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Original Article

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Polycyclic Aromatic Hydrocarbons Exposure Assessment in a Refractory Brick Production

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Abstract

In a refractory brick manufacturing company a qualitative and quantitative determination of the sources of occupational exposure to polycyclic aromatic hydrocarbons (PAHs) was obtained in order to validate targeted hygiene measurements. The study included the assessment of PAHs contamination of work surfaces by wipe-sampling, cutaneous exposure by hand washing, contamination of personal protective equipments (gloves) by extraction in solvent, and airborne PAHs concentration in atmospheric samples. Biomonitoring was also carried out by measurement of urinary 1-hydroxypyrene (1-OHPU) in three groups of workers (packaging, production, and controls). The surface contamination sampling was performed in production, packaging, and in other departments (external area) in theory less contaminated by PAHs. Two different areas were identified within the production, one included surfaces that were regularly cleaned (A area) and one included data from non-cleaned surfaces (B area). To confirm the source of exposure, a clear correspondence was observed between the percentage of the single compounds in the binder and those measured in wipes and air samples. As far as the wipes are concerned, the concentrations of phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)pyrene (BaP), and the total PAHs mixture were higher in the B area than the A area of production. The same happened between the A area and the other two departments. According to results of the statistical analysis, these differences were significant. These results were confirmed by the hand washing data and the analysis of PPE. On the other hand, a marked difference does not exist between the packaging department and the external area. In air samples, the differences were much less evident with only higher concentrations of anthracene and total PAHs between production as a whole and the other two departments. Biological monitoring

showed 1-OHPU values significantly higher in production workers than in packaging workers. In conclusion, the analysis of the wipes demonstrated that the production B area has a higher surface contamination compared to the production A area and the packaging department. In the absence of a significant difference in air concentrations of PAHs between A and B areas, this is attributable to surfaces not subject to cleaning. Results confirm that the measurement of surface contamination represents a valid tool for the assessment of sources of exposure to PAHs in the workplace.

Keywords: biological monitoring; benzo(a)pyrene; occupational exposure; pyrene; wipes

Introduction

In the last decade, emerging data has led to a reconsideration of the toxicological characteristics of polycyclic aromatic hydrocarbons (PAHs). In particular, the International Agency for Research on Cancer (IARC) not only reassessed the carcinogenicity of some industrial processes, but also assigned for the first time the *sufficient evidence* in humans for the carcinogenicity of occupational exposures (Group 1) to a single polycyclic aromatic compound (i.e. benzo(a)pyrene—BaP) (IARC, 2010, 2012). A number of papers took into consideration dermal exposure to PAHs generally demonstrating the importance of the cutaneous route (Jongeneelen *et al.*, 1988; Van Rooij *et al.*, 1992, 1993, 1994; Sobus *et al.*, 2009). Despite the toxicological interest in these compounds, there is a lack of occupational hygienic assessments taking into account both the sources of cutaneous and respiratory exposure, in particular, the measurement of surface contamination at the workplace. In the absence of exposure data, it is not possible to formulate a correct risk management (i.e. prevention and protection measures).

The study follows a previous research aimed at defining the main route of exposure to PAHs in workers involved in the production of refractory bricks (Sartorelli *et al.*, 2018). In the present study, a qualitative and quantitative determination of the sources of occupational exposure to PAHs was obtained by carrying out measurements on surfaces, in air, on personal protective equipment (PPE), and on skin, combined with biomonitoring. The aim was to validate targeted occupational hygiene measurements in order to allow a reduction of skin contamination and absorption in the studied population.

Materials and methods

The study included the assessment of PAH contamination of work surfaces by wipe-sampling, cutaneous exposure by hand washing, contamination of PPE (gloves) by extraction in solvent, and airborne PAH concentration in atmospheric samples. Biomonitoring was also carried out by the determination of urinary 1-hydroxypyrene (1-OHPU).

Together with biological monitoring, the contaminant removal from surfaces which can come into contact with workers' skin for estimating the dislodgeable portion is considered as an indirect method of assessing cutaneous exposure (EU CEN/TR 15278, 2006; EU ISO/TR14294, 2011).

Background

In 2010, two Pubmed search string determinants (one more specific, the other more sensitive) were proposed to retrieve information on the possible association between occupational risk factors and some pathologies (Mattioli *et al.*, 2010). Using *polycyclic aromatic hydrocarbons* AND *wipes* in June 2018, only 16 papers were found with the specific string (10 highly pertinent) and 28 with the sensitive one (15 relevant of which 10 were already found with the specific string). The pertinent articles mainly concerned the measurement of dermal exposure to PAHs by collecting wipe samples from skin or the assessment of surface contamination in chemotherapy workstations. No study was made of the measurement of PAH surface contamination by wipe sampling. Even if the problem has been known for a long time, there is a lack of information on the possible role of transfer from surfaces as a source of skin contamination by PAHs.

Occupational exposure to PAHs was studied in a refractory brick manufacturing company where a binder is used in its liquid form for the production of carbon-bonded doloma bricks. The binder is a formulation of pitch (heat treated residue from the distillation of high temperature coal tar) and anthracene oil (distillate from the fractional distillation of coal tar of bituminous coal). The concentration of BaP in pitch and in anthracene oil triggers the classification of these mixtures as carcinogenic for themselves as BaP is classified 1B carcinogen according to the CLP Regulation (Harmonized classification—Annex VI of Regulation EC No 1272/2008). In the production department, the binder is added to the ground mineral in mixers in a percentage of about 5% at temperatures above 100°C. The mixture is transported

to the presses and then to the ovens. These operations are automated and controlled in a confined workstation (the Synoptic Office), but at the presses there is a quality control station in which the manual measurement and weighing of some bricks are carried out. At the quality control station, tools used for cleaning and maintenance (like shovel and broom) are also stored.

Over the past years, the reduction of BaP in the binder was considered by the Risk Management of the plant as a significant step towards enhanced safety at the workplace. While the concentration of airborne PAHs could be classified as very low, the average values of 1-OHPU in a number of workers were relatively high (i.e. 1-OHPU >2 μg/g creatinine). This finding could depend on the fact that pyrene should mainly be taken up via skin. Moreover, the reduction of the BaP in the binder did not match that of the pyrene.

In a previous study, respiratory and cutaneous uptake of PAHs was estimated in a population of 13 workers from the production and packaging departments (Sartorelli *et al.*, 2018). During the work shift, personal samplings were carried out to measure the airborne concentrations of PAHs. Skin contamination was measured using 10 pads positioned at the level of the neck, chest, groin, ankle, and on work clothes at the level of chest and groin, as well as hand washes. Twelve PAHs were analyzed including pyrene and BaP. The estimate of the respiratory uptake of the pyrene was based on the method proposed by Van Rooij *et al.* (1993). Skin uptake was assessed by using the pyrene absorption constant (Kabs) obtained experimentally (Sartorelli *et al.*, 2001). The results showed that in the studied population the dermal uptake of pyrene was significantly greater than the respiratory one.

Surface contamination sampling and analysis

The surface contamination sampling was performed in the production and packaging departments. In the production area, four wipe samples were obtained from the quality control station surfaces (push-button panel, desks, pc keyboard) and five on the tools used in weighing and cleaning (weighing facilities, shovel, broom, caliber, gantry crane touch). Theoretically the contamination of surfaces subject to cleaning operations (quality control station surfaces) and non-cleaned surfaces (tools used in weighing and cleaning) could be different. This led to a further definition of two different areas within the production, one included surfaces that were regularly cleaned (A area) and one included data from non-cleaned surfaces (B area). In the packaging department, eight wipe samples were obtained from various equipment (push-buttons panel, pc keyboard, pallets). Moreover, three wipe samples were obtained from surfaces outside the two departments, in theory less contaminated by PAHs (all inside the factory with no traffic exhaust contamination: AS control panel of tempera ovens, beverage distributor, desk of the Synoptic Office).

The surface removal technique used was wipe-sampling with gauzes soaked in solvent. The surfaces were literally cleaned with the wipes by rubbing them first horizontally and then vertically and on the edges. The wipe removal technique was performed using gauzes (Universal Wipes©, CHELA Ltd, Enfield UK) wet with isopropyl alcohol and carefully squeezed. The surface on which the removal was carried out was 10 × 10 cm (100 cm^2). Frames with an inner surface of cm 10 × 10 were used for wipe-sampling. When it was not possible to use the frame (non-flat surfaces) the surface area was calculated in cm^2 depending on the shape of the object subjected to removal. The technique complies with the American Society for Testing and Materials (ASTM, 2016, 2017).

The PAHs were extracted from wipe samples in an ultrasonic bath with a mixture of dichloromethane and acetonitrile (20:80) and the extract filtered and injected into the HPLC system (series 1200 Agilent Technology Inc.) equipped with a fluorimetric detector. The chromatographic analysis was performed on a LC-PAHs Supelcosil column with acetonitrile-water gradient at the flow of 2 ml/min. The fluorimetric detector worked with a change program of λ ecc and λ em. Calibration was performed by injecting extracts of gauze fortified with known amounts of PAHs in a range of concentrations compatible with those of the samples. From the determination of the amount of PAHs in the extraction liquids, it was possible to ascertain the surface contamination expressed per surface unit (cm^2).

Air sampling and analysis

In the production department, nine static air samples were obtained at the quality control stations (six from A area and three from B area). Nine static air samples were obtained in the packaging department and three outside the two departments. Static air sampling was conducted in the areas corresponding to surface contamination sampling.

The measurement of airborne PAHs was conducted by two-stage sampling. To sample the airborne particulate, a sampler fitted with a fiber glass membrane pre-conditioned at 400°C was used as substrate, while for sampling the vapor phase an Amberlite-XAD 2 sorbent tube (Orbo 43 Supelco) was fitted in line directly after the sampling head. Air samples were collected at

a sampling rate of 2 l/min and frozen at –20°C until analysis. The samples were analyzed in HPLC with fluorimetric detection according to method 5506 published by US National Institute for Occupational Safety and Health (NIOSH).

Hand washing and gloves contamination

Contamination of the skin was assessed in 14 workers (6 in the production and 8 in the packaging department) by washing hands at the end of the workshift. For the purpose, 250 ml of 95% ethanol was slowly poured over workers' hands while they rubbed them together. The ethanol was collected in a disposable aluminium basin and the worker was allowed to soak hands and nails in the solution for 30 s. The procedure was performed twice in order to ensure the complete removal of PAHs from hands.

Six pairs of used gloves were analyzed (three in the quality control station of the production department and three in the packaging department). Heat-resistant cotton gloves were used in the production department while light nitrile gloves were used in packaging. Both light nitrile and heat-resistant gloves were soaked with ethyl alcohol, but the latter were subjected separately to a washing of both the outer and the inner surface using the same procedure of hand washing (for this purpose, the two types of gloves were worn by an operator before washing). The extracts obtained using commercial ethyl alcohol were frozen at –20°C until analysis. Before the analysis, the extracts were subjected to sonication for 10 min, then an aliquot was filtered and injected into the HPLC. The chromatographic analysis was performed as described in the surface contamination paragraph. Calibration was performed by injecting ethyl alcohol solutions fortified with known amounts of PAHs in a range of concentrations compatible with those of the samples. From the determination of PAHs in the extracts, it was possible to calculate their amount on the hands by multiplying the obtained concentration by the volume of the sample.

Chemical analysis of PAHs

In the different types of samples, the concentrations of the following 13 PAHs were analyzed: acenaphthene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, BaP, dibenzo(a,h)anthracene, benzo(ghi)perylene, and indeno(1,2,3cd)pyrene. The analytical methods are described above.

The limits of detection (LoDs) were calculated in all type of samples on the basis of a signal three times the background noise. To evaluate precision, six spiked samples were prepared at two different concentrations. The recovery of analytical methods was on average 100% for all compounds because calibrations were performed by addition of known amounts of PAH mixtures to the different matrices. Data are reported in Table 1.

Performance of sampling and analytical procedures

The precision and LoDs for the different measurements are shown in Table 1. Methods used for the sample extraction were fast and gave good recovery of analytes and precision. LoDs were sufficiently low to enable detection of PAHs in most samples.

The effectiveness of hand washing in the decontamination of workers' hands was not assessed. However, the high volume of ethanol used (rubbing during pouring), the hand bath in washing liquid and the second wash with 250 ml ethanol ensure the maximum removal of PAHs from skin at least for the portion not yet absorbed through the skin. The same consideration is valid for the two types of gloves analyzed that were subjected to the same washing procedure. Hand and glove washing were preferred to wiping (used for surfaces) in order to remove contaminants from all parts of hands/gloves including fingernails and interdigital areas.

The effectiveness of surface wiping was not assessed systematically. However, a second wipe test performed in six different location and analyzed separately did not reveal detectable concentration of PAHs in any sample. This demonstrated that the removal effectiveness was almost total.

Biological monitoring

Eleven packaging workers (mean age 43 years) and 15 production workers (mean age 49 years), all males and non-smokers, were included in the biological monitoring programme. Concentration of 1-OHPU was measured in a urine sample at the end of the workshift/end of the working week which did not always correspond to Friday. The workshift included 6 days of work and 2 days off with the following schedule: first shift 6 am–2 pm, second shift 2–10 pm, third shift 10 pm–6 am. The shift sequence was third/second/first. Urine samples were kept in a container duly shielded from light and frozen at –20°C until analysis. In the same time, 1-OHPU was measured in a urine sample of 37 workers (all males, mean age 47 years) not professionally exposed to PAHs and non-smokers (controls). Analysis of 1-OHPU was performed in HPLC with fluorimetric detection after enzymatic hydrolysis and Solid Phase Extraction (SPE) purification on C18 columns (Jongeneelen *et al.*, 1987). LoDs and precision are reported in Table 1.

Table 1. LoD and precision [coefficient of variation (CV%)] of the analytical methods on the different sample types.

Compound	Sample type	LoD	Concentration	Within-series precision (CV%)
Acenaphthene	Wipes	0.37	100	10.2
Phenanthrene	(ng/cm^2)	0.085	0.12–10	9.9
Anthracene		0.006	0.12–10	6.7
Fluoranthene		0.022	0.24–20	9.8
Pyrene		0.006	0.12–10	7.5
Benzo(a)anthracene		0.009	0.12–10	8.3
Crysene		0.010	0.12–10	4.5
Benzo(b)fluoranthene		0.023	0.24–20	4.7
Benzo(k)fluoranthene,		0.003	0.12–10	7.0
Benzo(a)pyrene		0.008	0.12–10	8.7
Dibenzo(a,h)anthracene		0.018	0.24–20	9.2
Benzo(ghi)perylene		0.013	0.24–20	13.1
Indeno(1,2,3cd)pyrene		0.083	0.12–10	10.4
Acenaphthene	Air samples	0.40	0.59–72	15.8
Phenanthrene	(µg/m^3)	0.10	0.22–83	23.8
Anthracene		0.10	0.12–7.7	25.0
Fluoranthene		0.02	0.040–3.8	15.4
Pyrene		0.0056	0.025–1.6	16.1
Benzo(a)anthracene		0.0014	0.0014–0.019	18.2
Crysene		0.011	0.011–0.15	21.4
Benzo(b)fluoranthene		0.0059	0.0059–0.022	22.8
Benzo(k)fluoranthene,		0.0009	0.0009–0.0070	21.7
Benzo(a)pyrene		0.0011	0.0011–0.017	18.0
Dibenzo(a,h)anthracene		0.0028	0.0028–0.025	28.1
Benzo(ghi)perylene		0.0058	0.0058–0.075	24.8
Indeno(1,2,3cd)pyrene		0.0029	0.0029–0.025	25.6
Acenaphthene	Handwashes and gloves	1.9	100	9.3
Phenanthrene	(ng/ml)	0.14	10–200	5.7
Anthracene		0.029	0.63–10	8.4
Fluoranthene		0.16	10–200	9.4
Pyrene		0.070	10–200	7.6
Benzo(a)anthracene		0.090	0.63–10	4.7
Crysene		0.12	0.63–10	11.6
Benzo(b)fluoranthene		0.18	1.2–20	5.8
Benzo(k)fluoranthene		0.026	0.63–10	5.4
Benzo(a)pyrene		0.071	0.63–10	5.9
Dibenzo(a,h)anthracene		0.14	1.2–20	7.4
benzo(ghi)perylene		0.16	1.2–20	10.9
Indeno(1,2,3cd)pyrene		0.76	0.63–10	11.2
1-hydroxypyrene	Urine (µg/l)	0.015	0.050–50	6.9

The recovery of analytical methods is on average 100% for every compound because calibrations were performed by addition of known amounts of PAH mixtures to the different matrices.

Statistical analysis

The statistical target was focused on seven variables (i.e. the concentration of phenanthrene, anthracene, fluoranthene, pyrene, BaP, total of all 13 PAH, and the pyrene/BaP ratio) which were measured in four different working areas (packaging, production A area, production B area, area outside the two departments) according to two different detection tools (wipes and environmental air samples). In the 4 working areas, 20 surface contamination measurements were globally

carried out by wipes as well as 21 atmospheric samples obtained in the areas corresponding to surface contamination sampling. Therefore, the appropriate methodology for carrying out the inference on this dataset was a MANOVA (i.e. the multivariate analysis of variance based on the seven considered variables) with two factors (represented by the four working areas and the two detection tools, respectively). However, the ordinary application of the MANOVA testing procedure based on the classic Hotelling statistic did not seem suitable in the present setting, owing to the small sample sizes of the factor groups, to both the complexity of the analysis (which includes 13 compounds for each sample) and to the work organization (staff mobility). Thus, a nonparametric approach to MANOVA was preferred. More precisely, the permutation strategy suggested by Pesarin and Salmaso (2010) was adopted, since it avoids the normality assumption and allows for the simultaneous testing of the variables which eventually led to the rejection of the global null hypothesis (i.e. no effect of the two factors). The methodology stems on the non-parametric combination of a set of dependent partial tests and it assumes that a global testing hypothesis may be subdivided into a set of sub-hypotheses. Each sub-hypothesis is assessed by means of a suitable permutation test statistic and these sub-hypotheses are jointly analyzed in order to control the underlying dependence relations, as well as to achieve simultaneous significance levels (Bretz *et al.*, 2010; Dickhaus, 2014). Permutation testing procedures were previously used in Occupational Hygiene (Sartorelli *et al.*, 2007).

With regard to PAH concentrations in handwashes and gloves, the statistical comparison was carried out by means of the Mann–Whitney test between the two homogeneous groups of exposure.

A further analysis was considered on biological monitoring. The concentration of 1-OHPU was considered on 58 samples according to 3 groups of workers (packaging, production, controls) and hence an ANOVA procedure was suitable for inference. In such a case, the Jonckheere–Terpstra test for ordered alternative hypothesis was adopted (Hollander *et al.*, 2014).

Results

Surface contamination and air sampling

Table 2 shows the concentration of phenanthrene, anthracene, fluoranthene, pyrene, and BaP, the total concentration of the 13 PAHs and the pyrene/BaP ratio in the wipes carried out on the surfaces of the different areas. To confirm the source of exposure, a clear correspondence was observed between the percentage of the

Table 2. Single PAHs and total PAHs (sum of 13 PAHs) concentration values and pyrene/BaP ratio in the wipes (ng/cm^2) and in the air samples (µg/m^3) carried out in the different sampled areas—median (mean ± SD).

	Packaging		Production A		Production B		External area	
	Wipes (ng/cm^2)	Air samples (µg/m^3)	Wipes (ng/cm^2)	Air samples (µg/m^3)	Wipes (ng/cm^2)	Air samples (µg/m^3)	Wipes (ng/cm^2)	Air samples (µg/m^3)
Phenanthrene	6.0 (12 ± 14)	3.8 (5.9 ± 4.9)	63 (97 ± 113)	13 (12 ± 2.2)	6957 (5894 ± 2689)	14 (17 ± 7.0)	14 (17 ± 5.2)	6.5 (7.2 ± 2.5)
Anthracene	0.61 (1.1 ± 1.3)	0.41 (0.51 ± 0.33)	5.1 (7.4 ± 8.2)	1.1 (1.0 ± 0.16)	589 (601 ± 296)	1.0 (1.1 ± 0.20)	1.2 (1.5 ± 0.46)	0.50 (0.65 ± 0.32)
Fluoranthene	3.7 (9.7 ± 12)	0.31 (0.41 ± 0.32)	24 (30 ± 31)	0.35 (0.40 ± 0.10)	1646 (1527 ± 625)	0.65 (0.62 ± 0.28)	5.9 (5.5 ± 1.3)	0.41 (0.58 ± 0.35)
Pyrene	1.8 (5.2 ± 6.5)	0.14 (0.18 ± 0.11)	15 (19 ± 21)	0.12 (0.12 ± 0.04)	788 (767 ± 312)	0.24 (0.21 ± 0.10)	2.4 (2.3 ± 0.56)	0.19 (0.22 ± 0.10)
BaP	0.10 (0.21 ± 0.35)	0.002 (0.002 ± 0.001)	0.26 (2.5 ± 4.5)	0.003 (0.003 ± 0.002)	13 (26 ± 18)	0.003 (0.003 ± 0.002)	0.04 (0.04 ± 0.01)	0.003 (0.003 ± 0.002)
Total PAHs	13 (31 ± 37)	6.0 (8.4 ± 6.6)	113 (181 ± 218)	29 (30 ± 11)	10,114 (9475 ± 4181)	29 (33 ± 7.7)	25 (27 ± 7.5)	8.8 (12 ± 1.7)
Pyrene/BaP	27 (50 ± 65)	74 (100 ± 89)	39 (37 ± 27)	48 (71 ± 55)	23 (39 ± 27)	70 (74 ± 31)	59 (56 ± 4.6)	94 (156 ± 155)

Table 3. Relative mean percentage of the single compounds in the mixture of 13 PAHs in the binder and in wipes.

	Phenanthrene	Anthracene	Fluoranthene	Pyrene	BaP
Binder	50%	4.4%	18%	11%	0.90%
Wipe samples	49%	4.9%	22%	11%	0.50%

Table 4. Comparison between single PAHs and total PAHs (sum of 13 PAHs) concentration values and pyrene/BaP ratio measured in four different working areas (packaging, production A area, production B area, area outside the two departments) according to two different detection tools (wipes and static air samples)—results of nonparametric statistical analysis (permutation test *P*-values).

	Phenanthrene	Anthracene	Fluoranthene	Pyrene	BaP	Total PAHs	Pyrene/BaP	*Combined P-values*
Wipes	0.0010*	0.0005*	0.0004*	0.0004*	0.0032*	0.0004*	0.9592	0.0005*
Air samples	0.0040*	0.0076*	0.5614	0.4614	0.7523	0.0001*	0.5986	0.0050*
Combined P-values	0.0001*	0.0002*	0.0021*	0.0011*	0.0171*	0.0001*	0.8912	0.0001*

*Statistically significant values.

Table 5. Comparison between single PAHs and total PAHs (sum of 13 PAHs) percentages and pyrene/BaP ratio measured in four different working areas (packaging, production A area, production B area, area outside the two departments) according to two different detection tools (wipes and static air samples)—results of nonparametric statistical analysis (permutation test *P*-values).

	Phenanthrene %	Anthracene %	Fluoranthene %	Pyrene %	BaP %	*Combined P-values*
Wipes	0.0002*	0.7124	0.0010*	0.0022*	0.3204	0.0016*
Air samples	0.0026*	0.0362*	0.0002*	0.0001*	0.0388*	0.0001*
Combined P-values	0.0001*	0.1194	0.0001*	0.0001*	0.0696	0.0001*

*Statistically significant values.

single compounds in the binder and those measured in wipe samples (Table 3).

Table 2 also shows the concentration of phenanthrene, anthracene, fluoranthene, pyrene and BaP, the total concentration of the 13 PAHs, and the pyrene/BaP ratio in the athmosphere of the different areas.

As to the hypothesis testing, the suggested permutation procedure gives rise to the *P*-values reported in Tables 4 and 5. It should be remarked that the marginal and combined *P*-values jointly hold since the procedure is simultaneous—as previously emphasized in the Statistical Analysis sub-section.

According to these *P*-values, the lack of effect of two factors (i.e. the different working areas and detection tools) on PAH concentrations should be jointly and marginally rejected. Indeed, the overall *P*-value and the combined *P*-values turn out to be <0.05 in all the cases (see the combined *P*-values in the last row and column of Table 4), with the exception of the Pyrene/BaP ratio. The combined *P*-value is actually the output of the non-parametric combination of the dependent partial tests for each marginal sub-hypothesis (i.e. the sub-hypothesis corresponding to the homogeneity of the expected values in the four working areas for each analyzed variable and for each detection tool). If the target variables (concentration levels of PAHs, total PAHs and pyrene/BaP ratio) are jointly considered, the expected values of these quantities are significantly different for the four working areas (see the global *P*-value = 0.0001, as given by the entry in last row and column of Table 4). In turn, this conclusion also holds when the marginal significances for the wipes (combined *P*-value = 0.0016, as given by the entry in the last column of Table 4) and for the air samples (combined *P*-value = 0.0001, as given by the entry in the last column of Table 4) are considered, i.e. the expected values of the target quantities

Table 6. Pyrene, BaP and total PAHs (sum of 13 PAHs) contamination values (μg) and pyrene/BaP ratio on hand washes and gloves—median (mean ± SD).

	Pyrene	BaP	Total PAHs	Pyrene/BaP
Thin gloves (packaging)	1.9 (5.0 ± 5.5)	0.060 (0.16 ± 0.19)	36 (65 ± 67)	34 (39 ± 18)
Thicker gloves (inside)	7.4 (16 ± 16)	0.21 (0.40 ± 0.34)	205 (468 ± 528)	36 (38 ± 5.1)
Thicker gloves (outside)	184 (238 ± 15)	5.6 (6.4 ± 4.1)	2785 (4497 ± 3228)	38 (38 ± 5.1)
Hand washes (packaging)	1.7 (2.7 ± 1.6)	0.050 (0.060 ± 0.040)	16 (27 ± 26)	39 (41 ± 21)
Hand washes (production)	8.7 (10 ± 8.0)	0.18 (0.25 ± 0.24)	100 (103 ± 68)	46 (43 ± 15)

are significantly different for the four working areas for each detection tool, when individually considered. Similarly, an analysis for each PAH, for the total PAHs and for the pyrene/BaP ratio may be marginally carried out. As an example, if phenanthrene is solely considered, the expected value of its concentration level is significantly different for the four working areas (combined P-value = 0.0001, see the first column of Table 4) and the same statement individually holds for each detection tool (P-values given by 0.0002 and 0.0026, respectively, see the first column of Table 4). Thus, solely the expected concentration of the pyrene/BaP ratio does not significantly differ in the four working areas when individually considered. Similarly, there is no marginal rejection for the fluoranthene, pyrene, and BaP variables if the air sampling is solely considered (Table 4). Again, it is worth remarking that, when the target variables are jointly considered, their expected values significantly differ in the four working areas, even if some marginal tests do not reject this hypothesis. All these conclusions simultaneously hold, since the dependence of the marginal tests is captured by the adopted permutation procedure. Analogous comments may be provided for the global and marginal analysis of the percentage of the single PAHs and total PAHs (Table 5).

In addition, on the basis of the analysis of the medians (Table 2), it is evident the pattern of the contamination of the surfaces in the various areas. In particular, the concentrations of the four PAHs considered and of the total PAHs were higher on the surfaces of the production B area than on those of the A area. Moreover, the PAH concentrations in wipes carried out on the surfaces of the A area were higher than those in wipe samples obtained in the packaging department, while there was no clear difference between the packaging and the external area.

Both contamination of surfaces and air pollution are qualitatively similar considering the pyrene/BaP ratio which is always very high compared to other workplaces such as coke ovens (Jongeneelen, 2014).

Air concentrations of phenantrene, anthracene, and total PAHs are higher in the production department than in packaging (P-value <0.01), without a clear difference between A and B areas.

Hand washing and gloves contamination

Table 6 shows concentrations of pyrene, BaP, and pyrene/Bap ratio on hand washes and gloves. With regard to total PAHs in hand washes, the statistical comparison carried out by means of the Mann–Whitney test showed significantly higher concentrations in the production department than in packaging (P-value <0.05). Also with respect to pyrene and BaP concentrations, a statistically significant difference was observed between the two groups, with greater contamination of both in the production department (P-value <0.05).

Biological monitoring

In Table 7, 1-OHPU values in workers and controls are summarized. The concentration of 1-OHPU was significantly higher in the urine of production workers than in those of packaging workers (Jonckheere–Terpstra test, P-value <0.05). Moreover packaging workers had higher 1-OHPU concentrations than controls (Jonckheere–Terpstra test, P-value <0.05).

Discussion

Observing the median concentrations of PAHs, the differences between the various departments are evident. As far as the wipes are concerned, the concentrations of phenanthrene, anthracene, fluoranthene, pyrene, BaP, and the total PAH mixture were higher in the B area than the A area of production. Concentrations of these compounds were also higher in the A area with respect to the other two departments. According to the findings of the statistical analysis these differences are significant. The results are confirmed by the hand washing data, significantly higher in production than in packaging. The analysis of PPE also reflects this trend showing that gloves of the quality control workers are the most contaminated. On the other hand, a marked difference does not exist between the packaging department and the external

Table 7. 1-hydroxypyrene urinary concentrations (μg/g creatinine) in workers and controls (all males non-smokers).

	Mean ± SD	Median	Range
Packaging workers	0.50 ± 0.36	0.31	0.22–1.1
Production workers	0.96 ± 0.77	0.59	0.14–2.8
Controls	0.16 ± 0.16	0.12	0.040–0.92

area. In air samples, the differences are much less evident with only higher concentrations of phenantrene, anthracene, and total PAHs between production as a whole and the other two departments, while pyrene and BaP concentrations are at the same level at the different areas. Biological monitoring showed 1-OHPU values significantly higher in production workers than in packaging workers, confirming the importance of dermal absorption of PAHs since 1-OHPU represents a marker of carcinogenic PAH exposure (Yamano *et al.*, 2014), deriving however from pyrene which is not recognized as a carcinogen.

In conclusion, the analysis of the wipes demonstrated that the production B area has a higher surface contamination compared to the production A area and the packaging department. In the absence of a significant difference in air concentrations of PAHs between A and B areas, this is attributable to surfaces not subject to cleaning. It can be explained by the greater deposition of the airborne pollutants on the already dirty working tools, which are not regularly cleaned.

Both contamination of surfaces and airborne PAHs are qualitatively similar since there are no significant variations in the pyrene/BaP ratio in the different departments, nor between wipes and air samples. As dermal uptake is the main route of exposure, surfaces appear the main source of exposure. The pyrene/BaP ratio is high even in the air samples and should be taken into account for biological monitoring interpretation.

Currently, in many production activities, biological monitoring is used for assessing control of exposure to PAHs. Due to the lack of a dose–response relationship, for many years, no limits have been proposed for 1-OHPU by International Agencies. In 2005, HSE introduced a Biological Monitoring Guidance Value for 1-OHPU (Unwin *et al.*, 2006) and in 2006, ACGIH recommended 1-OHPU as biomarker of PAHs exposure with the notation Nq (not quantitative), while the Scientific Committee on Occupational Exposure Limits (SCOEL) proposed a general population value of 0.5 μg/g creatinine of 1-OHPU with sampling advise (Heederik *et al.*, 2016). Jongeneelen (2014) proposed a *no observed genotoxic effect level* (NOGEL) in PAH-exposed workers as a point of departure for setting the limit value. Nine cross-sectional studies were selected based on the early genotoxic effects in white blood cells of PAH-exposed workers (i.e. chromosomal aberrations, sister chromatid exchanges, micronuclei, comet assay, DNA adducts) related to 1-OHPU concentration at the end of the shift/end of the week urine samples. In four out of nine studies, the NOGEL could be derived and the lowest level was 1 μmol/mol creatinine (equal to 1.93 μg/g creatinine). This limit was recommended in workplaces where the pyrene/BaP ratio was 2.5 as observed on average in coke ovens where the studies had been conducted (Jongeneelen, 2014). Actually, the key study for the Lowest Observed Genotoxic Effect Level (LOGEL) reported a pyrene/BaP ratio of 2.2 (Popp *et al.*, 1997). For workplaces with a different PAH profile an adjustment of the 1-OHPU limit based on the pyrene/BaP ratio was suggested. Considering that in the key studies the pyrene/BaP ratio ranged from 1.5 to 4.5, it was recommended that this range was used as a boundary for adjustment and not to apply adjustment beyond this range. The pyrene/BaP ratio adjusted limit value of 1-OHPU for a work environment with a pyrene/BaP ratio of 4.5 is 1.60 μmol/mol creatinine (3.09 μg/g creatinine). The general consensus regarding this methodology led ACGIH (2017) to recommend a Biological Exposure Index (BEI) for 1-OHPU that can be adjusted on the basis of the specific pyrene/BaP ratio.

Both Jongeneelen (2014) and the BEI Committee (ACGIH, 2017) recommended that the measurement of pyrene and BaP be carried out on air, skin, or surface samples, depending on which way of exposure the Industrial Hygienists consider as prevalent. In the light of the results in the present study the measures to be taken into consideration are those relating to surface contamination which in terms of pyrene/BaP ratio in practice do not differ from that found in air samples. A previous survey (Sartorelli *et al.*, 2018) showed the prevalence of the percutaneous absorption pathway and these results demonstrate that surface contamination is much greater than the concentration of airborne PAHs. The pyrene/BaP ratio is always very high (the median varying from 23 to 59 in the wipes of the various departments) as expected considering the content of the two compounds in the binder.

1-OHPU is considered as the most comprehensive carcinogenic biomarker of exposure to PAHs (Yamano *et al.*, 2014). On the other hand, the composition of the PAH mixtures varies considerably in the different workplaces. Thus, the 1-OHPU values do not directly reflect the carcinogenicity of the different PAH mixtures. Therefore, the BaP is often used as an indicator

of the carcinogenicity of PAH mixtures and the pyrene/BaP ratio is of crucial importance in the interpretation of PAH biological monitoring. Unwin *et al.* (2006) showed a good correlation between 1-OHPU and airborne BaP levels if samples from workers using respiratory protection or with significant dermal exposure were excluded. When pyrene/BaP ratio is constant, as in the studied workplace, 1-OHPU values could correlate with BaP even in dermal PAH exposures. However, dermal exposure is difficult to assess because skin contamination varies in the different skin regions as well as percutaneous penetration. These difficulties over time have hampered the introduction of dermal occupational exposure limits (DOELs). Currently, biological monitoring may be recommended when dermal exposure can contribute substantially to the body burden as in the case of PAHs.

A more accurate method for risk assessment is the use of Toxic Equivalency Factors (TEF). This approach uses BaP as a reference compound to which a TEF = 1 is assigned, while non-carcinogenic compounds have TEF = 0 (EPA, 1984; Petry *et al.*, 1996). Nisbet and LaGoy (1992) proposed a set of TEF values for various PAH based on of the existing literature that was integrated more recently for other compounds. In this way, the toxic equivalence (TEQ) of the mixture can be calculated in terms of equivalent concentration of BaP (BaPeq) as follows:

$$\text{BaPeqi} = \text{PAHi} * \text{TEFi};\ \text{and}\ \text{TEQ} = \sum \text{BaPeqi}$$

where:
PAHi = concentration of the PAHs congener i
TEFi = toxic equivalent factor for the PAH congener i (Zhu *et al.*, 2014).

Based on the measurements of the various PAHs, the carcinogenic properties of the mixture present in the workplace can be characterized in terms of BaP concentration/m^3.

The current limitation of this approach, because TEF has not been used for years, is not to have a dose–effect relationship based on TEQ. This hampers the identification of health based limits. It is not possible to simply report the values of BaPeq to those of BaP, given that there is no data in humans concerning exposure to the latter compound alone. Therefore, presently, the TEQ have some importance in the characterization of the PAH mixture, but for the assessment of the carcinogenic risk the 1-OHPU values adjusted for the pyrene/BaP ratio represent the most valid approach, as proposed by Jongeneelen and ACGIH.

In light of the above, 1-OHPU levels in workers producing refractory bricks, and consequently their exposure to PAHs, cannot be considered particularly high as they are always below the limit proposed by Jongeneelen (2014) in the specific case (3.09 µg/g creatinine) that is more conservative than the adjusted BEI (ACGIH, 2017). The latter in practice corresponds to the pyrene/BaP ratio of the mixture which is very high in the studied workplace. Nevertheless, preventive action is needed that should be directed towards reducing the contamination of surfaces in the production department, especially by improving the cleaning operations of work tools.

Conclusions

The obtained results confirm that the measurement of surface contamination represents a valid tool for the assessment of sources of exposure to PAHs in the workplace. In the specific case the correspondence between the percentage of the single compounds in the binder and in wipes clearly indicates that the main source of exposure to PAHs is represented by the binder itself. The study allows direct preventive interventions towards a better organization of the cleaning operations of the quality control station surfaces as well as a better interpretation of the biological monitoring data.

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Conflict of interest statement

The authors designed and executed the study and have sole responsibility for the writing and content of the manuscript.

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