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Environmental and biological monitoring of PAHs exposure in coke-oven workers at the Taranto plant compared to two groups from the general population of Apulia, Italy

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Key words

Polycyclic aromatic hydrocarbons; 1-hydroxypyrene; coke-oven workers; Benzo[a]pyrene, exposure

Parole chiave

Idrocarburi policiclici aromatici; 1-idrossipirene; lavoratori di cockeria

Summary

Background: *Polycyclic aromatic hydrocarbons (PAHs) exposure in the coke industry poses a risk for workers' health as well as for subjects living in the plant vicinity.* **Objectives:** *To assess PAHs exposure in coke-oven workers (CW) at the Taranto plant, Apulia, and in subjects from the general population living near (NC) and far away (FC) from the plant.* **Methods:** *Exposure was assessed by personal air sampling and urinary 1-hydroxypyrene (1-OHP) measured in 100 CW, 18 NC and 15 FC.* **Results:** *Median airborne benzo[a]pyrene (BaP) levels were 152, 1.5, and 3.6 ng/m^3 in CW, NC, and FC, respectively. In CW, median 1-OHP increased from 1.45 to 1.96 μg/g creatinine (crt) during the work shift (p>0.05); in NC and FC, 1-OHP levels were 0.56 and 0.53 μg/g crt. No significant differences between NC and FC for both air and urinary indices were found. BaP exposure in CW exceeded the recently proposed German acceptable (70 ng/m^3) and tolerable (700 ng/m^3) risk-based limit values in 82 and 11% of subjects, respectively. In NC and FC, BaP exposure exceeded the European target value for ambient air (1 ng/m^3) in 67 and 60% of subjects, respectively. Biomonitoring showed that 21% of CW had 1-OHP levels higher than the proposed biological limit value for the coke-oven industry (4.4 μg/g crt), while 93% of FC, and 88% of NC, had 1-OHP levels exceeding the Italian reference value (0.3 μg/g crt). Among non-smokers, a linear regression between 1-OHP and BaP (Pearson value r=0.65, p<0.05) allowed us to estimate levels of 1.2 and 1.9 μg/g crt for 1-OHP end-of-shift corresponding to acceptable and tolerable limit values.* **Conclusions:** *Although lower than in the past, PAHs exposure in the coke plant still poses a health risk for workers and the general population and requires further efforts to improve workplace conditions.*

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Riassunto

«Monitoraggio ambientale e biologico dell'esposizione a IPA nei lavoratori della cokeria di Taranto e in due gruppi della popolazione generale pugliese». **Introduzione:** *L'esposizione a idrocarburi policiclici aromatici (IPA) provenienti dall'industria della produzione del coke pone un potenziale rischio per la salute dei lavoratori e per i soggetti della popolazione generale.* ***Obiettivi****: Valutare l'esposizione a IPA in lavoratori della cokeria (CW) di Taranto, Puglia, e in soggetti della popolazione che vivono nelle vicinanze dell'impianto (NC) o a distanza da esso (FC).* **Metodi:** *L'esposizione a IPA è stata studiata mediante campionamento personale ambientale e misura di 1-idrossipirene urinario (1-OHP) in 100 CW, 18 NC e 15 FC.* **Risultati:** *L'esposizione mediana a benzo[a]pirene (BaP) è risultata 152, 1,5, e 3,6 ng/m³ rispettivamente nei CW, NC e FC. Nei CW, 1-OHP aumentava durante il turno di lavoro da 1,45 a 1,96 μg/g creatinina (crt) (p>0,05); nei NC e FC, i livelli 1-OHP erano 0,56 e 0,53 μg/g crt. Non è stata trovata alcuna differenza tra NC e FC, sia per l'esposizione ambientale che per i valori di 1-OHP. L'esposizione a BaP superava il valore limite di rischio accettabile (70 ng/m³) e tollerabile (700 ng/m³) proposti recentemente in Germania, nell'82 e nell'11% dei CW. Il 67% e il 60% dei NC e FC superava il valore obiettivo europeo per la qualità dell'aria (1 ng/m³). Il 21% dei CW aveva livelli di 1-OHP più alti del valore limite biologico proposto per l'industria del coke (4,4 μg/g crt), mentre il 93% dei FC e l'88% dei NC aveva valori di 1-OHP eccedenti i valori di riferimento della popolazione italiana (0,3 μg/g crt). Nei soggetti non fumatori, la regressione lineare tra 1-OHP e BaP (r=0,65, p<0.05) ha permesso di calcolare livelli di 1,2 e 1,9 μg/g crt per 1-OHP (fine turno) corrispondenti ai valori limite di rischio accettabile e tollerabile.* **Conclusioni:** *Benché più bassa che in passato, l'esposi-zione a IPA derivante dalla cokeria pone ancora un rischio per la salute dei lavoratori e della popolazione generale e richiede ulteriori sforzi per migliorare le condizioni igieniche di questo ambiente di lavoro.*

Introduction

Polycyclic aromatic hydrocarbons (PAHs), which are chemical compounds produced by combustion of organic materials, are widespread in the environment. PAHs are recognized as persistent pollutants, and 17 compounds (of more than 100 congeners) have been classified as priority pollutants by the U.S. Environmental Protection Agency (U.S. EPA) (35). PAHs are generally toxic, and they have been associated with negative reproductive, neurodevelopmental and cardiovascular effects (10, 11, 42). Moreover, some PAHs have been classified as known, probable, or possible carcinogens for humans by several agencies and governmental bodies (13, 23, 32). Some occupational activities associated with very high exposure to PAHs, including coke production, coal gasification, coal tar distillation, aluminium production, or coal tar pitch paving and roofing, have also been classified as carcinogenic to humans (23). Due to the well-known health effects of PAHs, there is growing concern about PAH exposure of both workers and the general population living in the vicinity of highly polluted industrial areas (27, 39).

Given the complexity of PAHs exposure (multiple compounds, different combustion processes, different occupational settings), different indexes of environmental exposure (coal-tar pitch volatiles, single selected PAH, particulate PAHs, or the sum of total measured PAHs), and different occupational exposure limit (OEL) values have been proposed by regulatory agencies and countries.

Among single PAH, only exposure to naphthalene (NAP) and benzo[a]pyrene (BaP) are currently regulated. Naphthalene, the most volatile PAH, is always present in large amounts in PAH mixtures; it is classified as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC) (23). Occupational exposure to naphthalene is regulated in many countries, with OEL in the 20-53 mg/m³ range (22). Specifically, the American Conference of Governmental Industrial Hygienists (ACGIH) recommends a threshold limit value, as time weighted average (TLV-TWA) of 52 mg/m³, but lists naphthalene among the intended changes, with a proposed TLV-TWA

of 10.5 mg/m^3 (1). Guidelines to protect the general population from naphthalene exposure have been proposed by the Wold Health Organization, with a guideline for indoor air quality annual average concentration of 0.01 mg/m^3 (41).

As benzo[a]pyrene (BaP) is classified as a known human carcinogen (IARC, Group 1) (23), occupational exposure to this compound is regulated in many countries, with limit values for airborne BaP in the 550-5000 ng/m^3 range (8-hour TWA) or in the 2000-20000 ng/m^3 range for the short-term limit value (22). However, OEL set for BaP are based on practical considerations about technical feasibility, so they should be regarded as technical guide values.

In recent years new risk-based limit values have been proposed to protect workers' health from exposure to carcinogens. In Germany, the Committee for Hazardous Substances (AGS Committee) proposed an acceptable risk of 4:10000 (4:100000 as of no later than the year 2018) and a tolerable risk of 4:1000 (6). These refer to a working lifetime of 40 years and continuous exposure every working day. For BaP, the proposed tolerable risk corresponds to a concentration of 700 ng/m^3, and the preliminary acceptable risk to a concentration of 70 ng/m^3 (7 ng/m^3 from 2018) (5). These risk limits and the respective substance concentrations in workplace air will not be regarded as legally binding limit values until the end of a testing phase (expected in 2015) when they should replace the former German technical guidance value (TRK Technishe Richtkonzentration) for BaP, set at 5000 μg/m^3 for the coke industry and 2000 μg/m^3 for other industries (7).

A similar approach has been taken in the Netherlands, where the Committee on Occupational Standards (DECOS) derives the health-based calculated-occupational cancer risk values (HBC-OCRVs), associated with excess mortality levels of 4:1000 and 4:100000, as a result of working life exposure to substances that have been classified by the European Union or DECOS as genotoxic carcinogens. For BaP, the HBC-OCRVs are 550 and 5.7 ng/m^3 for an excess cancer mortality level of 4:1000 and 4:100000, respectively (20).

To protect the health of the general population, 1 ng/m^3 is the target level for the total BaP content in the PM_{10} fraction, averaged over a calendar year, to be met by 31 December 2012 in the European Union (12).

Since exposure to PAHs is possible through different routes (respiratory, dermal, or gastrointestinal), biological monitoring is considered a gold standard to determine total PAHs intake. Urinary 1-hydroxypyrene (1-OHP), a pyrene metabolite, has been proposed as a biomarker of PAHs exposure (26), and it has been used in different environmental and occupational exposure studies (17). Despite this, a biological limit value for 1-OHP has not yet been set. So far, there are only proposals for some specific industrial settings or guidance values. For example, ACGIH lists 1-OHP among the biological exposure indices, with the NQ (non quantitative) notation (1), and suggested, in a draft document, 1.0 μg/L as a benchmark value to be considered as a post-shift level that indicates occupational exposure (23). The UK Health and Safety Executive (HSE) lists 4.0 μmo/mol creatinine (7.72 μg/g creatinine) as a biological monitoring guidance value (BMGV), where this value represents the 90th percentile of measurements taken from industries deemed to have good control (19). In 2001, Jongeneelen proposed a 3-level benchmark value based on literature review and health effects: the first level, 0.24 μmol/mol creatinine (0.46 μg/g creatinine), corresponds to the lowest reported level in non-smokers and non-occupationally exposed controls; the second level, 1.4 μmol/mol creatinine (2.7 μg/g creatinine), is the lowest level for which no genotoxic effect was observed in occupationally exposed subjects; the third level, 2.3 μmol/mol creatinine (4.4 μg/g creatinine), is the lowest reported level in the coke-oven industry for 1-OHP, equalling the TWA-TLV of 200 μg/m^3 as benzene soluble matter (BSM) or the TRK of 2000 ng/m^3 BaP (25).

For non-occupationally exposed and non-smoker individuals, reference 1-OHP values have been reported: in Italy, the reference range is 0.03-0.3 μg/g creatinine (33); in Germany the reference value is 0.5 μg/L (36); in Finland 0.65 μg/L (14); in the US, 0.424 μg/g creatinine (8).

The aims of this study were to assess PAHs exposure in coke-oven workers at the Taranto plant

in the Apulia Region of Italy, using environmental and biological monitoring, and to compare their exposure with that of two groups of the general population living within 2 km of the plant (near controls or NC) or approximately 50 km from the plant (far controls or FC). The NC group was included to evaluate the influence of living close to the coke plant on PAHs exposure, and the second as a reference group used as a regional control.

Materials and methods

Subjects and sample collection

The study was conducted between July and October 2005. The study population included 100 coke-oven workers (CW), and 33 subjects from the general population living at various distances from a steel plant situated in the town of Taranto, southern Italy. Eighteen subjects lived in the Tamburi district, within 2 km of the plant (NC), while 15 lived in Alberobello, a rural village (11000 inhabitants) located about 50 km from the plant (FC). The plant is the largest in Europe, covering 15 km^2, with coke production of 3200 Ktons/year and steel production of 15000 ktons/year (21). For each subject, data regarding personal characteristics, job description and smoking habits were collected through a questionnaire administered by trained interviewers.

Personal exposure to airborne PAHs was assessed by personal air samples collected with active samplers worn by the subjects (all subjects from the general population and a subgroup of 45 CW), in the respiratory zone during an 8-hour work shift in the second part of the work week. Urine spot samples were collected at the beginning (BS) and at the end of the work shift (ES) for CW and as a second morning void for NC and FC on the same day as the ambient exposure sampling. Samples were coded for blind analysis and delivered to the laboratory where they were stored at -20°C until analysis.

The study was approved by the Ethics Committee of the University of Bari. All participants gave their written consent.

Personal exposure to PAHs

Air samplers consisted of PTFE filters (37 mm diameter, 2 μm pores), to collect PAHs in particulate matter, connected in series with XAD-2 sorbent tubes (200 mg), to collect PAHs present in the vapour phase. Air was pumped through the samplers at 2 L/min. PAHs were desorbed with acetonitrile and analysed by HPLC with fluorimetric detection according to the U.S National Institute for Occupational Safety and Health (NIOSH) method 5506 (28). Fifteen priority PAHs, listed by the US Environmental Protection Agency (EPA), were quantified [naphthalene (NAP), acenaphthene (ACE), fluorene (FLE), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLU), pyrene (PYR), chrysene (CHR), benzo[a]anthrancene (BaA), benzo[k]fluoranthene (BkF), benzo[b]fluoranthene (BbF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (dBA), benzo[g,h,i]perylene (BPE), and indeno[1,2,3-cd]pyrene (IPY)]. Analytical limits of detection (LOD) were calculated for an 8 h sampling period with an average air volume collection of 0.48 m^3.

Summary indexes of exposure were also calculated including the total amount of airborne PAHs (Σ15 PAHs), the sum of seven out of the nine PAHs classified as known, probable or possible human carcinogens by IARC (BaA, CHR, BaP, dBA, BkF, BbF, and IPY; Σ7PAHs), and the sum of five out of the seven compounds classified as known, presumed or suspected human carcinogens by EU (BaA, CHR, BaP, dBA, and BkF; Σ5PAHs).

1-Hydroxypyrene

Urinary 1-OHP was analysed by HPLC with fluorimetric detection after enzymatic hydrolysis. Briefly, urine samples (2 ml) were incubated overnight at 37°C with β-glucoronidase. Samples were then purified by solid phase extraction (C18 cartridge) and the acetonitrile resulting solutions were analysed by HPLC equipped with a reverse-phase Supelcosil - C18 column (20 mm length, 4.6 mm internal diameter, 5 μm particle size). The flow of the mobile phase was 2 ml/min. The wavelengths used for quantification were 242 nm for ex-

citation and 388 nm for emission. The LOD of the method was 0.044 μg/L.

Urinary cotinine and creatinine

Urinary cotinine in BS samples was measured to detect smoking. Cotinine was detected by HPLC and UV detection as described previously (29). The detection limit of the procedure was 50 μg/L. Subjects with cotinine level below 100 μg/L were classified as non-smokers (19).

Creatinine (crt) was determined using Jaffe's colorimetric method. The creatinine value was used to assure sample validity, excluding samples with excessive physiologic dilution or concentration according to the 0.3 g/L ≤ creatinine ≤ 3.0 g/L range. (40).

Statistical analysis

Statistical analysis was performed using the SPSS 17.0 package for Windows (SPSS Inc., Chicago, IL, USA). For descriptive analysis (median, 5^{th} and 95^{th} percentile), results are presented as ng/m^3 for environmental exposure data, and as μg/g crt and μg/L for 1-OHP values. A value corresponding to one-half of the quantification limit was assigned to measurements below analytical quantification. For further statistical analysis, data were decimal log transformed to assure normal distribution. Comparisons were performed with Student's t-test for independent samples (smokers vs. non-smokers), Student's t-test for paired samples (BS vs. ES samples) and one-way ANOVA with Bonferroni correction (differences between job titles). Pearson's correlations were used to test the associations between variables. A two-sided p value of less than 0.05 was considered significant.

Results

Subjects

The main characteristic of the study subjects are reported in table 1. While CW were all males, NC and FC were predominantly females, moreover, CW were younger. CW were more frequently smokers, even if their median cotinine excretion was lower than that of the general population groups. Based on the questionnaire, the following job titles were identified in the CW group: top-oven workers (19 subjects; 10 smokers), side-oven workers (39 subjects; 26 smokers), and maintenance operators (39 subjects; 24 smokers).

Personal exposure to PAHs

Details of personal exposure to airborne PAHs (as the sum of the vapour and particle phases) were available for 15 FC, 18 NC and 45 CW. Each measured analyte, as well as Σ15 PAHs, Σ7PAHs

Table 1 - Selected characteristics of study subjects

	General population living far away from the plant (FC)	General population living near the plant (NC)	Coke-oven workers (CW)
N	15	18	100
Gender			
Male, N (%)	3 (20%)	3 (17%)	100%
Female, N (%)	12 (80%)	15 (83%)	0%
Age (years)			
mean ± SD	42±12	42±12	34±8
Smoking habit*			
Smokers, No. (%)	4 (27%)	1 (6%)	62 (62%)
Urinary cotinine(μg/L), median (min-max)	1614 (299-2948)	1352	1206 (104-4522)

* according to urinary cotinine classification, N= number

and Σ5PAHs are shown in table 2. The concentrations of most analytes were above the LOD in a high percentage of samples in each subgroup.

In FC and NC, 12 out 15 PAHs were present in at least 60% of samples, the analytes present at lowest levels were dBA, BPE and IPY. The compound most heavily present was NAP, contributing to the total amount by 49% and 69% in FC and NC, respectively. In FC, high FLE levels (contributing 15% to the total amount) and ACE (11%) were also found, while in NC the second most heavily present compound was ACE (5%). Comparing FC and NC, only ANT was significantly higher in FC. No differences were found for either total exposure (Σ15PAHs) or exposure to carcinogenic compounds (both Σ7PAHs and Σ5PAHs) between the two subgroups of the general population. The median BaP level was 3.6 and 1.5 ng/m^3, in FC and NC respectively, contributing 0.2 and 0.05% to the total amount. BaP levels were higher than the 1 ng/m^3 European target value in 60% of FC and 67% of NC subjects (figure 1).

In CW, 12 analytes were found in at least 78% of samples. The PAHs present at the lowest levels

Table 2 - Limit of detection (LOD) and personal exposure (ng/m^3) to PAHs in the study subjects

		General population living far away from the plant (N=15)		General population living near the plant (N=18)		Coke-oven workers (N= 45)	
	LOD ng/m^3	N ≥ LOD (%)	Median (5th-95th percentile)	N ≥ LOD (%)	Median (5th-95th percentile)	N ≥ LOD (%)	Median (5th-95th percentile)
NAP	7.4	12 (80%)	986 (<7.4-2657)	16 (89%)	2055 (<7.4-4607)	45 (100%)	53523 (3266-194171) [A, B]
ACE	2.6	15 (100%)	228 (118-1684)	16 (89%)	164 (<2.6-343)	45 (100%)	1486 (247-42141) [A , B]
FLE	1.5	14 (93%)	302 (<1.5-780)	17 (94%)	76 (<1.5-945)	45 (100%)	4550 (573-68030) [A, B]
PHE	0.1	15 (100%)	39 (16-108)	18 (100%)	34 (2-219)	45 (100%)	1968 (55-10847) [A , B]
ANT	4.3	13 (87%)	107 (<4.3-322) [C]	9 (50%)	15 (<4.3- 1551)	45 (100%)	3184 (278-34348) [A, B]
FLT	1.4	15 (100%)	35 (2-186)	14 (78%)	15 (<1.4-5125)	43 (96%)	1160 (43-139190) [A, B]
PYR	0.7	11 (75%)	7.1 (<0.7-143.6)	12 (67%)	5.0 (<0.7-140.6)	45 (100%)	471 (79.3-6380) [A, B]
CHR	1.1	9 (60%)	8.3 (<1.1-28.4)	11 (61%)	7.2 (<1.1-25.0)	44 (98%)	300 (63.2-1506) [A, B]
BaA	0.1	15 (100%)	4.8 (0.7-23.6)	18 (100%)	4.2 (0.4-21.6)	45 (100%)	196 (41.5-1831) [A, B]
BkF	0.1	14 (93%)	2.2 (<0.1 -32.0)	15 (83%)	1.8 (<0.1 -33.2)	45 (100%)	85 (3.8-1052) [A, B]
BbF	0.4	10 (67%)	5.1 (<0.4-61.4)	11(61%)	4.0 (<0.4-52.1)	45 (100%)	205 (42.3-2998) [A, B]
BaP	0.1	11 (73%)	3.6 (<0.1-36.3)	14 (78%)	1.5 (<0.1 -34.7)	44 (98%)	152 (4.8-2013) [A, B]
dBA	0.6	7 (47%)	<0.6 (<0.6- 72.5)	8 (44%)	<0.6 (<0.6-234.4)	35 (78%)	85 (<0.6-708) [A, B]
BPE	0.2	7 (47%)	<0.2 (<0.2- 91.0)	8 (44%)	<0.2 (<0.2-53.1)	19 (42%)	<0.2 (<0.2-2979)
IPY	2.1	3 (20%)	<2.1(<2.1- 33.7)	0 (0%)	<2.1	14 (31%)	<2.1 (<2.1-1883)
Σ15PAHs	-	-	1995 (460-4295)	-	2985 (48-6229)	-	83021 (11584-473321) [A, B]
Σ7PAHs	-	-	29.0 (3.8-250)	-	25.5 (3.5-322)	-	1319 (358-11560) [A, B]
Σ5PAHs	- -		18.5 (2.5-188)	-	22.4 (2.2-316)	-	1083 (274-6680) [A, B]

[A]=coke oven> general population living near the plant (p<0.05); [B]=coke-oven> general population living far away from the plant (p<0.05); [C]=general population living far away from the plant >general population living near the plant (p<0.05); LOD=Limit of Detection; N ≥LOD=number (percentage) of samples higher than the LOD

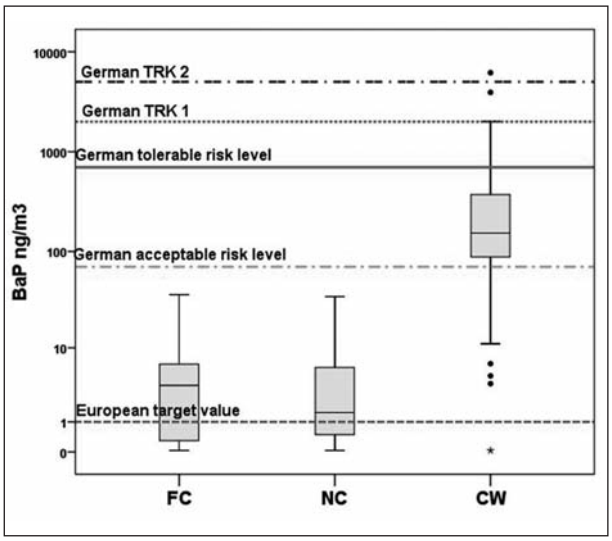

Figure 1 - Levels of airborne BaP (ng/m^3) in study subjects. **German TRK2:** German Technical guidance value for exposure to airborne BaP, 5000 ng/m^3 as an 8 h TWA for coke industry; **German TRK 1:** German Technical guidance value for exposure to airborne BaP, 2000 ng/m^3 as an 8 h TWA for an industry other than the coke industry; **German tolerable risk level:** 700 ng/m^3 corresponding to a tolerable risk of 4:1000 excess mortality; **German acceptable risk level:** 70 ng/m^3 corresponding to an acceptable risk of 4:10000 excess mortality; **European target value:** 1 ng /m^3

were BPE and IPY. The most abundant compound was again NAP (contributing 65% to the total amount), followed by FLE (5%). The median BaP level was 152 ng/m^3, contributing 0.2% to the total amount. The levels of each single airborne PAHs and exposure to carcinogenic compounds (Σ7PAHs and Σ5PAHs) were always higher in CW than in FC or NC (P<0.001), with the sole exception of BPE which did not differ between groups. When we considered different job titles, PAHs levels were somewhat higher in top-oven workers than in side-oven or maintenance operators, but statistical significance was reached only for ANT (10897 μg/m^3 in top-oven vs. 2357 μg/m^3 in side-oven workers, p<0.05) and BkF (282 μg/m^3 in top-oven vs. 60 μg/m^3 in side-oven workers, p<0.05). Median BaP levels were 360, 101 and 152 ng/m^3 in top-oven, side-oven and maintenance workers, respectively.

For BaP, 98% of CW were exposed to BaP levels higher than 1 ng/m^3, the European target value for environmental exposure, while as many as 82% were exposed to BaP levels higher than 70 ng/m^3, and 11% to levels higher than 700 ng/m^3, the German acceptable and tolerable risk limit values (figure 1). There was also a difference between CW with different job titles, with 75%, 72% and 95% of top-oven, side-oven and maintenance workers exposed to BaP levels higher than 70 ng/m^3, and 38, 6 and 5% of top-oven, side-oven and maintenance workers exposed to a BaP levels higher than 700 ng/m^3, respectively.

Measurement of 1-hydroxypyrene

Results of 1-OHP are shown in table 3. Data were available for 15 FC, 18 NC and 99 CW for before-shift samples and for 94 CW for end-of-shift samples. 1-OHP was found above the limit of detection in all samples.

No differences were found between FC and NC, while coke-oven workers had 1-OHP levels higher than both FC and NC. Higher 1-OHP values were found in smokers than in non-smokers for CW but not for FC. In CW, a significant difference between BS and ES samples was found only when 1-OHP levels were corrected for urinary creatinine, in both smokers and non-smokers. When comparing the 1-OHP levels among CW with different job titles, top-oven workers had BS values of 1.85 μg/g crt (3.19 μg/L) and ES values of 2.00 μg/g crt (5.59 μg/L), side-oven workers had BS values of 2.07 μg/g crt (4.07 μg/L) and ES values of 3.87 μg/g crt (3.35 μg/L), and maintenance workers had BS values of 1.04 μg/g crt (1.75 μg/L) and ES values of 1.49 μg/g crt (2.16 μg/L). Significant differences were found between the different job titles, with 1-OHP levels generally higher in top-oven and side-oven workers than in maintenance workers.

In general population subjects, as many as 93% of FC and 88% of NC had 1-OHP levels higher than the Italian reference value for non-smokers (0.30 μg/g crt) (33) (figure 2).

In coke-oven workers, 83% of ES samples had 1-OHP concentrations higher than the ACGIH benchmark level (1 μg/g crt) (1). Among these subjects, 7% had 1-OHP levels higher than the UK-HSE BMGV (7.72 μg/g crt) (19), 21% had 1-

Table 3 - Urinary 1-hydroxypyrene (μg/L and μg/g crt) in study subjects divided according to smoking habit

		General population living far away from the plant Median (5th-95th percentile), N	General population living near the plant Median (5th-95th percentile), N	Coke-oven workers Median (5th-95th percentile), N
1-OHP BS μg/L	All	0.73 (0.56-1.68), 15	0.69 (0.16-1.09), 17	2.29 (0.37-19.83) [A, B], 99
	Non-smokers	0.69 (0.56-1.68), 11	0.66 (0.16-1.09), 16	1.69 (0.14-19.83) [A, B], 36
	Smokers	0.79 (0.59-0.96), 4	0.79, 1	2.99 (0.51-19.36) [B], 62
1-OHP BS μg/g crt	All	0.56 (0.43-1.29), 15	0.53 (0.13-0.84), 17	1.45 (0.21-10.38) [A, B], 98
	Non-smokers	0.53 (0.43-1.29), 11	0.51 (0.13-0.84), 16	1.05 (0.19-9.32) [A, B], 36
	Smokers	0.61 (0.46-0.74), 4	0.61, 1	1.71 (0.32-10.38) [B], 62
1-OHP ES μg/L	All	-	-	2.97 (0.36-21.93), 94
	Non-smokers	-	-	2.72 (0.30-18.18), 34
	Smokers	-	-	3.63 (0.47-23.44), 60
1-OHP ES μg/g crt	All	-	-	1.96 (0.36-12.82), 94
	Non-smokers	-	-	1.79 (0.29-5.15), 34
	Smokers	-	-	2.21 (0.46-12.97), 60

N=number

[A]=coke-oven>general population living near the plant

[B]=coke-oven>general population living far away from the plant

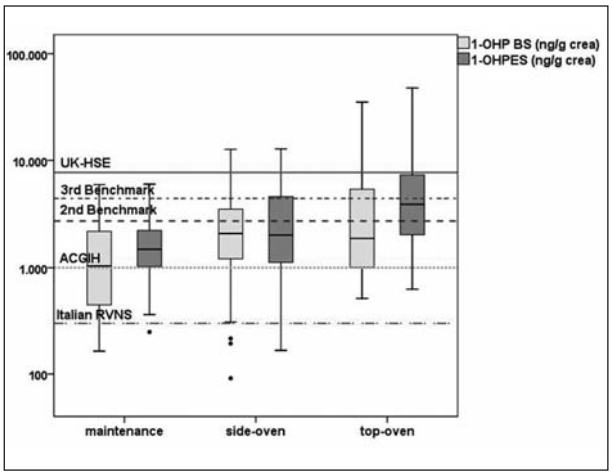

Figure 2 - Urinary 1-OHP (μg/g crt) levels in study subjects. **UK-HSE:** 7.7 μg/g crt biological monitoring guidance value according to the UK-HSE; **3rd Benchmark level:** 4.4 μg/g crt proposed by Jongeneelen; **2nd Benchmark level:** 2.7 μg/g crt proposed by Jongeneelen; **ACGIH:** 1 μg/L; **Italian RVNS:** 0.3 μg/g crt Italian reference value for non-smokers

OHP levels higher than Biological Exposure Level equivalent to an airborne exposure of 0.2 mg/m^3 (third benchmark guideline level of 4.40 μg/g crt according to Jongeneleen) (25), and 35% had 1-OHP levels higher than the no observed genotoxic effect level (second benchmark guideline level of 2.7 μg/ g crt according to Jongeneleen).

Correlations and linear regression analysis

Pearson's correlation coefficients between selected indices of airborne exposure to PAHs and 1-OHP level at the different moments of urine sampling are shown in table 4 for all the study subjects and separately for smokers and non-smokers. Selected PAH were considered: NAP, due to its abundance and toxicological relevance; PHE, representative of the 3-ring PAHs and due to its abundance; PYR, representative of the 4-ring PAHs, due to its abundance and because urinary 1-OHP is a product of its metabolism; BaP, representative of the 5-ring PAHs, due to its toxicological relevance; plus Σ15 PAHs, Σ7PAHs and Σ5PAHs.

Considering only airborne PAHs exposure, significant correlations were found between all analytes, and between each considered airborne compound and Σ7PAHs or Σ5PAHs.

Table 4 - Correlations, as Pearson's coefficient *r*, between urinary 1-OHP (μg/L and μg/g crt), and selected airborne PAHs (ng/m^3) in all subjects and in subjects divided according to smoking habit

		1-OHP ES	NAP	PHE	PYR	BaP	Σ15PAHs	Σ7PAHs	Σ5PAHs
1-OHP BS	All (N=77)	0.49**	0.39**	0.50**	0.49**	0.49**	0.55**	0.52**	0.54**
(μg/L)	Non-smokers (N=41)	0.40*	0.27	0.41**	0.38*	0.42**	0.52**	0.46**	0.51**
	Smokers (N=35)	0.54**	0.29	0.40*	0.50**	0.48**	0.40**	0.47**	0.46**
1-OHP BS	All (N=77)	0.40**	0.38**	0.50**	0.49**	0.52**	0.55**	0.53**	0.55**
(μg/ g crt)	Non-smokers (N=41)	0.40*	0.22	0.40*	0.34*	0.40*	0.45**	0.41**	0.46**
	Smokers (N=35)	0.38**	0.27	0.35**	0.47**	0.49**	0.40**	0.46**	0.45**
1-OHP ES	All (N=44)		0.11	0.13	0.35*	0.17	0.29	0.34*	0.33*
(μg/L)	Non-smokers (N=14)		0.03	0.15	0.28	0.47	0.49	0.40	0.42
	Smokers (N=30)		0.15	0.12	0.37*	0.20	0.31	0.34	0.31
1-OHP ES	All (N=44)		0.11	0.13	0.35*	0.17	0.29	0.34*	0.33*
(μg/g crt)	Non-smokers (N=14)		0.12	0.42	0.37	0.65*	0.51	0.41	0.53
	Smokers (N=30)		-0.01	0.21	0.25	-0.00	0.18	0.31	0.29

*=p<0.05
**=p<0.01

Significant correlations between 1-OHP BS and the different indices of airborne exposure to PAHs were found, while 1-OHP ES correlated significantly with PYR, Σ7PAHs, and Σ5PAHs. The lack of correlation between 1-OHP ES and the other airborne analytes and the weaker correlations observed may be due to the lower number of subjects with end-of-shift samples available. Generally, correlations were weaker or lacking when only smokers or non-smokers were considered, with the notable exception of the significant positive correlation between 1-OHP ES (μg/g crt) and BaP observed in non-smokers.

A linear regression analysis was performed with 1-OHP BS and ES (μg/g crt) as dependent variables and BaP (ng/m^3) as independent variable in non-smokers, with correlation coefficients of 0.40 and 0.65 (p<0.05). Based on these analyses, 1-OHP levels corresponding to selected BaP values were estimated (table 5). As expected, the regression slope resulted higher in ES samples than in BS samples. Notably, the intercept value was the same in both regression analyses and corresponded to the 1-OHP BS value found in the general population subjects.

Table 5 - Parameters of the linear regression analysis predicting 1-OHP (μg/g crt) from airborne BaP (ng/m^3) as independent variable (non-smokers) and calculated 1-OHP (μg/g crt) values corresponding to selected BaP (ng/m^3) concentrations

Dependent variables	N	Independent variable	Slope (SE)	Intercept (SE)	r	P
Log 1-OHP BS (μg/g crt)	41	Log BaP (ng/m^3)	0.135 (0.049)	-0.270 (0.078)	0.40	0.009
Log 1-OHP ES(μg/g crt)	14	Log BaP (ng/m^3)	0.191 (0.065)	-0.270 (0.153)	0.65	0.013

Calculated 1-OHP (μg/g crt) values corresponding to selected BaP (ng/m^3) concentrations:

BaP (ng/m^3)	1-OHP BS (μg/g crt)	1-OHP ES (μg/g crt)
1	0.5	0.5
70	1.0	1.2
700	1.3	1.9

Discussion

Exposure to airborne PAHs and comparison with limits

In this study, environmental and biological monitoring were performed to assess exposure to PAHs in subjects working and/or living at various distances from the Taranto coke plant. In coke oven workers, NAP was the most abundant compound, with a median level of 53523 ng/m^3. This level was one thousandth of the 50 mg/m^3 adopted in many countries as OEL and also far lower than 10 mg/m^3 proposed by ACGIH (1). Comparing BaP levels with the new German acceptable and tolerable risk limit (figure 1), we found that a high percentage of workers exceeded these limits, while if the 5000 μg/m^3 TRK value had been considered, only 2% of CW exceeded this limit. The German risk-based limits are stricter than the former German technical guidance values and make it possible to better define exposure situations that may endanger workers' health. The comparison based on job titles shows that, even if there were few significant differences between the different jobs, signifying a generally polluted work environment, nevertheless top-oven workers experienced the highest personal exposure.

The Taranto coke plant has been studied for many years, as it is the largest in Europe and represents a major source for pollution. For PAHs, in particular, this plant contributes to 95.8% of the total emissions for Italy, with 32240 kg/year (24), and various improvements have been made over the years to reduce emissions (16, 21). Currently, a median BAP level of 152 ng/m^3 was found in our study, while BaP levels of 2820 ng/m^3 were reported in the past (table 6).

As regards general population subjects, median BaP levels found in NC were in good agreement with those reported for the summer values collected in Tamburi, the district where our study was also conducted (38) (table 6). Moreover, BaP levels up to 34.7 ng/m^3 are consistent with previous data

Table 6 - Summary of PAHs exposure and urinary end-of- shift 1-OHP in selected published papers for the same coke plant and the general population living in the vicinity

Year of the study	N of subjects	Σ15 PAHs (μg/m^3)	BaP (ng/m^3)	1-OHP μmol/mol crt	1-OHP μg/g crt	Reference
A) Studies on this coke plant						
1992	76 CW	27.1 (3.6-62.6)[a]	2820 (450-4840)[a]	0.98 (0.040-5.587)[a]	1.89 (0.08-10.8)[a]	4
1) 1993-94 2) 1999-2000	1) 50 CW 2) 27 CW	1) 1.1-442.9[b]	1) 500-52600[b] 2) <520-11200[b]			16
2001-2002	355 CW			1.05 (0.01-31.04)[a]	2.03 (0.02-59.90)[a]	3
2005	100 CW	83 (11-473)[c]	152 (4.8-2013)[c]	0.98 (0.19-6.63)[c]	1.9 (0.36-12.8)[c]	This study
B) Studies on general population living near this coke plant						
1992	18 controls			0.068 (0.018-0.56)[a]	0.13 (0.04-1.08)[a]	4
1999-2000	Environmental exposure study performed in the town area near the plant		Summer 1999: 1.2 (<0.1-6.1)[a] Winter 2000: 0.2 (<0.1-11)[a]			38
2005	1) 15 far controls 2) 18 near controls	1) 1.99 (0.05-0.43)[c] 2) 2.98 (0.05-6.23)[c]	1) 3.6 (<0.1-36.3)[c] 2) 1.5 (<0.1 -34.7)[c]	1) 0.38 (0.29-0.87)[c] 2) 0.36 (0.08-0.56)[c]	1) 0.73 (0.56-1.68)[c] 2) 0.69 (0.16-1.09)[c]	This study

[a]=median (min-max); [b]=min-max; [c]=median (5th-95th percentile); N=number

showing that, in Tamburi, seasonal changes in PAH exposure are associated with wind direction, with higher PAH levels (up to 40 ng/m^3) registered when the wind was blowing from the north, the location of the industrial area (2). Surprisingly, similar BaP levels were also found in FC for whom no industrial emission exposure is known. As the village of Alberobello is situated 50 km north-east of the plant, the emissions from the coking plant do not reach it. The hypothesis that may explain this result is that these subjects were exposed to PAHs from domestic heating and cooking sources, given the fact that open fireplaces are common in rural settings and barbecuing is very popular. Unfortunately, no detailed information on this source of PAHs exposure was collected so at the moment this hypothesis cannot be further supported. On the other hand, the number of general population subjects involved in the study was very limited and not properly matched with the exposed subjects for personal characteristics, so other data are needed to confirm our results.

1-Hydroxypyrene and comparison with limits

Median 1-OHP levels, corrected for urinary creatinine, in control subjects and in coke-oven workers were compared with the reference values for the general population or with the proposed biological limit values for occupational exposure (figure 2). The different limit values were exceeded by several workers. Comparing BS and ES samples, a higher percentage of workers (83% vs. 67%) exceeding the ACGIH benchmark level was observed in ES samples, as a result of daily work activity. It is noteworthy that the majority of BS samples were higher than the ACGIH benchmark level, which is consistent with the slow kinetics for 1-OHP excretion (estimated to be 5 - 40 h) leading to an accumulation of this chemical in the human body during the work week, as previously observed (31,32).

In control subjects, no difference in the 1-OHP level was detected when far and near control subjects were compared. Such results, although unexpected, show a good agreement between environmental and biological monitoring. Most of these subjects had 1-OHP levels higher than the Italian reference value for non-smokers (0.30 μg/g crt) (33) (figure 2). This is consistent with the relatively high environmental exposure detected for these subjects. However, considering that the Italian reference value for the general population was lowered in recent years passing from 0.65 μg/g crt proposed in 2003 (30) to 0.3 μg/g crt proposed in 2011, and that our study was performed in 2005, we conclude that our results are consistent with past exposure in the Italian general population.

Comparing 1-OHP ES levels with those reported in previous studies (table 6) (3, 4, 16), it was noted that median and range were similar. This is in contrast to the airborne BaP data, that was significantly lowered compared to the past, and may be explained by the fact that inhalation is only one of the possible exposure routes for these individuals; for example dermal absorption was estimated to account for as much as 50% of the total PAHs intake in coke over workers (15, 37).

Correlations and linear regression analysis

Significant correlations were found between 1-OHP and airborne PAHs, in particular between 1-OHP, BaP and the different indexes of exposure to carcinogenic compounds. On this basis, in non-smokers, a linear regression analysis with 1-OHP BS and ES was performed (table 5). The resulting correlation coefficients were not particularly high (r=0.40 and 0.65), which may be due to both the limited number of subjects eligible for analysis and to the contribution of other sources to the total intake of PAHs, and particularly to the role of dermal absorption, as mentioned above (15, 37), and the role of diet, especially relevant for the general population subjects (23). We estimated 1-OHP BS and ES values corresponding to BaP levels at the European target value, the German acceptable and tolerable risk levels. These were 0.5, 1.0 and 1.3 μg/g crt and 0.5, 1.2, and 1.9 μg/g crt for 1-OHP BS and 1-OHP ES, respectively. These may be proposed as biological exposure equivalents for occupational exposure to BaP in the coke-oven industry. These values are lower, although in the same order of magnitude, than the previously proposed occupational biological limit values, which

support current proposals to lower occupational limit values. A value of 0.5 μg/g crt, calculated for BaP at 1 ng/m^3 is also consistent with the reference values proposed for non-smoking subjects by different regulatory bodies (ranging from 0.3 to 0.5 μg/g crt).

Conclusion

In conclusion, in the study environmental and biological monitoring results for coke-oven workers are in good agreement and show a similar and limited percentage of subjects exceeding the technical airborne and biological limit values suggested by German and UK regulatory bodies (2 and 7% for air BaP and 1-OHP ES, respectively). Furthermore the comparison with previous studies shows a significant decrease in exposure levels, consistent with improved industrial hygiene measures taken at the studied workplace over the years. Conversely, 11 and 82% of CW workers exceeded the recently proposed German tolerable and acceptable risk limit values, showing that, following the more rigorous environmental limits, further preventive actions are required to improve workplace conditions and reduce the risk for health. To improve exposure assessment, taking into consideration all exposure routes, efforts need to be made to confirm our results and add further evidence for the identification of a risk-based biological limit value for 1-OHP.

No potential conflict of interest relevant to this article was reported

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