



Hydroxypyrene Excretion as Indicator of Cancer Risk in Street Sweepers and Polycyclic Aromatic Hydrocarbons (Pahs) Exposure

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Abstract

Exposure to polycyclic aromatic hydrocarbons (PAHs) is associated with possible carcinogenicity, according to European directives. Studies helped to clarify the contributions to the total dose resulting from environmental and occupational exposure, by tobacco smoke, by diet and skin absorption. Recently, it was shown that levels of 1-Hydroxypyrene U (1-OHPu) may be predictive of individual dose, environmental and employment exposure, proportionately even though indirectly expressing the concentration of Benzo[a]pyrene (Bap) and of pyrene (Pyr), too, since the latter is associated to Bap with stable proportionality. The 1-OHPu, metabolite of Pyr, which proved to be mutagen for photochemical effect, can be considered the better indicator of internal dose not only for recent exposure, but also as an indicator of accumulation and diffusion in the human body, given the greater solubility in water of Pyr compared to Bap. Moreover, it is more sensitive than 3-Hydroxybenzo[a]pyrene (3-OHBAp) for the estimation of total exposure to PAHs. Measurements of the concentration of 1-OHPu in the urinary excretions of street sweepers at the end of their daily working period showed an increase of the 1-OHPu geometric mean concentration both forever smokers - never smoking and/or selectively affected by chronic bronchitis (all smokers). These workers are exposed in the urban environment to pollution due to diesel engines, domestic heating and industrial PAHs emissions from petrol and coal combustion in power stations. The continuous increase of the number of workers affected by bronchitis ask for experimental studies to test the ability of Pyr to remain in the epithelium and in sub-bronchial epithelium, apparently to a greater extent for its greater dilution in the inflamed tissue, slowing down its biotransformation in water-soluble metabolites. In this way, a reserve of pre-toxic PAHs accumulates at pulmonary level and determines a stronger urinary elimination of 1-OHPu. The presence of 1-OHP in urinary excretions is, then, an indicator of PAHs exposure.

Evidence of ILCR (incremental life-time cancer risk) is demonstrated in association with ambient Bap concentration, smoke and chronic bronchitis vs. control group (ILCR_{inhalation} range 5, 6-9, 13×10^{-6}), and a historical similarity with chimney sweepers is remembered.

Abbreviations

CYP: Cytochrome P450, 1-OHPu: Urinary 1-hydroxypyrene; PAHs: Polycyclic aromatic hydrocarbons, PYR: Pyrene, Bap: Benzo[a]pyrene, ILCR_{inhalation}: Incremental lifetime cancer risk inhalation, CSF: Cancer slope factor, Searly: Early shift, Send: End shift.

Introduction

It is well known that exposure to PAHs like Dibenzo(a,h)anthracene (DBA), Benzo(a)pyrene (BaP), Pyrene (Pyr), Benzo(a)anthracene (BaA), Benzo(b,j,k)fluoranthene (BbF), Benzo(k)fluoranthene (BkF) is bound to possible carcinogenicity, as highlighted in the statements of phrases R45/R49/H350 according to European directives [1-4]. The concentration of PAHs in the respiratory system increases with age (e.g., for BaP, BkF) according to the diagram men > women, smokers >> never-smokers, cancerogenous lung cells > not cancerogenous ones [5]. On the other hand, the liver ability to proceed to biotransformation by cytochrome 4501A1, activates in many cases the same toxicity and carcinogenesis. So, if the same PAHs are the major air pollutants as a result of combustion processes, many data indicate in the feeding and gastro-enteric tract an additional quota of dose, because, although they are lipophilic compounds, some of them are present in varying concentrations in water and in various types of food, including vegetables [6,7], US-EPA, SCF, JECFA classification of PAHs pollution in environment and food [6].

The study of DNA polymorphisms of genes like CYP1A1, GSTM1, mEH, GSTP1 and GSTT1 clarified that individual variability in levels of 1-OHPu is a consequence of higher metabolic capacity in "CYP1A1 Ile/Val genotype" and "GSTM1 null genotype" individuals: in children CYP1A1 and CYP1B1, NQO1 (activation) and GSTM (disable) are identified [8,9]. Many environmental factors, such as temperature, light, oxygen, ozone and pollutant concentrations, oxidation, photochemical reactions with NO₂, SO₂ and SO₃, influence the chemical reactivity. Bioavailability is an important factor to determine the possibility that a higher or lower quantity of PAHs enter into cellular systems. For instance, PAHs present in the vapor phase (e.g., gas from coke-fuelled ovens), is of high bioavailability, while PAHs from urban environments (heating, transport) is not [10]. However, given the difficulty of estimating the total "intake/uptake" from the air (environment/employment/exposure smoke), diet intake and skin absorption, the best choice for assessing the total exposure to PAHs is a biomarker study. We have taken into account that 1-OHP, although not specific to genotoxicity like 3-OHBAp [11], can be chosen as a good indicator of internal exposure dose not only for PAHs (since it is the metabolite of Pyr, which among PAHs is the

chemical compound more representative and more diffused in the urban and working environment and represents 90% of the urinary excretion) even for different categories of persons [12-14]. It may be a sign of greater exposure to different risk stratifications, with urinary concentrations up to 2500 times higher than the 3-OHBP (12, 16.17). It is also useful compared to Pyr, which is able to become mutagenic after exposition to sunlight [Pyrene (84) 129 00 - - 0 + + 8926 ± 3111205 ± 42 (0.03) Bacteria death] [15]. In this sense, we cannot exclude the synergy with the most powerful carcinogens and in particular with Bap [6]. Keeping also into account that Pyr, for its water solubility characteristics (7.2 × 10⁻⁴ mmol/l), is able to pass through the lung into the blood and undergo biotransformation, Pyr solubility has to be compared to the one of Bap (1.5 × 10⁻⁵ mmol/l), about 48 times lower. Moreover, the latter presents a vapor density of 8.4 × 10⁻⁷, about thousand times lower than that of Pyr, which is 8.86 × 10⁻⁴. Then 1-OHPu can be considered as a better predictive model of PAHs concentration in different conditions of risk. A lifetime risk of respiratory cancer per ng/m³ was calculated [16,17].

Materials and methods

In this study we evaluated the urinary excretion of 1-OHPu in 31 healthy "street sweepers" and in 8 with a bronchitis process all smokers, exposed mainly to urban and industrial air pollution (they use semiautomatic systems to empty garbage road containers and/or manually collect bags with materials for recycling, but the exposure is also due to fumes from diesel engine combustion and to other environmental pollutants in areas where the production of PAHs is due to the operation of coal-fired power stations, lime furnaces, petrochemical candles, combustion of part of olive trees - Salento country), as an indicator of internal dose for exposure to fumes from the combustion of diesel and internal combustion engines, but also of domestic heating systems and industrial emissions of PAHs from petrochemical factories and coal-fuelled power stations) [18]. All the

39 "street sweepers", living in the same operational area (Brindisi-Italy), were male. Among the healthy (non bronchitic) "street sweepers", the smokers were found to be 12 (26%). For every worker the 1-OHPu urinary concentration was measured in samples collected at the beginning and at the end of their working periods for at least three consecutive days of work. The measurements were made by using the method of Jongeneelen (1987) [19], after enzymatic hydrolysis of β-glucuronides and/or sulphate conjugates, followed by analysis using high-performance liquid chromatography (HPLC), combined with a fluorimetric detector. The limit of detection of the method is 25 ng/l. The concentrations of urinary 1-OHPu were expressed in ng/g creatinine.

Results

Environmental exposure values are reported in table 1. The geometric averages of the measured 1-OHPu concentration values are shown in table 2 and table 3, and histograms in figure 1, figure 2 and figure 3. The above values were compared with the ones of a group of 18 workers involved in indoor activities, in order to evaluate the usefulness of 1-OHPu assay as part of health monitoring processes. In figure 4 is reported ILCR_{inhalation} in PAHs exposure and in figure 5 the same data on a logarithmic scale.

The concentration values associated to never smokers (NS) and forever smokers (S) and exposure (years) are shown in table 2 and figure 2, at the beginning and end of working shift.

ILCR calculated by PAHs exposure with benzo[a]pyrene concentration expressed in ng/m³ ILCR = {C_a [CSF (BW/70)^{1/3}] dayyear⁻¹·year/BW·AT}·C_f [20,21] where:

ILCR is incremental life-time cancer risk [22]

C_a is PAH concentration as Bap ng/m³ in working shift

CSF(3,1) is cancer slope factor ng/m³ (WHO₂₄); (mgKg⁻¹day⁻¹)⁻¹ U.S.EPA, 2001

Table 1: Environmental exposure value.

Total PAH		Benzo[a]pirene		PM10
ng/m ³	Total ng/exposed person/working shift	ng/m ³	ng"intake"/exposed person/working period	µg/exposed person/working period
33-75	495-1125	0.089-0.55	1.33-8.25	450

Table 2: 1OHP_u shift_{early} never smokers (S_{early} NS) and smokers (S_{early} S) and Shift_{end} never smokers (S_{end} NS) and Shift_{end} Smokers (S_{end} S) - t-test (*).

Table 1 Risk	Smoke	N°	Geom.-mean ng/gr creat	-DS	+DS	
Exposed Shift _{early}	NS	19	316	158	630	
	S	12	1000	398	2511	P < .001 vs. NS and contra NS (*)
Exposed Shift _{end}	ES-NS	19	1259	398	3989	P < .001 vs NF I T (*)
	ES-S	12	1981	629	6237	P < .001 vs NF IT (*)
Control non exposed	NS	9	230	103	513	
	S	9	425	151	1138	

Table 3: Concentration of 1-OHP_u associated to exposure(y) in never smokers (NS) and forever smokers (S), at the beginning (S_{early}) and end (S_{end}) of working shift -t-test (*)

Risk exposure (y)	Shift/Smoke	N°	Geom.mean ng/gr creat	-DS	+DS	
Exposed < 15	S _{early} NS	10	251	158	398	
Exposed > 15	S _{early} NS	9	436	173	1096	
Exposed < 15	S _{end} NS	10	1071	446	2570	
Exposed > 15	S _{end} NS	9	1031	333	3184	
Exposed < 15	S _{early} S	8	794	316	1995	
Exposed > 15	S _{early} S	4	1000	254	3987	
Exposed < 15	S _{end} S	8	1995	501	7761	P < 0.01 comparing y < 15 - y > 15 NS S _{early} (*)
Exposed > 15	S _{end} S	4	1584	316	7913	
Exposed broncostr. all smokers	S _{early} S	8	1412	870	2290	
	S _{end} S	8	2137	1513	3019	P < 0.01 comparing y < 15 - y > 15 NS S _{early} (*)
Smokers < 15s	S _{early}	4	1819	1713	1931	
	S _{end}	4	3133	824	11712	
Smokers > 15s	S _{early}	8	794	316	1995	
	S _{end}	8	1584	501	5011	

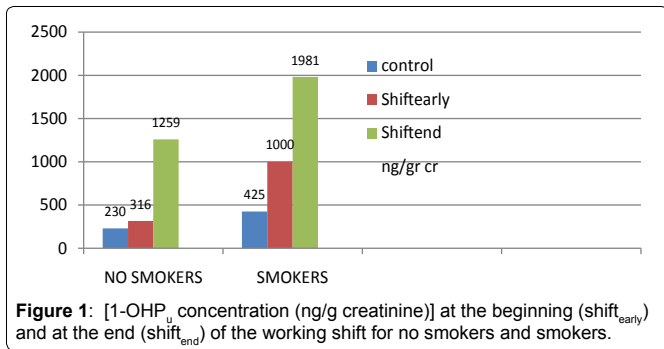


Figure 1: [1-OHP_u concentration (ng/g creatinine)] at the beginning (shift_{early}) and at the end (shift_{end}) of the working shift for no smokers and smokers.

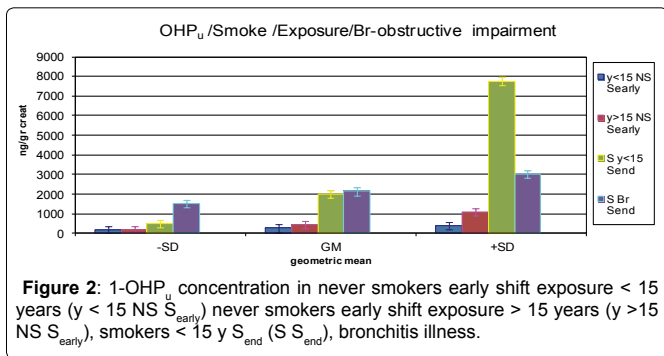


Figure 2: 1-OHP_u concentration in never smokers early shift exposure < 15 years (y < 15 NS S_{early}), never smokers early shift exposure > 15 years (y > 15 NS S_{early}), smokers < 15 y S_{end} (S S_{end}), bronchitis illness.

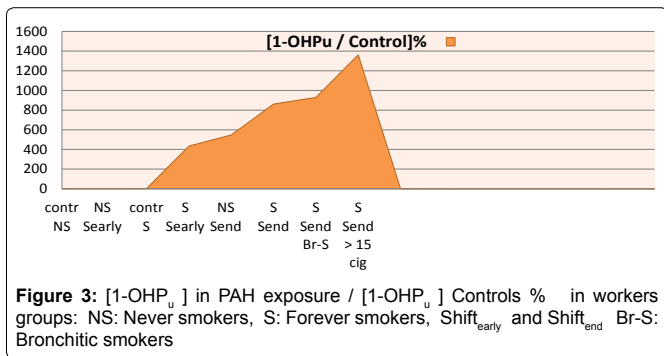


Figure 3: [1-OHP_u] in PAH exposure / [1-OHP_u] Controls % in workers groups: NS: Never smokers, S: Forever smokers, Shift_{early} and Shift_{end} Br-S: Bronchitic smokers

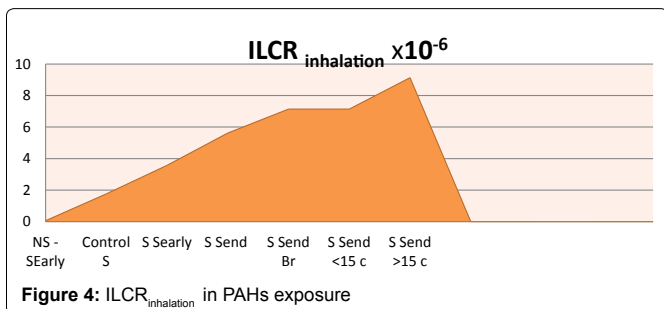


Figure 4: ILCR_{inhalation} in PAHs exposure

BW = body weight (75Kg)

40 year of life in work

Dayyear⁻¹ - days of work for year of work exposure (210)

AT = average time in work (40)

C_f = 10⁻⁶

For evaluating risks from inhalation exposure, MDH recommends a Health Risk Value (HRV) of 0.001 ng/m³ for B[a]P [23], which is based on a slope factor of 7.3 (mg/kg-day)⁻¹. This slope factor is the geometric mean of the B[a]P slope factor range used by the U.S. Environmental Protection Agency (U.S. EPA, 2001).

The geometric mean concentration of 1-OHPu among the exposed workers at beginning of their working shift is greater in forever smokers (S) than for never smokers (NS) and for never smokers controls (control NS). But, the most significant data is that the exposed workers, both never smokers and forever smokers, at the end of their working shift (S_{end} NS: S_{end} S) show higher 1-OHP_u

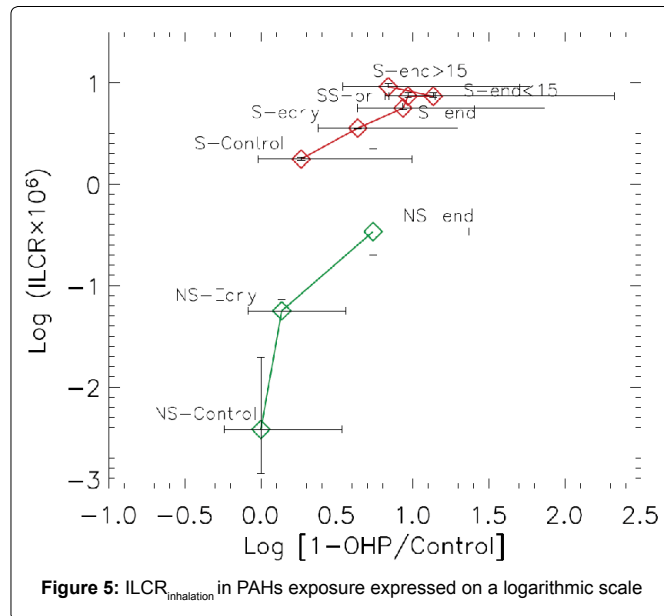


Figure 5: ILCR_{inhalation} in PAHs exposure expressed on a logarithmic scale

concentration values than that of the same workers at the beginning of their working shift and of the control non-exposed workers. Tables and figures also demonstrate the smoke influence, associated with the exposure, i.e. the greater values of urinary excretion of 1-OHPu against the ones of never-smokers. More, the 1-OHP_u concentration is significantly higher in forever smokers with more than 15 years of exposure, than in never smokers at the beginning of their working shift (S_{early} NS). Cigarette smoke factor is significant for smokers in conjunction with less than 15 years of exposure at the end of working shift (S_{end} shift < 15 y exposure), as well as for the control group. The same happens for those with bronchial obstructive impairment.

In general 1-OHP_u concentration behaves as an interesting variable, associated not only to recent exposure, but also to the effects of accumulation, since the increase in concentration is influenced by the years of exposure as well as by unhealthy breathing ways, likely to inflammation and edema accumulation.

As regards the application of preventive rules, the characteristics of smoking behavior are to be studied. Behaviors depend on the technology used by the worker. Sweepers attached to the moving medium with hook switch of dumpsters to the moving car, can hardly smoke during the service period, but they can smoke very much, assuming the related risk, at the beginning and end of their workday.

Discussion

Our study showed an increase in the urinary excretion of 1-OHP at end of working shift with respect to the one at the beginning, in smokers and never smokers and in suffering from chronic bronchitis, exposed to pollution sources such as urban traffic, combustion fumes from diesel engines, home heating systems and emissions of PAHs from petrochemical factories and gas and coal combustion in power stations [18]. The smoking of tobacco is confirmed, among exposed to vehicular traffic, to be an important additional factor of increase of urinary excretion of 1-OHPu [1]. However, the contribution to the increase of global dose due to eight working hours is not negligible, due to the accumulation characteristics demonstrated by PAHs. Urinary excretion of 1-OHPu, as an indicator of internal dose of PAHs, proves to be not only an indicator of recent exposure, but also of accumulation in the past. It can be used as a signal to identify the groups at greatest risk, although its proved photochemical mutagenic effect is not considered to be specific for genotoxicity, as for 3-OHBP [11]. However, it can be anyway considered as the best indicator of internal dose for exposure to PAHs for several categories of persons [12,13], taking also into account that Pyr is much more soluble in water and able to pass through the lung to blood and undergo biotransformation with respect to Bap [16,24]. Urinary excretion of 1-OHPu, as an indicator of the internal dose exposure to fumes from

diesel engine combustion and to other environmental pollutants in areas where the production of PAHs is due to the operation of coal-fired power stations, lime furnaces, combustion of part of olive trees, petrochemical candles involves a careful evaluation of the risk factors in the context of the working procedure of street sweepers. The prevention of this kind of risk might be reduced by the use of electric and/or natural gas fuelled cars especially in waste sorting house by house.

The evidence of a greater concentration in bronchitic men ask for experimental studies to test the ability of Pyr to remain in the epithelium and in sub-bronchial epithelium, apparently to a greater extent, for greater dilution in the inflammatory process, and then to undergo a biotransformation activity slowdown in water-soluble metabolites. It establish a reserve as a pre-toxic pulmonary factor, similar to the genotoxic action of compounds like BaP [25], indicating accumulation phenomena well known for PAHs, since when attached to inhaled particles at the alveolar level are quickly released if placed at the surface of these particles, while associated to deeper penetration ones, like nanoparticles [26], are released more slowly [10] or involved in uptake endocytosis [27].

It could be useful to calculate the “cancer risk assessment” in our group and similar ones [28]. In this contest, the calculation of ILCR (incremental life-time cancer risk inhalation) in PAHs exposure as a factor of risk evaluation for lung cancer demonstrates a good association with 1-OHP_u concentration, but only for smokers with exposure after finishing work and subjects with br-obstructive impairment (ILCR_{inhalation} range 5, 6-9, 13×10^{-6}), condition that contributes to the total risk with skin contact and ingestion [29]. We are in agreement with recent study [30] about the characteristics of variability of 1-OHP_u excretion in different groups of workers exposed or in people with urban or industrial pollution, but in addition to environmental and behavioral factors are to be considered as important parameters as surface, size, clustering that affect in the process of uptake, intake and biotransformation in different types of atmospheric particulate in much part of nanoparticle size.

Conclusions

The possibility of implementing prevention is based on simple and effective principles and on the utilization of useful methods. In our case, the street sweepers in the performance of their work are exposed to a mixture of PAHs of some carcinogenic action. In the grading of risk, this exposure appears to be important, in addition to traditional influencing factors such as the time of exposure, cigarette smoking, food intake and the effects of accumulation within the working day, associated also to possible respiratory diseases. The 1-OHP_u urinary concentration test appears useful to identify groups of persons exposed to greater risk of carcinogenicity. It is also a biological indicator of exposure to Benzo[a]pyrene, which among the PAHs is the most dangerous agent, according to ILCR values (ILCR_{inhalation} range 5, 6-9, 13×10^{-6}), and accumulates in the body. Jongeneelen (1992), proposed an occupancy limit of 1-OHP_u urinary concentration of 2.3 μmol/mol creat for an environmental concentration of 2 μg/m³. Further studies on the concentrations in air show that Pyr values of 49 ng/m³ corresponding to 0.85 ng/ml, with considerable lowering of values [31].

The findings of the present study are linked to the beginnings of toxicology involving chimney sweeps, namely the early work by Sir Percival Potts in the late 1770s. His work contributed to the emerging science of epidemiology as well as toxicology and lead to one of the first public health laws in Europe, namely the Chimney Sweepers Act of 1788.

We believe that further results in the analysis of the permissible limits of carcinogenic risk, waiting for epidemiological projections, often late in terms of prevention, are key to the reduction of the negative effects, DNA damage and the development of cancer.

Conflict of interest

The authors declare no conflict of interest

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