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Environmental and biological monitoring of occupational exposure to polynuclear aromatic hydrocarbons during highway pavement construction in Italy

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ABSTRACT

Objectives: We performed a cross-sectional study with the main aim of evaluating occupational exposure to polycyclic aromatic hydrocarbons (PAHs) in workers involved in the pavement construction of a new highway in Northern Italy, where modified bitumen was used as binder for Hot Mix Asphalt.
Methods: We applied a combined approach of air and biological monitoring. Both the aerosol and vapour phases of bitumen were collected applying the NIOSH 5506 method. The 16 PAHs listed as high priority by EPA were determined by HPLC-UV. End-of-shift urine samples were collected from 144 workers to determine 1-hydroxypyrene (1-OHP) and 2-naphthol (2-NAP) concentrations after enzyme digestion and HPLC-UV analysis. Sociodemographic and lifestyle information was collected by a questionnaire.
Results: Paving workers were actually exposed to PAHs, including carcinogenic compounds, that were measurable only in the aerosol phase. Higher exposure as well as dose levels were measured for the paver group. Biological monitoring confirmed that 1-OHP was less affected by smoking habits as compared to 2-NAP and showed a higher association with occupational exposure.
Conclusion: Carcinogenic PAH compounds were detectable only in the aerosol phase and this must be taken into account in the adoption of preventive measures. Biomonitoring supported the superiority of 1-OHP as compared to 2-NAP in assessing the internal dose in such workers.

1. Introduction

Bitumen is an organic binder currently used for several engineering applications; it is mainly used for construction of transportation infrastructures and roofing materials and treatments. Nowadays, bitumen is produced by a number of distillation processes of crude oil during petroleum refining. Chemically, it is a complex mixture of naphtenic, aliphatic and/or aromatic hydrocarbons, polycyclic aromatic hydrocarbons (PAH) and heterocyclic compounds containing sulphur, nitrogen, oxygen and metals (IARC, 2013). Bitumen is quite different from coal-derived products, such as coal-tar and coal-tar pitch, which contain much higher concentrations of PAH (particularly in the 3- to 7-ring size range), whereas bitumen contains higher concentrations of paraffinic and naphthenic hydrocarbons (Puzinauskas and Corbett, 1978). Hot Mix Asphalt (HMA) and bituminous mixture are the terms currently used for a mixture of coarse and fine aggregates, sand, filler and bitumen (normally added at about 5% of total weight) that is used as bearing layer in the multilayer system composing a transportation infrastructure (road, airport, railway sub-ballast, harbour, *etc.*). Because of the temperature dependency of bitumen, it is necessary to increase the temperature of the mixture with the goal of reducing bitumen viscosity and to make the HMA able to form a layer having a suitable bearing capacity in the pavement multilayer system. In the short period in which the HMA is at high temperature, few hours from plant production to the end of laying and compaction, hot bitumen releases vapours of volatile organic compounds, which condensate into aerosol in the air. Depending on the technological process used to produce and lay down the bituminous mixture, paving bitumen (CAS No. 8052-42-4)

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must be heated at temperatures ranging from 100 to 130 °C (Warm Mix Asphalt) to 160–180 °C (HMA). The higher temperature range (160–180 °C) is normally used to reduce the viscosity of a particular kind of bituminous binder, named modified bitumen, currently used in Europe for heavy load applications (*e.g.* highways, airports, harbours, *etc.*). In the refinery, it is obtained by blending a standard bitumen with some additives including polymers, crumb rubber, elastomers, sulphur, polyphosphoric acid and other products (IARC, 2013), with the aim to increase the binder performance. Such additives can variously affect the composition of bitumen fumes. Warm-mix asphalt showed 30–60% reduction of total organic matter in the workers' breathing zone as compared to HMA (Kriech et al., 2011). The addition of waste recycled latex and natural bitumen produced better quality bitumen with lower PAH emissions than conventional bitumen (Rasoulzadeh et al., 2011). On the other hand, higher aldehyde concentrations were measured in fumes from asphalt containing waste plastic and talloil as compared to conventional asphalt (Väänänen et al., 2006). Such fumes were also more genotoxic (Lindberg et al., 2008). Apart from additives, the concentration of PAHs in bitumen emissions is a function of the mixture's working temperature (Cavallari et al., 2012; Binet et al., 2002), even if field studies have demonstrated that bitumen emissions are not simply predictable according to the chemical composition and temperature application (Watts et al., 1998; Kriech et al., 2004).

During construction works, laying and compaction of HMA are performed by worker teams that include pavers, screed operators, raking workers and rollers. Pavers drive the paver machine, which receives the HMA from the delivery truck and distributes it to form a layer on the ground. Screed operators and rakers follow the paver machine, controlling the spread and levelling of the HMA's layer. Rollers drive roller compactors and have the mobility to work at varying distances from the paving machine (NAPA and EAPA, 2011).

Bitumen fumes have recently been classified as Group 2B carcinogens by the IARC (2013). Carcinogenic compounds present in bitumen fumes include PAH mixtures containing Benzo[*a*]pyrene (BaP), which have recently been classified by the European Union (EU) Scientific Committee of Occupational Exposure Limits (SCOEL) as group A carcinogens, *i.e.* genotoxic carcinogens for which no health based occupational exposure limit is applicable (SCOEL, 2016). The US Environmental Protection Agency (EPA) has identified 16 PAH compounds as High Priority Pollutants (EPA_{HPP}): Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benz[*a*]anthracene, Chrysene, Benzo[*b*]fluoranthene, Benzo[*k*]fluoranthene, Benzo[*a*]pyrene, Dibenz[*a,h*]anthracene, Benzo[*ghi*]perylene and Indeno[1,2,3-*cd*]pyrene. The EU classifies Benz[*a*]anthracene, Benzo[*b*]fluoranthene, Benzo[*j*]fluoranthene, Benzo[*k*]fluoranthene, BaP, Chrysene and Dibenz[*a,h*]anthracene as 1B carcinogens (presumed to have carcinogenic potential for humans), whereas Naphthalene is classified as a group 2 carcinogen (suspected of having carcinogenic potential for humans) (Regulation (EU) No, 1272/, 2013).

In the occupational setting, the workers' exposure to PAH mixtures should be periodically assessed by environmental and/or biological monitoring. The urinary concentration of 1-hydroxypyrene (1-OHP) is the most used biomarker of internal dose, as it is the most reliable and practical marker for monitoring PAH exposure at the individual or group level (IARC, 2010; Dor et al., 1999). A Biological Exposure Index (BEI®) of 2.5 μg/l [about 1.79 μg/g creatinine (creat.), assuming an average urine creatinine (creat.) concentration of 1.4 g/L] at the end of shift, end of workweek, adjusted for the pyrene- to-BaP ratio of the air mixture the workers are exposed to, has been first proposed in 2017 by the American Conference of Governmental Industrial Hygienists (ACGIH, 2017). The Agency also mentions 3-hydroxybenzo[*a*]pyrene with the note Nq, which means that it is recommended to measure it but no BEI® can be given for it yet. For 1-OHP, SCOEL has proposed a biological guidance value (BGV) of 0.5 μg/g creat., representing the 95th percentile of 1-OHP in general (occupationally unexposed) population. Again, SCOEL cites 3-hydroxybenzo[*a*]pyrene as a possible future biomarker for PAH exposure (SCOEL, 2016). In UK, a biomonitoring benchmark value for occupational exposure of 4 μmol/mol creat. (about 7.7 μg/g creat.) is in effect for 1-OHP, based on the 90th percentile of levels measured in workers in good workplaces (Unwin et al., 2006). 1- and 2-Naphthol (NAP) are the main phase 1 metabolites of naphthalene and are excreted by urine in similar amounts (Preuss et al., 2003). 1-naphthol can derive also from the insecticide carbaryl (Sams, 2017). ACGIH proposes the sum of urinary concentration of both metabolites as biomarkers of exposure to naphthalene but yet without indicating a BEI® value (ACGIH, 2017). According to the Italian Society for Reference Values (Italian Society for Reference Values (SIVR, 2011), the 95^{th} centile of reference values for 1-OHP is set at 0.3 and 0.7 μg/g creat. for non-smokers and smokers, respectively, whereas for 2-NAP the corresponding value is set at 8 μg/L, irrespective of smoking habits.

The present cross-sectional study was performed during a three-year period (2013–2015) with the main aim of characterising the occupational exposure to the 16 EPA_{HPP} PAH of workers involved in the construction of a new highway in Northern Italy by a combined approach of air and biological monitoring. For the latter purpose, the end-of-shift urine concentrations of 1-OHP and 2-NAP were determined as biomarkers of internal dose.

2. Methods

2.1. Study design, setting and subjects

We performed a cross-sectional study in the context of a prevention programme on the road paving sector by the local Health Protection Agencies. The programme involved 14 companies working on the paving of a new highway in Northern Italy. The safety data sheets of raw materials indicated that modified bitumen was used as binder for the HMAs. One-hundred and forty-four male workers were enrolled, with a mean age of 44 ± 10 (range 22–62) years and employment duration of 14 ± 9 (range 0.25–40) years. Each worker was interviewed by a skilled physician, using a structured questionnaire to collect information about occupational and non-occupational exposure to PAHs, from smoking habits, residence (urban *vs* rural; traffic), diet (grilled food in the last 3 days) or drug consumption. The worker sample included 63 current smokers, smoking on average 8 ± 11 cigarette/day (range 3–40). A spot urine sample was collected from each worker at end-of-shift (ES) after at least three work-shifts. Biological samples were collected in the context of the workers' mandatory health surveillance programme and in agreement with the Helsinki Ethical Principles for Medical Research Involving Human Subjects. All enrolled subjects gave their written informed consent.

2.2. Air sampling and analysis

Air sampling of vapours and aerosols of HMA application and subsequent laboratory analyses were performed following strictly the NIOSH 5506 method (available at: https://www.cdc.gov/niosh/docs/2003-154/pdfs/5506.pdf). Air sampling lasted at least 4 h. Four sampling campaigns were performed between March 2014 and July 2015, collecting overall 9 stationary and 8 personal air samplings. Data collected during tunnel paving (1 personal and 3 stationary samplings) were excluded from the analysis because of problems significantly affecting the quality and reliability of collected data (high wind speed -see below- and very low working time). Four stationary samplers were fixed to the paver machine and the remaining two were placed in open field sufficiently far from the working area (background air sampling). Personal samplers were worn by 3 pavers, 3 ground operators and 1 roller. During each sampling day, meteorological variables were measured, including temperature (15.6–25.4 °C range), relative humidity (0.25–0.69% range) and wind speed (0.46–4.50 m/s range). The limit of detection (LOD, absolute weight) of the analytical method varied according to the different compounds, as follows: 500 ng for

Acenaphthylene, 25 ng for Naphthalene, 20 ng for Acenaphthene, 5 ng for Fluorene, 0.40 ng for Indeno[1,2,3-*cd*]pyrene, 0.20 ng for Fluoranthene, 0.10 ng for Phenanthrene, Anthracene, Pyrene and Chrysene, 0.050 ng for Benz[*a*]anthracene and Benzo[*b*]fluoranthene, 0.04 ng for Dibenz[*a,h*]anthracene, 0.03 ng for Benzo[*ghi*]perylene, 0.02 ng for Benzo[*k*]fluoranthene and BaP. In the days of air sampling, there was no concomitant traffic from vehicles different from those of the working area.

2.3. Biological monitoring

One-hundred and forty-four ES spot urine samples were collected during 11 collection rounds between September 2013 and July 2015. 1-OHP was determined according to a published method (Jongeneelen et al., 1985) with minimal changes. Briefly, samples underwent enzymatic hydrolysis with beta-glucuronidase aryl-sulfatase (from Elix pomatia, Merck KGaA, Darmstadt, Germany) at 37 °C ON and concentration on SPE C18 3 ml columns with methanol (Supelco, Bellefonte, PA, USA). About 20 ml of the eluate was analysed by high-performance liquid chromatography with fluorimetric detection (λ excitation 242 nm; λ emission 388 nm) using Supelcosil LC-18 DB columns (Supelco, Bellefonte, PA, USA).

The method for 2-Naphtol differed from the previous one for the addition of acetonitrile and centrifugation after enzymatic digestion and for the characteristics of fluorimetric detection (λ excitation 227 nm; λ emission 355 nm). For both biomarkers, the ClinCheck® control-*lyophilised human urine for toxic organic compounds* (Recipe, Munich, Germany) was used for accuracy and precision of the analytical technique, the latter being less than 9% (as coefficient of variation) in both cases. The LODs for 1-OHP and 2-NAP were 0.05 and 0.5 μg/l, respectively. Urinary creatinine was determined by the method of Jaffe (Kroll et al., 1986). Fifteen samples were excluded from subsequent analysis as urine creatinine concentration did not meet the WHO exclusion criteria for very diluted (creatinine concentrations lower than 0.3 g/L) or very concentrated (creatinine concentration higher than 3.0 g/L) urine samples (World Health Organization (WHO, 1996). In order to allow the comparisons with the respective Italian reference values (Italian Society for Reference Values (SIVR, 2011), the 1-OHP levels were normalised to creatinine whereas 2-NAP levels were not normalised at all.

2.4. Statistical analysis

The statistical analysis was performed using the IBM SPSS Statistics 23 for Windows™. A value corresponding to half the analytical LOD was attributed to undetectable air or urine concentrations. In some cases (reported in Table 1), PAH concentrations could not be determined, owing to analytical interferences on the chromatographic run. The variable distributions were evaluated by the Kolmogorov-Smirnov test: most of the PAHs showed a log-normal distribution, whereas the biomarkers showed an abnormal distribution also after logarithmic transformation. Either the Mann-Whitney *U* or the Kruskal-Wallis test were respectively used to evaluate differences between two or more groups, whereas the Spearman's rank correlation test was applied to evaluate the relationships among variables. Analysis of covariance (ANCOVA) was performed to study the relationships among 1-OHP or 2-NAP as dependent variables and smoking habits, sampling month and job-task as independent variables. 1-OHP and 2-NAP concentrations were transformed into dummy variables (higher than and within the 95th centile of the Italian reference levels for the respective biomarker) and were set as dependent variables into bivariate logistic regression models, considering the job-task and smoking extent (no. of cigarette/day) as independent variables. Such results were further tested by generalised liner models (fixed effects). Then, the dependence of both 1-OHP and 2-NAP (dependent variables) on current smoking habits [either the dichotomous (yes *vs* no) or the continuous (no. of cigarette/day) variables] and the job-task was tested by IBM SPSS Neural Network analysis following the Multilayer Perceptron (MLP) procedure while performing a sensitivity analysis in order to compute the importance of each predictor in determining the neural network. For all analyses, a two-sided *p* value < 0.05 was considered as significant to reject the null hypothesis.

3. Results

3.1. Air monitoring

The distributions of PAH air concentration levels are reported in Table 1. Overall, PAH air levels were higher in the stationary (nearby the paver machine) as compared to personal samplings, the difference being statistically significant for anthracene, BaP and dibenz[*a,h*]anthracene ($p < 0.05$). PAH concentrations in the working area were constantly higher than background environmental concentrations. The pyrene-to-BaP ratio was lower in stationary (paver machine) than personal samples, whereas the sum of PAH concentrations showed an opposite trend. PAHs in the vapour phase were always higher than in the aerosol one and EU carcinogenic PAHs were detectable only in the particulate phase. Table 2 summarises the distributions of PAHs measured by personal samplings. On average, the overall PAH exposure was higher for pavers. Fig. 1 shows the relationships between the sum of PAHs and both pyrene (Fig. 1A) and naphthalene (Fig. 1B) air concentrations. Both regressions are significant, with a higher strength for the first.

3.2. Biological monitoring

Seventy-three out of 129 samples exceeded the 95th centile of the Italian RVs for 1-OHP (0.7 μg/g creat. and 0.3 μg/g creat. for smokers and non-smokers, respectively), most of them (55%) being ground workers, and 70 samples (44% ground workers) the 95th centile of the Italian RVs for 2-NAP (8 μg/L, irrespective of smoking habits). Fourteen workers exceeded the ACGIH BEI® value for 1-OHP of 2.5 μg/L, most of them (57%) being again ground workers. However, only one paver exceeded the BEI® value of 7.34 μg/l resulting from adjustment by the pyrene-to-Bap ratio, considering the worst exposure scenario in our context. Fifty-eight workers exceeded the SCOEL BGV, most of them (67%) being again ground workers. As expected, both 1-OHP and 2-NAP concentrations were significantly affected by smoking habits with average values about 2- (medians 1.23 *vs* 0.55 μg/g creat.) and 6-fold (median 26.90 *vs* 4.30 ng/l) higher in smokers than non-smokers ($p < 0.0001$, for both the biomarkers, Mann-Whitney U test), respectively. Both biomarkers, in particular 2-NAP, were significantly correlated to cigarette/day number (Spearman's *rho* 0.72 and 0.34 for 2-NAP and 1-OHP, respectively; $p < 0.0001$ for both). 1-OHP but not 2-NAP concentrations were significantly affected by the season (medians of 1.05 *vs* 0.69 μg/g creat., in spring-summer *vs* autumn-winter samplings, $p < 0.01$, Mann-Whitney U test), the finding being supported also by the inverse relationship between 1-OHP concentrations and the sampling month as ordinal variable (Spearman's *rho* -0.18, $p < 0.05$). No other significant effect was found for variables collected by the questionnaires. Table 3 shows the biomarker distributions in workers stratified by the job-task, as they result from the univariate Kruskal-Wallis analysis and by ANCOVA models. In the latter, the biomarker is set as dependent variable, the job-task as fixed factor and the cigarette/day number and the creatinine concentration as covariates. In ANCOVA models, the effect of season on both the biomarkers was not significant hence it was excluded from the models. Both analyses show a significant trend of reduction of $1-$OHP values across jobs in the following order: paver driver > ground worker > roller-driver > truck-driver/other. ANCOVA shows that 1-OHP values are influenced by the job-task ($p < 0.0001$) more than smoking ($p = 0.002$ for cigarette/day no.). On the other hand, 2-NAP values are not significantly affected by

Table 1
Geometric means (GM) and standard deviations (GSD) of PAH air concentrations (ng/m^3) in the working area and in the general environment.

PAHs	Stationary samplers (N = 4)				Personal samplers (N = 7)				Environment	
	> LOD	N[#]	GM	GSD	> LOD	N[#]	GM	GSD	Sample 1	Sample 2
Naphthalene	4	–	1229.88	3.28	7	–	759.82	1.9	23.50	158.40
Acenaphthene	3	1	245.7	12.31	6	1	285.93	14.37	10.00	66.70
Acenaphthylene	1	2	539.96	5.59	0	1	250	1	< LOD	< LOD
Anthracene	4	–	40.12*	10.06	7	–	20.39	2.69	0.05	0.20
Phenanthrene	4	–	519.83	6.52	7	–	578.85	5.36	6.30	18.40
Fluorene	1	2	82.11	10.71	1	1	4.58	4.4	< LOD	< LOD
Benz [*a*] anthracene	4	–	147.19	25.55	7	–	202.22	4.47	0.30	0.60
Chrysene	3	1	42.39	10.74	2	5	5.65	3.36	0.05	0.40
Fluoranthene	4	–	33.38	89.07	7	–	91	8.83	0.20	16.30
Pyrene	4	–	168.41	13.23	7	–	182.43	4.7	1.10	1.90
Benzo [*a*] pyrene	4	–	22.93*	9.9	7	–	12.12	3.31	0.01	0.20
Benzo [*b*] fluoranthene	3	1	44.31	12.46	2	5	15.89	1.9	0.03	0.40
Benzo [*k*]fluoranthene	4	–	3.89	33.34	7	–	6.08	4.75	< LOD	< LOD
Dibenz [*a,h*] anthracene	3	1	7.16*	8.48	7	–	2.46	4.13	0.02	0.20
Benzo[*ghi*]perylene	3	1	15.44	4.43	7	–	6.17	4.43	0.02	0.30
Indeno[1,2,3-*cd*]pyrene	3	1	1.68	8.45	6	–	3.11	4.19	< LOD	< LOD
Pyrene/Benzo[*a*]pyrene			7.34	1.81			15.05	1.61	5.50	190.00
Σ PAH			5182.37	5.54			3490.13	2.88	368.18	442.81
Σ PAH $_{Aerosol}$			835.9	5.43			1572.22	3.51	277.19	278.08
Σ PAH $_{Vapour}$			2623.55	4.92			2340.42	2.38	366.52	439.15
Σ PAH $_{2\text{-}3\ rings}$			3270.71	4.18			2506.76	3.01	349.05	439.50
Σ PAH $_{4\text{-}6\ rings}$			734	18.78			810.82	3.35	3.31	19.13
Σ PAH $_{Ca.\ EU}$			355.57	16.33			280.14	3.14	0.72	1.51
Σ PAH $_{Ca.\ EU\ Aerosol}$			128	14.43			353.49	2.88	0.72	1.41
Σ PAH $_{Ca.\ EU\ Vapour}$			< LOD	< LOD			< LOD	< LOD	< LOD	< LOD

Σ: sum; *ca.* EU: carcinogenic according to the EU classification.
[#] No. of samples in which the compound could not be determined because of analytical interferences.
* $p < 0.05$, concentrations in stationary *vs* personal samples.

Table 2
Distributions of the air concentrations of PAHs (ng/m^3), measured by personal samplers on seven workers. GM = geometric mean; GSD = geometric standard deviation; Σ = sum.

PAHs	Pavers (N = 3)		Ground workers (N = 3)		Roller (N = 1)
	GM	GSD	GM	GSD	Values
Benzo[*a*]pyrene	6.66	2.95	20.3	4.34	15.6
Pyrene/Benzo[*a*] pyrene	12.33	2.05	16.8	1.25	19.65
Σ PAH	2412.8	3.14	4870.1	3.64	3,887.9
Σ PAH $_{Aerosol}$	895.19	3.49	2344.04	5.02	1,463.4
Σ PAH $_{Vapour}$	1800.06	4.37	2658.24	2.31	2,700.3
Σ PAH $_{2\text{-}3\ rings}$	1556.69	3.02	3876.72	3.8	2,830.0
Σ PAH $_{4\text{-}6\ rings}$	622.2	5.52	966.96	3.1	1,057.9
Σ PAH $_{Ca.\ EU}$	147.97	4.99	401.04	1.54	647.9

ca. EU: carcinogenic according to the EU classification

the job-task in the univariate analysis whereas ANCOVA shows decreasing values in the order paver driver > ground workers > roller drivers. The truck-driver/other task group shows values similar to the ground worker but not significantly different from the roller-driver group. 2-NAP values are influenced by cigarette smoking ($p < 0.0001$) more than the job-task ($p = 0.004$). Table 4 summarises results of bivariate logistic regression models in which 1-OHP or 2-NAP values, dichotomised according to the 95th centile of Italian RVs, are set as dependent variables, and both job-task and cigarette/day number as independent variables. 1-OHP values exceeding the 95th centile of Italian RVs are significantly associated with different job-tasks, with a non-significant trend of values resembling that of ANCOVA and univariate analysis, but not with the extent of smoking habits. The likelihood of the model, correctly classifying 71% of samples, is supported by the Hosmer and Lemeshow test ($p = 0.128$). On the other hand, 2-NAP values higher than the 95th centile of Italian RVs are significantly associated in particular with the cigarette/day no. ($p < 0.0001$) and to

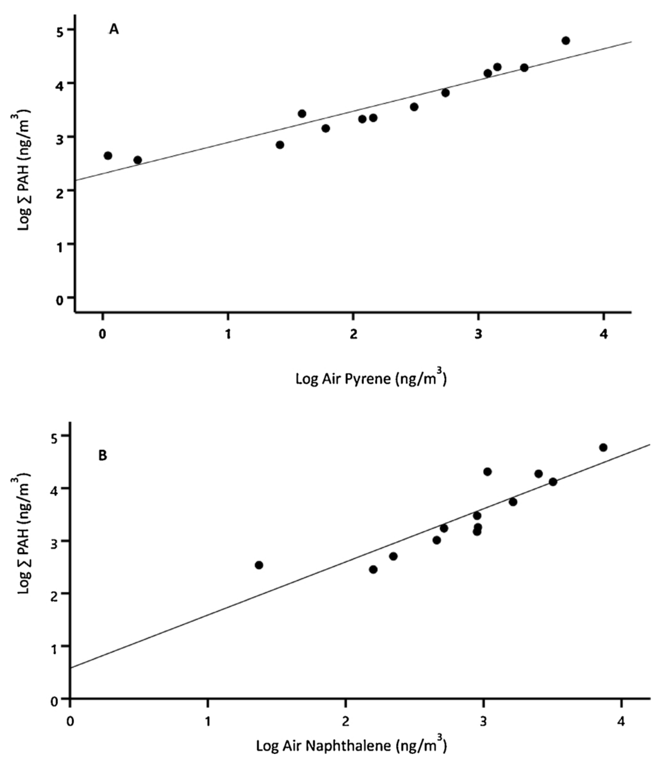

Fig. 1. Regression between the Log of the sum (Σ) of air PAH levels (less Pyrene in A and less Naphthalene in B) and A): Log Pyrene concentrations (equation: 2.31 + 0.58 X, $R^2 = 0.91$, $p < 0.0001$), B): Log Naphthalene concentrations (equation: 0.58 + X, $R^2 = 0.78$, $p < 0.0001$).

a lesser extent with the job-task. Again, the Hosmer and Lemeshow test ($p = 0.79$) supports the likelihood of the model, which correctly classifies 79% of the samples. Confirmatory results on such findings were

Table 3
Distributions of 1-idroxypyrene (1-OHP) e 2-Napthol (2-NAP) in workers stratified by job task. The marginal means (M) (and standard errors, SE) resulting from covariance analysis (ANCOVA) are shown in the right-hand columns.

Job task	1-OHP, µg/l		2-NAP, µg/l	
	Median (95thile; min-max)	M[c] (SE)	Median (95thile; min-max)	M[c] (SE)
Pavers (n = 21)	1.40 (7.31; 0.15–7.70)	1.59 (0.23)	18.40 (70.07; 2.30–70.90)	23.96 (2.46)
Ground workers (n = 61)	1.03 (3.83; 0.13–6.11)	1.50 (0.14)	8.30 (45.07; 0.20–63.10)	17.46 (1.45)[§]
Roll-drivers (n = 27)	0.63 (3.15; 0.04–3.38)	0.90 (0.21)[**]	10.00 (48.36; 0.30–55.0)	11.77 (2.19)[§§,Δ]
Truck-drivers/Others (n = 21)	0.26 (1.78; 0.01–1.79)	0.39 (0.24)[***]	7.50 (73.29; 1.20–74.90)	14.84 (2.45)[§]
P	[a] < 0.0001	[b] < 0.0001	NS	[b] < 0.0001

[a] Kruskal-Wallis analysis.
[b] ANCOVA.
[c] marginal means calculated at average covariate values of 8 cig./day and 1.68 g/l creatinine (covariance analysis).
*** $p < 0.0001$ *vs* ground workers and pavers.
** $p < 0.05$, *vs* ground workers and pavers.
§ $p < 0.05$ *vs* pavers.
§§ $p < 0.0001$ *vs* pavers.
Δ $p < 0.05$ *vs* ground workers.

Table 4
Results of binomial logistic regression, in which workers are stratified according to the 95th centile of Italian reference values for the investigated biomarkers (dependent variable in the models) and job-task, taking into account also the extent of tobacco smoking (independent variables in the models). Odds Ratios and 95% confidence intervals are shown (OR, 95% CI).

Variables		1-OHP		OR (95% CI)
		> 95th ile RV	≤95th ile RV	
Job-tasks	Truck drivers/others	19	1	Ref.
	Rollers	15	12	24.36 (2.81–211.04)*
	Ground Workers	40	21	40.95 (4.94–339.22)**
	Pavers	17	4	88.25 (8.75–890.12)***
Cigarette/day				1.02 (0.98–1.06)
Variables		**2-NAP**		**OR (95% CI)**
		> 95th ile RV	≤95th ile RV	
Job-tasks	Truck drivers/others	10	11	Ref.
	Rollers	15	12	1.66 (0.26–10.45)
	Ground Workers	31	29	3.02 (0.62–14.73)
	Pavers	14	7	6.25 (1.01–38.65)*
Cigarette/day				1.27 (1.15–1.40)***

*** $p < 0.0001$.
** $p = 0.001$.
* $p < 0.005$.

obtained by mixed models analysis (data not shown). The neural network analysis by Multilayer Perceptron models shows (Fig. 2) that 1-OHP values are mostly influenced by the job-task (0.56 importance) than by the cigarette/day (0.32 importance) and an opposite behaviour was found for 2-NAP (relative importance of 057 *vs* 0.31 for cigarette/day and job-task, respectively).

4. Discussion

In the case study here described of an HMA working area for a highway construction, in which modified bitumen was used as binder, the occupational inhalation exposure to PAH was of the same order of magnitude as the German Human Bitumen Study (Breuer et al., 2011), but lower than observed in a Finnish study on modified asphalt paving (Väänänen et al., 2006). The pyrene-to-BaP ratio values ranged from about 7 to 15, on average, and thus higher than the 2.5 value found in the air of coke ovens and in coal tar pitch binder used to produce aluminium reduction electrodes (IARC, 1985). SCOEL (2016) has calculated that a mean airborne 8h-TWA exposure over 40 working years in the order of 6 ng Bap per m^3 would lead to an excess lung cancer mortality rate of 4×10^{-5}. In 9 out of 13 air samples (69%), B[a]P concentrations exceeded 7 ng/m^3, a value that the German Federal Institute for Occupational Safety and Health (BAuA) has indicated as the concentration below which the occupational risk to develop cancer by inhalation exposure is *acceptable* (adjunctive risk of 4×10^{-5} lung cancers). In such samples the concentrations were in the range (upper limit 700 ng/m^3) corresponding to a *tolerable* risk level (adjunctive risk of lung cancer between 4×10^{-5} and 4×10^{-3}) (Committee on Hazardous Substances - AGS management – BauA, 2016). However, such results require caution and cannot be generalised, owing to the low number of air samplings and considering that they are limited to just one of the large number of engineering technologies currently available on the EU market for producing, laying and compacting bituminous layers for transportation infrastructure pavements. As a further study limitation, we have not adequately considered the possible confounding role of diesel exhausts from work vehicles that may have influenced air and biological samplings (IARC, 2014) and of diesel oil commonly used by paving workers as a cleaning agent (Weker et al., 2004). A useful preventive piece of information resulting from our study is that the EU carcinogenic PAH species were detectable only in the aerosol phase, hence in this context primary prevention interventions to reduce the inhalation of airborne particulate matter should be

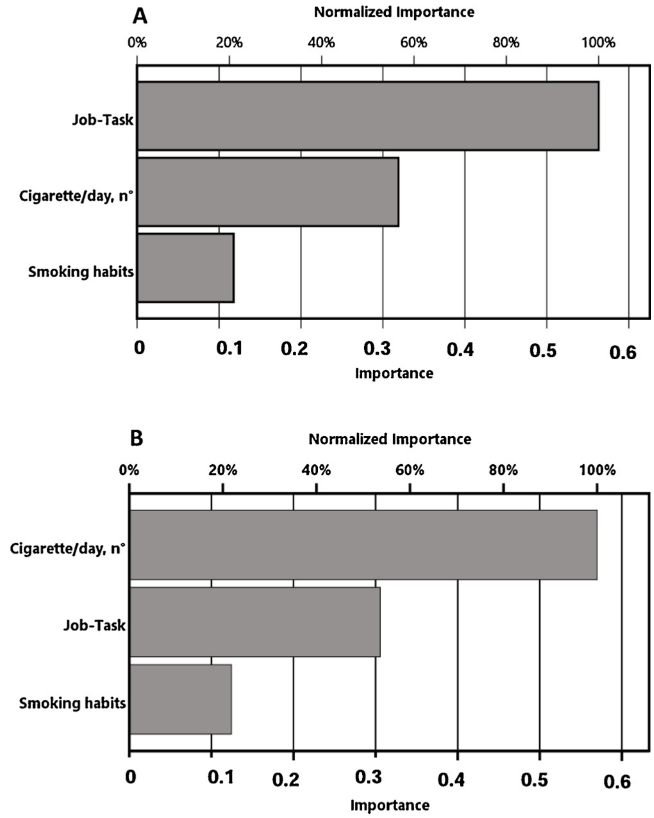

Fig. 2. Predictive models resulting from the Multilayer Perceptron procedure for 1-hydroxypyrene (2 A) and 2-naphthol (2B). The first is mainly influenced by the job-task, the other by the extent of tobacco smoking.

developed and adopted. Skin contamination, however, can be a main absorption route for PAHs (McClean et al., 2007) and in this regard biomonitoring can be more informative than air monitoring for the assessment of the internal dose that may result from a combination of both inhalation and dermal absorption. Despite the limitation of deriving from a non-carcinogenic parent compound, the validity of 1-OHP as a tracer internal dose biomarker in PAH exposed workers has long been recognised (Dor et al., 1999). In this study, we have associated the determination of 2-NAP (deriving from a 2-ring PAH compound), as PAH biomarker representative of more volatile PAH species. Owing to the low number of personal air samplings hindering the evaluation of exposure-dose relationships, we evaluated the regressions between the overall PAH concentrations and the levels of the biomarkers' parent compounds (pyrene and naphthalene) in the mixture, obtaining in both cases highly significant relationships that supported the biomarker choice. According to previous estimations on coke oven workers, where the pyrene-to-Bap ratio is on average 2.5 (Jongeneelen, 2001), an ES 1 − OHP value of 4.44 μg/g creat. was proposed as a benchmark guideline corresponding to an air BaP concentration of 2 $\mu g/m^3$. On the basis of such relationship, and taking into account that in our context the pyrene-to-BaP ratio was about six-fold higher (personal samplings), at an observed geometric mean value of 1-OHP of 0.48 μg/g creatinine, we can estimate a BaP concentration of about 30 $\mu g/m^3$, slightly higher than actually measured.

Observed 1-OHP values were slightly higher [on average, 0.55 (our study) *vs* 0.42 μg/l (Pesch et al., 2011), considering only non-smokers] than observed in a large German study on workers using mastic asphalt, lower than previously determined in the Finnish study on workers involved in modified asphalt paving [0.36 (our study) *vs* 0.89 μg/g creat. (Väänänen et al., 2006), considering only non-smokers] and comparable to values determined in a US study on HMA workers [geometric means of 0.77 (our study) *vs* 0.70 (McClean et al., 2012)]. The latter study demonstrated that dermal absorption of PAH was a main determinant of 1-OHP values in HMA workers (McClean et al., 2012). According to such results, we may hypothesise that also in our exposure scenario skin absorption of PAH would have greatly contributed to observed 1-OHP values.

In agreement with air determinations, biomonitoring, in particular 1-OHP data, showed that slightly more than half of the worker group considered in this study showed biomarker values higher than the upper limit of the Italian RVs and in most of the cases, they were ground workers. It is well known that both 1 − OHP and 2-NAP values are affected by strong confounding by smoking habits, but in the present study overall statistical analyses have demonstrated that 1-OHP is less affected as compared to 2-NAP by such interference. In fact, both univariate and multivariate analyses showed clear and consistent associations and trends between 1-OHP values and the job-task, a variable that we can consider a true marker of the occupational context. On the other hand, this could not be observed for 2-NAP. The distribution of 1-OHP values in workers performing different job tasks was quite consistent with the results of air monitoring and well compatible with the position of different workers in respect of the source of bitumen-related emissions. Pavers are exposed to fumes released from HMA that is discharged by the trucks into the paver machine; ground workers are exposed to HMA that has been laid down by the paver machine and the roller is exposed to a bitumen that is almost cooled after the previous processes. To the best of our knowledge, this is the first time that the Neural Network analysis has been applied in the occupational biomonitoring field. Using the MLP procedure, we could verify a lower dependence of 1-OHP from smoking habits, as compared to NAP-2 values.

Both SCOEL (2016) and ACGIH (2017) indicate 3-hydroxybenzo[*a*]pyrene (3-OHBap) as a biomarker for PAH biomonitoring, even if neither agency has yet proposed a specific biological value. To the best of our knowledge, there is only one study in literature measuring urinary 3-OHBap values in asphalt workers but the biomarker was undetectable (Szaniszló and Ungváry, 2001). Further research on this topic is advisable.

5. Conclusions

The present study confirms previous findings of significant exposure to PAHs among HMA workers involved in construction of transportation infrastructures. Exposure and dose levels were consistent with each other, showing higher levels on the paver machines and among pavers. PAHs classified as 1B carcinogens by the EU were detectable only in the particulate phase. BaP levels were constantly higher than the EU guideline value for the general environment and in most of the cases higher than the BauA standard corresponding to an acceptable risk. The biomonitoring results support a higher relevance of 1-OHP as compared to 2-NAP for the risk assessment of HMA exposed workers.

Conflict of interest statements

None to declare.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxlet.2018.06.005.

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