0.80 (<0.0001)	0.88 (<0.0001)	0.52 (<0.0001)	0.67 (<0.0001)
c-PAH (μg/m ³)		0.77 (<0.0001)	0.27 (0.0003)	0.38 (<0.0001)	0.11 (0.15)
Pyrene (μg/m ³)			0.69 (<0.0001)	0.54 (<0.0001)	0.43 (<0.0001)
Phenanthrene (μg/m ³)				0.52 (<0.0001)	0.73 (<0.0001)
1-OH-Pyrene (μg/g crea)					0.66 (<0.0001)

0.1 μg/m³) and graphite electrodes (1.0 and 0.1 μg/m³). The association between c-PAH and ΣPAH was significant ($r_s = 0.51$; $P < 0.0001$). ΣPAH contained high proportions of 2–4-ring PAH such as naphthalene, PHE, and PYR, whereas only low concentrations of 5- and 6-ring PAH such as B[a]P or benzo[ghi]perylene were detected. Naphthalene was the most abundant PAH detected in coke-oven and converter emissions, whereas phenanthrene was the most abundant PAH in the manufacture of graphite electrodes and refractory materials. PYR and PHE could be measured in 85.4 and 99.4% of all air samples, whereas c-PAH including B[a]P (73.1%), dibenz[*a,h*]anthracene (47.9%),

benz[*a*]anthracene (80.7%), benzo[*b*]fluoranthene (75.4%), benzo[*k*]fluoranthene (67.2%), chrysene (81.3%), and indeno[1,2,3-*cd*]pyrene (58.5%) were quantified in less samples and were frequently close to their respective LODs. Concentrations of c-PAH and naphthalene ranked among the highest in converter and in coke-oven emissions.

Internal PAH exposure

Median levels of 1-OHP and ΣOHPHE were 7.8 and 12.4 μg/g crea in exposed workers compared to 0.18 and 1.0 μg/g crea in referents (Table 2). Median concentrations

of 1-OHP and Σ OHPHE were highest in converter workers (13.5 and 37.2 $\mu\text{g/g}$ crea), followed by the production of graphite electrodes (9.7 and 15.8 $\mu\text{g/g}$ crea), refractory materials (8.4 and 13.0 $\mu\text{g/g}$ crea), and coke (3.8 and 6.8 $\mu\text{g/g}$ crea). An association was found between 1-OHP and Σ OHPHE in urine ($r_s = 0.66$; $P < 0.0001$, Table 3). Considerable differences in exposures depending on the site of the workplace were observed in coke-oven workers with top-oven workers ($n = 11$) having higher urinary concentrations of 1-OHP (median 17.4 $\mu\text{g/g}$ crea) than side-oven workers ($n = 15$, median 5.7 $\mu\text{g/g}$ crea). Coke-oven workers in other areas of the plant but adjacent to the coke-oven batteries ($n = 11$) had a median 1-OHP level of 5.4 $\mu\text{g/g}$ crea.

The correlations between air-borne PAH exposure and internal PAH exposure are shown in Table 3. With the exception of c-PAH and Σ OHPHE ($r_s = 0.11$; $P = 0.15$) external exposure and internal dose were associated with each other. Highly significant associations were found between Σ PAH and Σ OHPHE ($r_s = 0.67$; $P < 0.0001$) and PHE with Σ OHPHE ($r_s = 0.73$; $P < 0.0001$), while other associations were of weak to moderate significance with Spearman correlation coefficients of $r_s \leq 0.54$. After adjusting for smoking and type of industry, a doubling of PYR was associated with a 1.31-fold increase of 1-OHP (95% CI 1.24–1.39; $P < 0.0001$), whereas a doubling of PHE resulted in a 1.40-fold increase of Σ OHPHE (95% CI 1.32–1.48; $P < 0.0001$) (Table 4). Type of industry (workplace-specific factors) appeared to be a strong additional predictor of 1-OHP levels rather than external exposure to

PYR alone and resulted in 17.8- (coke production) to 51.8-fold (graphite-electrode production) higher concentrations when compared with referents after taking into account the association of 1-OHP with its parent compound PYR. Similarly, the type of industry was a significant and strong predictor of Σ OHPHE levels but with less pronounced differences between the industries. Current smoking was associated with a 1.77-fold higher concentration of 1-OHP (95% CI 1.36–2.31) but did not significantly influence Σ OHPHE ($P = 0.72$). Occupational exposure to PAH in all settings was the dominant source of PAH exposure rather than smoking. Non-smoking PAH-exposed workers had 20-fold higher median levels of 1-OHP than smoking referents (5.85 vs. 0.29 $\mu\text{g/g}$ crea).

DNA damage

Table 2 shows the distribution of 8-oxo-dGuo and DNA strand breaks between exposed workers and referents as well as by type of industry. The median level of 8-oxo-dGuo in exposed workers was 5.7/10⁶ dGuo nucleotides and significantly elevated compared to the referents (3.5/10⁶ dGuo). DNA strand breaks in terms of the OTM also were higher in exposed workers (2.3 vs. 1.3). Levels of 8-oxo-dGuo and DNA strand breaks were highest in graphite-electrode production (11.3/10⁶ dGuo and 3.0) followed by those in the manufacture of refractory materials (5.4/10⁶ dGuo and 2.1). Coke-oven workers had significantly elevated levels of DNA strand breaks (2.2) but not of adducts (4.9/10⁶ dGuo), whereas effects in form of 8-oxo-dGuo

Table 4 Effects of the parent PAH compound, type of industry, and current smoking on the urinary concentrations of 1-hydroxypyrene and Σ OH-phenanthrenes in 171 PAH-exposed workers and 48 referents

	1-OH-Pyrene ($\mu\text{g/g}$ crea)			Σ OH-Phenanthrenes ($\mu\text{g/g}$ crea)		
	Exp(β)	95% CI	P value	Exp(β)	95% CI	P value
Intercept ^a	0.15	0.11–0.20		1.01	0.78–1.31	
Exposure ^b						
Log pyrene ($\mu\text{g}/\text{m}^3$)	1.31	1.24–1.39	<0.0001			
Log phenanthrene ($\mu\text{g}/\text{m}^3$)				1.40	1.32–1.48	<0.0001
Reference group	1			1		
Type of industry						
Coke production	17.8	11.9–26.6	<0.0001	11.8	8.33–16.8	<0.0001
Refractory materials	39.8	28.7–55.1	<0.0001	13.2	9.98–17.4	<0.0001
Carbon electrodes	51.8	33.5–80.1	<0.0001	16.1	11.1–23.4	<0.0001
Converter workers	26.6	14.7–48.0	<0.0001	16.5	9.95–27.2	<0.0001
Current smoking						
No	1			1		
Yes	1.77	1.36–2.31	<0.0001	1.04	0.83–1.31	0.72

CI confidence interval

^a Estimated geometric mean of the dependent variable in non-smoking referents

^b Exposure variable log-transformed and centered by the median of the distribution in exposed workers

(4.4/10⁶ dGuo) and DNA strand breaks (1.4) were less pronounced in converter workers. DNA strand breaks were weakly associated with the air-borne concentration of Σ PAH in the regression model ($P = 0.03$), whereas no associations between 8-oxo-dGuo with exposure to PAH were observed (Table 5). All other associations between exposure and DNA damage were found to be negative on the individual level suggesting that the positive finding between Σ PAH and OTM can be a result from multiple testing. Current smoking was not associated with 8-oxo-dGuo or DNA strand breaks. No association was observed between 8-oxo-dGuo and DNA strand breaks ($r_s = 0.13$; $P = 0.08$).

Discussion

PAH are known to be genotoxic and carcinogenic in humans. Epidemiological studies have shown an excess of lung cancer in certain PAH industries including coke production (IARC Group 1). However, limited data on PAH exposure as well as genotoxic and carcinogenic effects have been available for graphite-electrode production and other occupational settings with potentially high exposures to PAH (Boffetta et al. 1997; IARC 2009). Here, a nationwide cross-sectional study in Germany was conducted to analyze exposure to PAH and DNA damage. Previous reports in our cohort focused on dose–response associations and the influence of polymorphic genes (Pesch et al. 2007; Rihs et al. 2005). We focused on PAH exposure and effects by occupational setting (type of industry). The results allow a direct

comparison of PAH exposure and DNA damage across typical settings of which one of them, coke production, is well studied and categorized as carcinogenic in humans by IARC. However, only limited data on graphite-electrode, refractory, and converter workers are available in the literature.

PAH exposure

The highest levels of PAH exposure were observed in workers recruited from the steel industry who performed brickwork lining of converters where pig iron is converted into crude steel. Graphite and other unwanted residues were burnt off by the injection of air. Workers were also required to clean the insides of the converters, a confined environment, by scratching off burned residues by hand. They were wearing overalls, leather gloves, and dust masks. Converter workers in steel industry experienced 20–40-fold higher concentrations of Σ PAH (576 $\mu\text{g}/\text{m}^3$) and three- to tenfold higher concentrations of c-PAH (12.7 $\mu\text{g}/\text{m}^3$) than workers recruited from the production of coke, refractory materials, and graphite electrodes. Internal dose in terms of 1-OHP (13.5 $\mu\text{g}/\text{g}$ crea) and Σ OHPHE (37.2 $\mu\text{g}/\text{g}$ crea) also ranked highest in converter workers compared to the other PAH industries. However, the differences of metabolite levels (two- to fourfold) were far less pronounced compared to those observed for air-borne exposures pointing to other routes of uptake rather than inhalation only. In addition, particle-bound PAH might be efficiently filtered by the dust masks. Available data on converter workers in steel industry exposure could not be traced in the literature, making it

Table 5 Effect of PAH exposure in air, type of industry, and current smoking on DNA damage in 171 PAH-exposed workers and 48 referents

	8-oxo-dGuo/10 ⁶ dGuo			Olive tail moment			8-oxo-dGuo/10 ⁶ dGuo			Olive tail moment		
	Exp(β)	95% CI	P value	Exp(β)	95% CI	P value	Exp(β)	95% CI	P value	Exp(β)	95% CI	P value
Intercept ^a	3.66	3.27–4.11		1.40	1.22–1.59		3.65	3.26–4.09		1.40	1.23–1.60	
Exposure ^b												
Log Σ PAH ($\mu\text{g}/\text{m}^3$)	0.98	0.96–1.01	0.19	1.04	1.00–1.07	0.03						
Log c-PAH ($\mu\text{g}/\text{m}^3$)							1.01	0.99–1.04	0.19	0.99	0.96–1.01	0.30
Reference group	1			1			1			1		
Type of industry												
Coke production	1.15	0.99–1.34	0.07	1.64	1.38–1.96	<0.0001	1.11	0.95–1.31	0.18	1.70	1.41–2.05	<0.0001
Refractory materials	1.52	1.34–1.72	<0.0001	1.58	1.37–1.82	<0.0001	1.50	1.33–1.70	<0.0001	1.60	1.39–1.85	<0.0001
Carbon electrodes	2.99	2.53–3.52	<0.0001	2.26	1.87–2.73	<0.0001	2.99	2.54–3.52	<0.0001	2.23	1.84–2.71	<0.0001
Converter workers	1.40	1.10–1.78	0.01	1.12	0.85–1.47	0.44	1.24	0.98–1.56	0.07	1.35	1.03–1.77	0.03
Current smoking												
No	1			1			1			1		
Yes	1.03	0.94–1.14	0.51	0.98	0.87–1.10	0.68	1.04	0.94–1.14	0.49	0.97	0.87–1.09	0.64

CI confidence interval

^a Estimated geometric mean of the dependent variable in non-smoking referents

^b Exposure variable log-transformed and centered by the median of the distribution in exposed workers

impossible to compare the present results with previous studies.

Workers in coke production revealed a wide variation of individual PAH exposures. Air-borne c-PAH exposure ($4.7 \mu\text{g}/\text{m}^3$) ranked second highest and concentrations were fivefold higher than those observed in the production of graphite-electrodes and refractory materials. However, no significant differences in ΣPAH concentrations between these settings were observed. Median internal exposures in terms of 1-OHP ($3.8 \mu\text{g}/\text{g}$ crea) and ΣOHPHE ($6.8 \mu\text{g}/\text{g}$ crea) in coke-oven workers were lower than those observed in all other industrial settings. The selection of certain areas at the coke plant for exposure monitoring highly influenced the levels observed, e.g., median 1-OHP in top-oven workers ($17.4 \mu\text{g}/\text{g}$ crea) were about threefold higher than those in side-oven workers ($5.7 \mu\text{g}/\text{g}$ crea) in agreement with previous publications (Yang et al. 2007; Lin et al. 2006). No differences between side-oven workers and employees working in the side-product area (e.g., ammonium sulfate production) were observed ($5.4 \mu\text{g}/\text{g}$ crea). Similar results as described for 1-OHP were found for ΣOHPHE . The results indicate that coke-oven emissions are first directed upwards (leading to higher exposures in top-oven workers), and are then evenly distributed over the area while affecting both, side-oven and side-product workers to a similar extent. Median personal PAH exposures at the German workplaces reported in this study (ΣPAH $22.5 \mu\text{g}/\text{m}^3$; 1-OHP $3.8 \mu\text{g}/\text{g}$ crea; ΣOHPHE $6.8 \mu\text{g}/\text{g}$ crea) were at the lower end of those concentrations previously reported in coke production. Van Delft et al. (2001) provided an overview of PAH exposure in the production of coke from European plants with average levels ranging from 15.9 to $241 \mu\text{g}/\text{m}^3$ and internal exposures in terms of 1-OHP between 0.8 and $11.6 \mu\text{g}/\text{g}$ crea. In recent years, studies were also carried out in Chinese coke-oven plants. There, median and average concentrations of ΣPAH were higher and reported to be between 63 and $515 \mu\text{g}/\text{m}^3$ (Chen et al. 1999; Zhang et al. 2000; Wu et al. 2002, 2003). Results also have shown higher internal exposures and 1-OHP concentrations in urine between 17 and $23.8 \mu\text{g}/\text{g}$ crea (Serdar et al. 2003; Lu et al. 2002; Leng et al. 2004, 2005). Technical and other reasons might explain the observed differences between exposures at European plants and those in China, because the methods of assessing exposures were similar between all studies. Only a few studies are available on the excretion of ΣOHPHE in coke-oven workers. Mean concentrations of $16.5 \mu\text{g}/\text{g}$ crea were reported from spot urine samples post shift and a total of $34 \mu\text{g}$ in 24-h-urine samples from German plants (Strunk et al. 2002; Grimmer et al. 1993), whereas volume-related exposures of $7.2 \mu\text{g}/\text{l}$ for the sum of 3- and 9-hydroxyphenanthrenes were observed in a Chinese plant (Serdar et al. 2003).

IARC recently assessed occupational exposures during carbon-electrode manufacturing for the first time and classified graphite-electrode production as probably carcinogenic to humans (Straif et al. 2005). Workers in the production of graphite electrodes were exposed to lower concentrations of air-borne ΣPAH but to similar levels of c-PAH as measured in the production of refractory materials. Concentrations of c-PAH, in both the production of graphite electrodes and refractory materials, were about $1.0 \mu\text{g}/\text{m}^3$ and close to the limits of detection of the individual compounds. Therefore, results on c-PAH exposure remain inconclusive. Relatively high 1-OHP levels were observed in production of graphite electrodes in our study ($9.7 \mu\text{g}/\text{g}$ crea) when compared with coke production ($3.8 \mu\text{g}/\text{g}$ crea). Earlier investigations in a German plant also revealed high levels of 1-OHP (approximately $20 \mu\text{g}/\text{g}$ crea) in the baking and impregnation area but levels below $3.0 \mu\text{g}/\text{g}$ crea in graphitization and conditioning (Angerer et al. 1997). Median concentrations measured in German plants, including those presented here, were higher than reported for an Italian graphite-electrode plant with $1.4 \mu\text{g}/\text{g}$ crea where protective measures were implemented (dell'Omio et al. 1998). The concentrations measured in our study were also at the upper end compared to graphite-electrode workers in a Dutch cohort in 1992 where urinary concentrations of 1-OHP could be observed in the range between 1.7 and $9.8 \mu\text{g}/\text{g}$ crea (Buchet et al. 1992). The corresponding average exposure to ΣPAH in Dutch workers was $223 \mu\text{g}/\text{m}^3$ and thus far higher than in the German settings presented here ($13.5 \mu\text{g}/\text{m}^3$). Our results indicate dermal uptake of PAH in graphite-electrode production as previously assumed (Angerer et al. 1997). Although air-borne PYR ($\sim 1 \mu\text{g}/\text{m}^3$) and PHE ($\sim 3 \mu\text{g}/\text{m}^3$) were similar in the production of coke and graphite electrodes, more than twice the amounts of urinary 1-OHP and ΣOHPHE were measured in graphite-electrode workers (9.7 and $15.8 \mu\text{g}/\text{g}$ crea) compared to coke-oven workers (3.8 and $6.8 \mu\text{g}/\text{g}$ crea). Similar results are seen for workers in the production of refractory materials although less pronounced for PHE. Therefore, our study also confirms additional dermal uptake of PAH in workers producing refractory materials as previously reported (Gündel et al. 2000). Because the concentrations of PAH metabolites were moderately correlated with their parent compounds and varied by type of industry, our study overall supports additional routes of PAH uptake rather than by inhalation alone. Additional routes of uptake are of particular importance in graphite-electrode and refractory production as well as converter workers but less in coke-oven workers. We observed that workers in refractory and graphite-electrode production often were wiping sweat off their skin (with or without using gloves) due to hot working environments, while this behavior was less observed in coke-oven workers (who mainly worked

outside). Therefore, biological monitoring has to be considered an important tool to accurately assess exposure in PAH industries. The association between PHE and Σ OH-PHE is stronger ($r = 0.73$) than between PYR and 1-OHP ($r = 0.54$). In addition, current smoking did not influence Σ OH-PHE, whereas it turned out to significantly confound internal exposure in terms of 1-OHP in our study and in line with literature (Qiu et al. 2007). Therefore, Σ OH-PHE might be a more accurate predictor of occupational exposure to high PAH levels rather than 1-OHP. A potential cause might be the higher volatility of PHE compared to PYR and the relative amounts of PYR and PHE taken up by smoking and via workplace exposure. Uptake of PHE and PYR by smoking (pure inhalation) and therefore the ratio between PHE and PYR can be considered constant for a particular smoker. However, at the workplace there is an additional uptake of PHE and PYR by inhalation on top of smoking. This additional uptake by inhalation is better represented by PHE rather than PYR because PHE shows higher volatility than PYR. Consequently, occupational exposure by inhalation can be assessed more suitably by Σ OH-PHE in urine rather than 1-OHP.

The median level of 1-OHP of the general population in Germany (smokers and non-smokers, $n = 573$) is $0.10 \mu\text{g/g}$ creatinine (Human Commission 2005). No data on Σ OH-PHE is available. Concentrations in exposed workers were found to be 100-fold higher for 1-OHP ($7.8 \mu\text{g/g}$), whereas concentrations in the referents ($0.18 \mu\text{g/g}$) were approximately twofold higher than those observed in the general population of Germany. The latter can be explained by a higher percentage of smokers (41 vs. 32%) and a potential background exposure to traffic exhaust in our reference group compared to the general population in Germany.

DNA damage

PAHs have been demonstrated to damage DNA. 8-oxo-dGuo, a highly mutagenic DNA lesion, and the Comet assay have been used in vitro to show that oxidative stress contributes to PAH-induced genotoxicity and lung carcinogenicity (Seike et al. 2004; Thielen et al. 2006; Park et al. 2008a, b). Previously published studies in humans reported significantly higher levels of DNA strand breaks and 8-oxo-dGuo in WBC of exposed workers than in referents (Zhang et al. 2003; Marczynski et al. 2006). In our study, significantly higher levels of both 8-oxo-dGuo and DNA strand breaks were found also in WBC of PAH-exposed workers compared to unexposed workers. However, the results varied by type of industry. Although PAH exposure was highest in converter workers, DNA damage was found at lower than average levels in these workers compared to other industries. Decreased bioactivation and tumor initiation of PAH by inhibiting CYP1A1 and CYP1B1 in a dose-dependent

manner after high co-exposures to particulate matter as recently observed in animal studies might serve as an explanation (Courter et al. 2007). These authors concluded that complex mixtures can alter PAH-induced carcinogenesis and directly influence PAH-induced genotoxic effects. Although we did not measure dust load in the different types of industries, high co-exposures to dust were observed at the workplaces, in particular for converter workers in steel industry and, to a lesser extent, in the production of refractory materials.

Highest levels of DNA damage in terms of DNA strand breaks and 8-oxo-dGuo in WBC were found in the manufacture of graphite electrodes and were about twice as high (3.0 and $11.3/10^6$) compared to the other industries. A recent study in Italian graphite-electrode workers employing the Comet assay also revealed an increased genotoxic risk (Moretti et al. 2007). However, again co-exposures to silica and carbon dusts were observed in those workers and excess mortality was high for silicosis (Merlo et al. 2004). Similar as for converter workers and in refractory material industry, the results for graphite-electrode workers show the significance of exposures to multiple compounds, their interactions, and the importance to discuss DNA damaging effects dependent on particular exposure circumstances at different workplaces.

In contrast to converter workers and employees in the production of refractory materials and graphite electrodes, more data could be traced in the literature for coke-oven workers. As previously reported in several studies in Chinese workers, our study employees in coke production had significantly elevated levels of DNA strand breaks compared to referents. Current opinion is that data from different laboratories cannot be compared with one another, but it has been shown that levels of DNA strand breaks, in terms of OTM, are similar between those studies where appropriate methods recommended by ESCODD are used (ESCODD 2003). Based on these protocols mean OTM levels of 2.6 (95% CI 2.1 – 3.3) in exposed and 1.0 (95% CI 0.8 – 1.2) in non-exposed workers were observed by Leng and co-workers (2004), whereas OTM levels of 2.5 ± 0.75 in high-exposed workers compared to 1.7 ± 0.69 and 1.6 ± 0.46 in intermediate and low-exposed workers were found by Yang et al. (2007). The differences between all three groups using a multivariate analysis of covariance with adjustment for age, length of work, pack-years smoked, and alcohol use were significant ($P = 0.019$). Although reports in coke-oven workers consistently show increased DNA strand breaks, there were no differences between exposed workers and referents for 8-oxo-dGuo in WBC in our study or in a previous study by Zhang et al. (2003).

The majority of previous publications on PAH exposure reported significantly higher levels of DNA damage in

terms of DNA strand breaks and 8-oxo-dGuo but usually at group level only and in line with our study (Leng et al. 2004; Marczynski et al. 2006; Yang et al. 2007). These levels were representative of grouped data. Together with previously published data from studies *in vitro* (Seike et al. 2004; Thielen et al. 2006; Park et al. 2008a, b) our results provide evidence that oxidative DNA damage is a relevant mechanism of PAH-induced genotoxicity in occupationally exposed workers and in the overall network of different mechanisms of PAH-induced genotoxicity.

However, group levels are difficult to interpret and can hardly be used to draw accurate conclusions of PAH-induced genotoxicity for an individual worker. Group levels also cannot be used to provide evidence whether or not a particular compound or group of compounds such as PAH specifically and exclusively cause genotoxic stress in humans. Therefore, we also studied the dose–response association on an individual level because statistical analyses of dose–response relations with larger sets of individual data from these industries are scarce. We could not find quantitative correlations between DNA strand breaks or 8-oxodGuo in WBC with any single shift measure of airborne PAH exposure at the individual level with the exception of a weak correlation between OTM and Σ PAH. No associations were also detected with internal exposure levels. Similar to our data, no association between 1-OHP in urine and 8-oxo-dGuo in blood could be found by Zhang et al. (2003). There are several reasons for the observed lack of associations between PAH exposure and DNA damage on an individual level in our study but also the study published by Zhang et al. (2003). The cross-sectional study design does not necessarily account for variability in exposures and long-term effects. A single shift measurement of air-borne PAH might not be sufficient to assess genotoxic effects. The assessment of internal exposure and effect also is based on single time points (all post shift) although post-shift sampling must be considered an appropriate time point to study oxidative DNA damage of PAH exposure because studies *in vitro* have shown an immediate increase of oxidative DNA damage (within hours) in terms of 8-oxo-dGuo and DNA strand breaks after exposure to PAH (Seike et al. 2004; Thielen et al. 2006; Park et al. 2008b). Nevertheless, a slight delay in response due to the *in vivo* situation cannot be ruled out completely. In addition, 1-OHP and Σ OHPHE in urine—although highly sensitive and specific—are derived by non-carcinogenic PAH and do not represent the internal dose of carcinogenic PAH compounds such as B[a]P. PAH are a complex mixture, several of them are complete carcinogens and their overall mode-of-action must be considered a conglomeration of different pathways within the exposure-disease continuum rather than a “one-way street” in terms of a particular mechanism such as oxidative stress. The complexity of the individual exposure

circumstances at the workplace, including co-exposures and varying routes of exposures (e.g., dermal exposure) are also possible reasons for the lacking association with individual data. With regard to co-exposures Courter et al. (2007) studied the confounding influence of particulate matter on CYP450 enzymes in a controlled animal experiment with PAH exposure. This experiment is of particular importance for the interpretation of epidemiological studies because high co-exposures to dust were observed at the workplaces in our study and previously in graphite-electrode workers by Merlo et al. (2004). Enzyme activities of CYP1A1, an important enzyme responsible for biochemical activation of PAH, were found to be inhibited in the presence of dust in a dose-dependent manner. This finding is remarkable keeping in mind that Courter et al. (2007) studied one single confounding factor only, whereas workers are exposed to a huge variety of different compounds which either can amplify or attenuate individual biological pathways of PAH-induced genotoxicity such as oxidative stress. Path analysis directly confirms the interpretation of our experimental results from a biological point of view (Qiu et al. 2007). Air-borne exposure to PAH is contributing about 60% to internal dose in terms of 1-OHP, whereas 1-OHP itself is contributing 9% only to the occurrence of DNA strand breaks in blood. The results by Qiu et al. (2007) and our results show that PAH exposure contributes to oxidative DNA damage but cannot be used exclusively as sensitive and specific predictor of oxidative DNA damage in humans and in varying types of industries. Co-exposures, as discussed above for converter and graphite-electrode workers, together with potential confounders increase the variation of the biological data and impede the assessment of associations between exposure to particular substances and biological effects on an individual level.

Summary

In conclusion, our analyses revealed high levels of external and internal PAH exposure in coke, refractory material, and graphite-electrode industry as well as in converter workers. Exposure at the workplaces by far exceeded exposure due to tobacco smoking. Internal dose in terms of 1-OHP and Σ OHPHE in relation to Σ PAH exposures indicated additional routes of exposure at various workplaces rather than inhalation only, in particular in the production of graphite electrodes, refractory materials, and in converter workers. We also observed increased levels of DNA damage in blood in exposed workers that were highest in the graphite-electrode production but no quantitative dose–response relations could be found on an individual level. The present study suggests the significance of exposures to multiple compounds, their interactions, and the importance to

discuss DNA damaging effects dependent on particular exposure circumstances at different workplaces. In addition, the underlying biological processes which are capable to influence pathways such as oxidative stress must be taken into account.

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