Polycyclic Aromatic Hydrocarbon Exposure in an Artificial Shooting Target Factory: Assessment of 1-Hydroxypyrene Urinary Excretion as a Biological Indicator of Exposure

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Five representative workers and two external observers were monitored by personal air and urinary 1-hydroxypyrene (PyOH) sampling for a four-shift working week in an artificial shooting target factory. The targets (clay pigeons), are made from petroleum pitch and molded at 190°C. No respiratory protective mask was worn. Atmospheric concentrations of pyrene and benzo (a) pyrene (BaP) ranged from 0.66 to 5.05 mmg/m³ and 0.037 to 0.270 mmg/m³ respectively with a mean pyrene/BaP ratio of about 20 and a correlation \( r = 0.51 \). Maximum PyOH urinary excretion ranged from 1.84 to 10.9 mmol/molCreat. This occurred at the postshift for the observers but often appeared later for workers: up to 10.75 h for the person with the apparently highest dermal exposure. The apparent PyOH excretion half lives ranged from 1.9 to 12.5 h with an arithmetic mean of 6.1 h. All these data were confirmed by additional measurements taken over a weekend after the postshift. The correlation between atmospheric pyrene and urinary PyOH concentrations (increase over the shift) was poor \( (r = 0.37) \). It improved greatly \( (r = 0.74) \) if the amount of pyrene inhaled over the shift and the corresponding amount of PyOH excreted were considered. The ratio of urinary excreted PyOH to the pyrene inhaled dose (with assumed retention of 100%), ranged from 0.18 to 0.70 (arithmetic mean = 0.34). This suggests that the respiratory tract is the main entrance route for pyrene (apart from the worker who handled crude targets without gloves). © 2000 British Occupational Hygiene Society. Published by Elsevier Science Ltd. All rights reserved.

Keywords: polycyclic aromatic hydrocarbons; 1-hydroxypyrene; petroleum pitch

INTRODUCTION

Until the end of the eighties, carcinogenic risk of workers exposed to polycyclic aromatic hydrocarbons (PAHs) was assessed by atmospheric monitoring, with determination of benzene (or cyclohexane) soluble matter and/or benzo(a)pyrene (BaP). Later 16 PAHs containing 2–6 rings were analyzed individually or summed.

In spite of the limitation and the uncertainties attached to the choice of a single indicator, BaP — for which a great deal of toxicity and occurrence data is available — seemed to be the most appropriate and was therefore most often used by industrial hygienists for estimating carcinogenic risk.

Biological monitoring, another type of approach to determine workers exposure to PAHs has been used for about 10 years. In this case, 1-hydroxypyrene (PyOH), a major metabolite of pyrene, has been suggested as a biological indicator of exposure (Jongeneelen et al., 1985, 1987). This approach has the advantage of taking into account all the exposure routes, particularly dermal absorption which could be very important: higher than 70% according to some authors (Van Rooij et al., 1993a; Quinlan et al., 1995).

However, there are several disadvantages:

- PyOH sometimes seems to indicate the absorption of only pyrene and other light PAHs which are not carcinogenic (Petry et al., 1996).
- The results of the various authors differ somewhat, and in some cases are conflicting: correlation between air concentration of pyrene
and PyOH, excretion half-lives, respective parts and bioequivalence of dermal and pulmonary routes.

The use of a BaP metabolite as an indicator of exposure to carcinogenic PAHs, for example 3OHBaP, would appear to be more appropriate. Nevertheless its determination in urine of exposed workers is rarely performed, particularly as the current methods are not sensitive enough for such small quantities of this metabolite (Ariese et al., 1994; Grimmer et al., 1997). So far, industrial hygienists have continued to use urinary PyOH as a biological indicator. Therefore, a great deal of data on urinary PyOH is available at the present time (Blaak et al., 1994; Elovaara et al., 1996). In comparison, there are few data concerning moderately polluted workplaces, with PAHs levels considered acceptable (C BaP < 0.5 μg/m³) (Boogaard and Van Sittert, 1994).

The plant in which our study was conducted belongs to this case: an artificial shooting target factory where the basic binder, namely coal tar pitch, has been substituted by a petroleum pitch that is less rich in PAHs. The aim of this study was to establish the continuous profile of the urinary PyOH of exposed people as well as the relationship between atmospheric and biological data. It was also to determine whether measurements of urinary PyOH levels are suitable to estimate exposures to low atmospheric concentrations of carcinogenic PAHs.

To achieve this, five workers of the manufacture and two external observers were monitored over a four-shift working week by means of atmospheric and urinary samples. Additional experiments were carried out to estimate the post shift elimination over a weekend.

MATERIALS AND METHODS

Plant and work description

The artificial targets are made of chalk (70–75%) and petroleum pitch (25–30%). After mixing, the paste is molded in non closed automatic presses at 190°C. The targets are then removed from the mold (the hardening time is about 20 s) and placed on a covered but non closed conveyor belt which leads to the manual packing station. When the study was conducted, there were seven production lines (press + conveyor belt), two equipped with a painting station.

Among the five monitored workers, three (two women) responsible for one or two lines effected the packing and minor interventions on the presses. The fourth was in charge of the maintenance of the presses and conveyor belts, and the last (a woman) was a foreman: her job consisted in truck driving, controlling, maintaining and replacing other workers. The two external persons were observers with a low level of physical activity. Nobody wore a respiratory protector. On the other hand, both on the lines wore gloves regularly.

The daily activities of each worker were recorded: splitting the work into elementary parts (standing, moving, carrying loads, etc.) allowed estimation of the mean respiratory flow-rate during the shifts. This evaluation was supplemented by cardiac frequency monitoring. The combination of the two approaches resulted in a quantitative assessment of the respiratory flow with a uncertainty of about 4 l/min (Horvat and Meyer, 1998).

Atmospheric monitoring

Sampling. Personal breathing zone air samples were taken for each worker and observer over the four shifts and on the additional working days (two 4-hour samplings per shift in preference to one 8-h, in order to minimize losses of matter during sampling).

The airborne material was sampled at a flow rate of 1 l/min (Gilian air samplers, Gilair model) on a 37 mm diameter glass-fiber filter (Whatman GF/C) for particulate matter, followed by an XAD-2 purified resin adsorption tube for gas phase and semi-volatile PAHs. The glass-fiber filter was placed in a polystyrene cassette (Millipore model) in closed configuration: cover plate with sampling aperture of 4 mm diameter corresponding to a suction velocity of 1.33 m/s.

After sampling, the cassettes and tubes were stored in closed boxes and analyzed over the following week.

Analysis. After the outer walls of the cassette had been cleaned carefully (to avoid any contamination), the filters were taken out and ultrasonically extracted twice with 10 ml of dichloromethane for 15 min. Because of the possible deposited PAHs (Lafontaine et al., 1999), the inner walls of the cassette were washed twice with 2 ml of methanol (other common solvents such as dichloromethane cannot be used because they attack the polystyrene of the cassette). The two extracts were gently concentrated under helium, adjusted to 1 ml by addition of methanol, and quantified separately (this cassette rinsing operation was not performed on the samples of the working week, but only those of the additional experiments).

The XAD-2 extraction was carried out for 15 min with 3 ml of toluene in an ultrasonic bath. To prevent losses of the volatile substances, the extract was analyzed immediately after adding of 3 ml of methanol.

Five μl of the final volume of each extract were injected in a Jasco 880 HPLC system: a column set
to 30°C (Chromspher Si-C18 100 × 4.6 mm, 3 μm), with a 85/15 methanol/water mobile phase at a flow rate of 1 ml/min. The PAH detection conditions were: excitation 335 nm, emission 385 nm for pyrene and excitation 365 nm, emission 420 nm for BaP (Hitachi F 1050 fluorimeter). In these sampling and analysis conditions, the detection limits were 5 ng/m³ for pyrene and 1 ng/m³ for BaP.

**Biological monitoring**

**Sampling.** All the voided urines were collected from Tuesday 13 h to Saturday afternoon. Over the working week the samplings were generally taken as follows: pre-shift (~13 h), during the shift (one or several times), post-shift (~21 h), bedtime, getting up, morning. For the additional experiments during the week end elimination, the sampling timing was free.

After the volume was measured, the urine samples were stored in the refrigerator after sampling, before being transferred into a cold room (at about 0°C).

**Analysis.** 1-PyOH was analyzed according to a slightly modified method of Jongeleenen (Jongeneelen et al., 1987): 5 ml of urine were adjusted to pH 5 with 5 ml of 0.4 M sodium acetate (pH 5) and a few drops of HCl 4 M. The mixture was then enzymatically hydrolyzed overnight at 37°C (25 μl of glucuronidase 30 U/ml/aryl sulfatase 60 U/ml helix pomatia, Merck).

The hydrolyzed urine was purified on solid phase C18 (500 mg/3 ml Varian Bondelut): after priming with 5 ml methanol, then 10 ml distilled water, the sample was drawn through the column at a rate of about 1 ml/min, next after washing with 3 ml of water, eluted with 3 ml hexane and finally PyOH and other metabolites were recovered with 5 ml dichloromethane.

This latter fraction was gently evaporated to dryness under nitrogen without heating and redissolved in 500 μl of a 50/50 acetonitrile/methanol. PyOH was separated and quantified by HPLC on a 150 × 4 mm C18 column (Lichrospher from Merck) with isocratic acetonitrile/water + 50 mg/l ascorbic acid (50/50) at a flow rate of 1 ml/min. The fluorescence detector (Hitachi F 1050) was set to 240 nm for excitation and 390 nm for emission. In these conditions, PyOH detection was 20 ng/l.

**Data analysis**

The urinary PyOH concentrations were corrected for creatinine; in the same way, the urinary excretion rates were adjusted to the mean daily creatinine excretion rate. Calculations for each day were based on a pharmacokinetic model with zero order absorption and first order urinary elimination rates. The residue from the previous day exposure was taken into account as was the background level. This was estimated by the last values of the previous week end after more than 48 h of elimination. This background results from the personal lifestyle (diet, smoking) and above all from the exposures of previous working weeks.

The partial amount of PyOH excreted and the corresponding elimination rate were calculated from equations (1) and (2) respectively. The apparent half life of the urinary PyOH excretion was calculated (equation 3) from the slope (K2) of the elimination rate decrease curve estimated according the Sigma Minus method (Ritschel, 1980). For additional experiments, the decrease of the excretion rate curves were fitted with the Kintool software (Qualilab, Orleans, France) using the simplex method. The choice of a mono or bicompartimental model was based on the lower Akaike index value.

The daily amount of excreted PyOH was determined by summing each partial amount corrected for the background excretion during the same period and the residues of the previous day (subtraction) and the day studied (addition). These residues were calculated from the area under the excretion curve from the beginning of the next exposure to infinity. The results of day two are illustrated by the area delimited by the thick continuous line (Fig. 1).

\[
\text{partial amount of PyOH} = C_i \cdot V_i \quad (1)
\]

\[
\text{PyOH rate} = C_i \cdot V_i / (t_i - t_{i-1}) \quad (2)
\]

\[
C_i = \text{PyOH concentration in urine sample } i \quad (\text{ng/ml})
\]

\[
V_i = \text{volume of urine sample } i \quad (\text{ml})
\]

\[
t_i = \text{voidance time of urine sample } i \quad (\text{h})
\]

\[
\text{half life} = t_{i/2} = 0.693/k \quad (3)
\]

\[
k = \text{apparent elimination rate constant} = 2.303 \cdot K
\]

**RESULTS**

**Atmospheric exposures**

The results of the four shift working week (inner wall deposits not taken into account), are presented in Table 1. With regard to the workers and observers, the mean of the BaP air concentration was low, about 0.1 μg/m³, less than the German TRK (2 μg/m³) and even less than the recommendation used in France (0.15 μg/m³).

These values are about 10 times lower than when coal tar pitch was used. The decrease results from the lower BaP content of the petroleum pitch (1.2 g/kg) compared to the coal tar pitch (7.5 g/kg). On the other hand, as the pyrene content was nearly the same in both binders,
its mean air values were unchanged and therefore the pyrene/BaP ratio is 10 times higher.

In order to evaluate the suitability of pyrene as representative of carcinogenic risk, correlations were determined with BaP using linear regression analysis. As seen in Fig. 2, pyrene and BaP are poorly correlated ($r = 0.51$).

The deposits on the inner walls of the sampling cassettes were determined only for the additional experiments (34 personal samplings). The results concerning these deposits and the distribution of pyrene and BaP between the cassette and XAD2 tube are summarized in Table 2 (no pyrene or BaP was found between filter and tube, no BaP was recovered on XAD2). The BaP and pyrene on the inner walls represent 15 and 17% respectively of the total sampled.

**Biological monitoring**

Table 3 summarizes the urinary PyOH concentrations (referred to creatinine, without background correction) for the five workers and the two observers for the four-shift working week. Only the pre-shift (13 h), postshift (21 h) and maximum (with time elapsed after postshift) values are reported.

The two observers F and G started with low PyOH values (about 0.1 μmol/molCreat) while the workers still continued to eliminate despite three non-exposure days, particularly worker C who generally had the highest values (about 10 μmol/molCreat). However if the variation from minimum (preshift) to maximum is considered, the difference is less significant.

Except for the observers, the maximum did not always correspond to the postshift: the longer the cutaneous contact with nude (unpainted) targets, the later the maximum seems to occur (more than 10 h for worker C whose PyOH urinary levels were virtually constant for several hours after the post-shift).

The increase in preshift PyOH values indicates an accumulation during the week for everybody, including the observers. However, a plateau seems to be reached after three days of exposure.

All this is also illustrated by the Fig. 3 which shows three entire typical profiles of the monitoring:

- workers C and D: they were in charge of a line, executed the same operations but C handled nude targets without gloves and D usually handled painted targets with gloves;
- observer F: generally situated between the packing station and the middle line.

**Table 1. Atmospheric pyrene, BaP levels (μg/m³), and pyrene/BaP ratio according to activities**

<table>
<thead>
<tr>
<th>Workers</th>
<th>Pyrene Range</th>
<th>BaP Range</th>
<th>Pyrene/BaP Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Packing</td>
<td>n = 8</td>
<td>1.03–4.70</td>
<td>2.56</td>
</tr>
<tr>
<td>B Maintenance</td>
<td>n = 8</td>
<td>1.38–5.05</td>
<td>2.90</td>
</tr>
<tr>
<td>C Packing</td>
<td>n = 7</td>
<td>1.90–3.33</td>
<td>2.70</td>
</tr>
<tr>
<td>D Packing</td>
<td>n = 8</td>
<td>1.59–3.34</td>
<td>2.80</td>
</tr>
<tr>
<td>E Foreman</td>
<td>n = 8</td>
<td>1.93–2.96</td>
<td>2.47</td>
</tr>
<tr>
<td>F</td>
<td>n = 8</td>
<td>0.66–2.46</td>
<td>1.65</td>
</tr>
<tr>
<td>G</td>
<td>n = 8</td>
<td>1.25–3.63</td>
<td>2.39</td>
</tr>
<tr>
<td>Total</td>
<td>n = 55</td>
<td>0.66–5.05</td>
<td>2.50</td>
</tr>
</tbody>
</table>
The other profiles agreed with that of D or F and are therefore not shown.

The values of apparent urinary excretion half lives during the four-shift week are presented in Table 4 (after corrections for the residues of the previous day and background). They ranged from 1.9 to 12.5 h with an arithmetic mean of 6.1 h (from 1.9 to 9.1 h with a mean of 5.6 h if worker E is dismissed). There was no apparent difference according to the exposed people (worker or observer) or the day of exposure. The values in the last column have not been corrected for residues and background. This illustrates the importance of the corrections which have a huge effect especially for the workers (reduction by about 30% of the values).

From the additional experiments (monitoring after the postshift and over a weekend without exposure) interesting results were observed:

- after 48 h the PyOH urinary levels were still higher than those of non-occupationally exposed people, except for the two observers and perhaps worker D although his urinary PyOH was still decreasing (Fig. 4).

Table 2. Distribution of pyrene and BaP in the different parts of the sampling device

<table>
<thead>
<tr>
<th></th>
<th>Pyrene %</th>
<th>BaP %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Walls/Total</td>
<td>Filter/Total</td>
</tr>
<tr>
<td>Mean</td>
<td>17.1</td>
<td>27.7</td>
</tr>
<tr>
<td>Range</td>
<td>4.4–38.3</td>
<td>6.8–62.5</td>
</tr>
</tbody>
</table>

Table 3. Urinary PyOH concentrations (μmol/molCreat.) determined over the four-shift working week: pre- and postshift values, maximum values, time between postshift and maximum

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Max</td>
<td>(Time)</td>
<td>Pre</td>
<td>Post</td>
<td>Max</td>
<td>(Time)</td>
<td>Pre</td>
<td>Post</td>
<td>Max</td>
<td>(Time)</td>
<td>Pre</td>
<td>Post</td>
<td>Max</td>
<td>(Time)</td>
</tr>
<tr>
<td>A</td>
<td>0.60</td>
<td>6.27</td>
<td>6.27</td>
<td>0</td>
<td>2.93</td>
<td>8.17</td>
<td>9.02</td>
<td>+1.25 h</td>
<td>4.12</td>
<td>9.19</td>
<td>9.52</td>
<td>+1.25 h</td>
<td>4.02</td>
<td>7.27</td>
<td>7.27</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0.86</td>
<td>4.37</td>
<td>4.97</td>
<td>+1.25 h</td>
<td>0.89</td>
<td>5.41</td>
<td>7.69</td>
<td>+1.25 h</td>
<td>2.88</td>
<td>7.84</td>
<td>10.81</td>
<td>+1.25 h</td>
<td>3.41</td>
<td>8.19</td>
<td>9.06</td>
<td>+1.25 h</td>
</tr>
<tr>
<td>C</td>
<td>4.51</td>
<td>9.02</td>
<td>9.13</td>
<td>+1.75 h</td>
<td>4.14</td>
<td>8.17</td>
<td>8.65</td>
<td>+10.75 h</td>
<td>6.02</td>
<td>10.94</td>
<td>10.94</td>
<td>0</td>
<td>6.14</td>
<td>7.93</td>
<td>8.78</td>
<td>+1.75 h</td>
</tr>
<tr>
<td>D</td>
<td>1.30</td>
<td>5.08</td>
<td>5.08</td>
<td>0</td>
<td>1.89</td>
<td>2.87</td>
<td>5.65</td>
<td>+2 h</td>
<td>2.02</td>
<td>5.25</td>
<td>6.26</td>
<td>+2 h</td>
<td>2.10</td>
<td>6.24</td>
<td>6.24</td>
<td>0</td>
</tr>
<tr>
<td>E</td>
<td>4.80</td>
<td>9.71</td>
<td>10.76</td>
<td>−2 h</td>
<td>3.68</td>
<td>9.61</td>
<td>10.21</td>
<td>+3.25 h</td>
<td>4.99</td>
<td>7.89</td>
<td>7.89</td>
<td>0</td>
<td>4.49</td>
<td>5.33</td>
<td>8.07</td>
<td>+3.25 h</td>
</tr>
<tr>
<td>F</td>
<td>0.07</td>
<td>1.97</td>
<td>1.97</td>
<td>0</td>
<td>0.14</td>
<td>1.84</td>
<td>1.84</td>
<td>0</td>
<td>0.65</td>
<td>2.05</td>
<td>2.05</td>
<td>0</td>
<td>0.84</td>
<td>2.04</td>
<td>2.04</td>
<td>0</td>
</tr>
<tr>
<td>G</td>
<td>0.16</td>
<td>2.55</td>
<td>2.55</td>
<td>0</td>
<td>0.43</td>
<td>2.52</td>
<td>2.52</td>
<td>0</td>
<td>0.71</td>
<td>2.11</td>
<td>2.11</td>
<td>0</td>
<td>0.70</td>
<td>2.23</td>
<td>2.23</td>
<td>0</td>
</tr>
</tbody>
</table>

Fig. 2. Relationship between atmospheric BaP and pyrene.
Fig. 3. Urinary PyOH excretion of two workers (C and D) and observer F over the four-shift working week (values not corrected for residues of previous days and for background).

Table 4. PyOH excretion half lives (h) corrected for previous residues and background. Means for the week with and without corrections

<table>
<thead>
<tr>
<th>Corrected apparent half lives (h)</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Mean</th>
<th>Not corrected Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Workers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>5.1</td>
<td>8.2</td>
<td>6.9</td>
<td>8.8</td>
<td>7.2</td>
<td>12.3</td>
</tr>
<tr>
<td>B</td>
<td>1.9</td>
<td>9.3</td>
<td>6.4</td>
<td>4.4</td>
<td>5.5</td>
<td>7.4</td>
</tr>
<tr>
<td>C</td>
<td>3.9</td>
<td>9.1</td>
<td>3.1</td>
<td>5.2</td>
<td>5.3</td>
<td>8.8</td>
</tr>
<tr>
<td>D</td>
<td>5.4</td>
<td>5.2</td>
<td>5.3</td>
<td>4.0</td>
<td>5.0</td>
<td>9.2</td>
</tr>
<tr>
<td>E</td>
<td>6.7</td>
<td>8.7</td>
<td>8.0</td>
<td>12.5</td>
<td>9.0</td>
<td>14.5</td>
</tr>
<tr>
<td>Observers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>3.1</td>
<td>5.9</td>
<td>6.4</td>
<td>3.8</td>
<td>4.8</td>
<td>5.4</td>
</tr>
<tr>
<td>G</td>
<td>5.3</td>
<td>5.9</td>
<td>5.6</td>
<td>5.9</td>
<td>5.7</td>
<td>6.2</td>
</tr>
</tbody>
</table>

Fig. 4. Excretion and excretion rate of urinary PyOH for worker D after one of the additional experiments (values not corrected for residues of previous day and for background).
the data suggest a monophasic form for three excretion rates and, as summarized in Table 5, a biphasic form in six cases: workers A, B, D (once), observers F (twice) and G (once). Figure 5 shows the two most representative excretion rate curves: worker B and observer F.

Table 5. Half lives of urinary excretion rates with a biphasic form

<table>
<thead>
<tr>
<th>Exposed person</th>
<th>B1</th>
<th>D1</th>
<th>F1</th>
<th>G1</th>
<th>A2</th>
<th>F2</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st phase</td>
<td>3.4</td>
<td>2.8</td>
<td>4.6</td>
<td>2</td>
<td>2.7</td>
<td>1.9</td>
<td>2.9</td>
</tr>
<tr>
<td>2nd phase</td>
<td>24.2</td>
<td>21.3</td>
<td>27.8</td>
<td>24</td>
<td>18.3</td>
<td>24.8</td>
<td>23.4</td>
</tr>
</tbody>
</table>

Relationship between atmospheric pyrene and urinary \( \text{PyOH} \)

The relationship between pyrene and \( \text{PyOH} \) was determined from different ways.

The correlation coefficient was \( r = 0.37 \) when the average atmospheric concentration of pyrene and the increase in \( \text{PyOH} \) concentration over the shift are considered (Fig. 6). This was \( r = 0.39 \) with the postshift \( \text{PyOH} \) values, and \( r = 0.48 \) with the increase in \( \text{PyOH} \) concentration between preshift and maximum.

The relationship was also determined from the amount of inhaled pyrene during the shift and the corresponding amount of \( \text{PyOH} \) excreted: from the preshift of one day to the preshift of the day after the next (after subtraction of the residues of the previous days, the background, and the amount derived from the second day of exposure).

The calculations were at first carried out by assuming a ventilation flow rate of 20 l/min for everybody and next with the assessment from the study of the activities and the cardiac frequency of each person during the shift: the rate was about 15 l/min for the observers and ranged from 22 to 27 l/min for workers. The correlation obtained in the first case (\( r = 0.51 \)) improved (\( r = 0.74 \)) when the flow rates were corrected (Fig. 7).

From the same corrected data, the ratio of urinary excreted \( \text{PyOH} \) to the inhaled dose of pyrene was also calculated (with the assumption of a retention of 100%). The values ranged from 0.18 to 0.70 (arithmetic mean = 0.34), the highest corresponding to the workers with cutaneous contact such as
worker C (range 0.39–0.70, mean 0.50); however, none exceeded unity.

**DISCUSSION**

Replacing the coal tar pitch by a petroleum pitch as a binder for manufacturing artificial targets has reduced the atmospheric BaP concentrations to very low levels (about 0.1 \( \mu \text{g/m}^3 \)), far below the limits generally used in European countries. In keeping with these good results, the inhalation exposures were considered reasonable with respect to the carcinogenic risk and no additional preventive measure (ventilation system for instance) was planned.

On the other hand, the PyOH urinary concentrations are high, in the same region as those observed in electrode or coke plants and greatly exceed the biological exposure limit (2.3 \( \mu \text{mol/mol Creat} \)) suggested by some authors for coke plants (Jongeneelen, 1992). Both this value and that for electrode plants (4.3 \( \mu \text{mol/mol Creat} \)) (Ny et al.,...
1993) seem unrealistic in the case of target manufacturing and other similar cases (Boogard and Van Sittert, 1994; Quinlan et al., 1995). The authors themselves propose a correction factor using the pyrene/BaP ratio, especially in situations where the PAH profiles are different from those observed in coke or electrode plants. Because of the poor correlation observed between atmospheric pyrene and BaP, such a correction seems difficult to apply to the target plant.

Therefore, in this case the determination of urinary PyOH does not permit a pertinent assessment of a potential risk and should only be used to measure exposure to light PAHs (di to tetra cyclic).

Such a contradiction between atmospheric and urinary data can occur in opposite circumstances: despite a very high BaP inhalatory exposure (60 μg/ m³), a chiseller exhibited urinary PyOH concentrations comparable with assemblers who were 1000 times less exposed (Helkkilä et al., 1995).

Atmospheric values

As can be seen in Table 1, the atmospheric exposure of workers was approximately the same despite their different activities; this is due to the fact that they generally operated in the same environment (except for worker B who often worked nearer to the presses) with low variations of the PAH levels. This is also valid for the observers whose exposure was only slightly lower although they were often further from the lines than the operators.

In the same way, the pyrene/BaP ratio hardly differs from the mean value (21.8) except for worker B (mean = 18) and observer G (mean = 27.2). These two extreme values, together with the fact that the inner wall deposits were not taken into account, partly explain the poor correlation observed between pyrene and BaP.

The atmospheric pyrene concentration occurs in the calculation of its representativeness with regard to other PAHs or BaP and above all in the assessment of the distribution between inhalatory and cutaneous absorption (Quinlan et al., 1995; Van Rooij et al., 1993a). Therefore, the accuracy of its determination is crucial.

Some authors sampled only the particulate fraction of pyrene and may have underestimated the values by about 50%, with a large variation range. At the present time everyone takes the volatile fraction into account but few are aware of the problem of deposits on the inner walls of the 37 mm sampling cassettes.

Although the influence of these deposits is relatively moderate in the case of the artificial targets (less than 20% of pyrene on the inner walls as seen in Table 2), it is greater for other work situations (Lafontaine et al., 1999) like cathode relining (pyrene in the deposits on average represents 38% of the total with a range from 14 to 68%), or the restoration of soils polluted by creosote (arithmetic mean 41%, range 25–72%) for which there may be more pyrene on the inner walls than on the filter. This problem is likely to exist for air samples from other workplaces such as coke, electrode or other plants. Disregarding the deposits leads in such cases to significant underestimation of the amount of pyrene inhaled.

The existence of deposits and the resulting underestimation is not specific to the Millipore cassette. Likewise, with a conic metallic sampling head similar to the GGP (Gesamstaub Gas Probenahmekopf) used by some authors (Petry et al., 1996; Van Rooij et al., 1993b; Jongeneelen et al., 1990), the deposits are much larger than with the Millipore model and are much more variable (Lafontaine et al., 1999).

Excretion half lives

In our study, the values of apparent urinary elimination half lives determined after the postshift are much lower than those observed in most other studies. The literature generally quotes values higher than 10 h with a mean of about 20 h. This difference may be due to various causes such as the kind of exposure (respiratory, oral, cutaneous), the variations between individuals, and above all the half lives determination modes:

- The calculations were generally made without background data corrections or with corrections based on less exposed controls. As seen previously in Table 4, the consequence may be a mean overestimation exceeding 30%.
- Most of the time in the other studies, for practical reasons, urine samples were collected just before and after the shifts. The half lives determined from these data are only approximate. In particular, this leads to an overestimation that is even greater the shorter the half lives values and the longer the time elapsed between the previous voidance and the postshift.

Furthermore, they are unavoidably overestimated when the maximum of excretion takes place some hours after the postshift. In the case of the target plant, the resulting variation is also about 30%. It would undoubtedly be greater with a higher dermal contamination like that found in creosote plants (Boogaard and Van Sittert, 1994).

In our study, the half lives were determined from the slope of the excretion rate decrease curves. Because this requires a great many samples and analyses, few industrial hygienists have the opportunity to work in this way. Hence, in these cases the values are also lower than 10 h: 9.8 h for 5 volunteers (Breznicki et al., 1997) and from 6 to 9 h for 9
workers (Vu Duc and Lafontaine, 1999) in an aluminum plant.

The additional data obtained from the week end follow up experiments enabled us to refine our results, particularly those concerning the shape of the excretion curves and the corresponding half lives. In three cases, the excretion rate decrease curve suggests a PyOH excretion with a monophasic form. In six cases, the PyOH excretion rate looks more biphasic in form, as already observed in a wood preserving plant (Heikkilä et al., 1995).

As can be seen in Table 5, the first elimination phase presents short half lives (2–4.6 hours), shorter than those (5–6 h) reported in the wood preserving plant, but near some experimental data obtained after oral controlled exposure: 3.1–5.9 hours (Buckley and Lioy, 1992). The second elimination phase presents longer half lives (18.3–27.8 h) particularly close to those observed in the creosote plant (22–24 h); this may be due to the delayed release from a deep compartment.

The half lives calculated from the additional experiments can be considered as more reliable than those of the four-shift working week. Indeed, for the latter, the time elapsed between the PyOH excretion maximum and the following preshift, is too short to appreciate the existence of a potential second phase with any degree of accuracy. This second phase may explain:

- the progressive increase in the daily preshift values observed for workers and observers as the week went on.
- the high base values observed for only the workers at the beginning of the four-shift working week: 48 h is not sufficient to recover the urinary levels of non-exposed people (contrary to what may be predicted by the mean half life — 6.1 h — determined for the working week). With regard to the slowness of this second elimination, the base value at the beginning of the week may be assimilated to a background rather than a residue of the previous day. Therefore, this justifies a posteriori our corrections for estimating the half lives and the daily amounts of PyOH excreted over the four-shift working week.

The PyOH elimination kinetic data are essential to calculate the BEL from the established pharmacological model which employees the half life values (Leung and Paustenbach, 1988) but also to determine the best time to take urine samples.

Industrial hygienists (ACGIH, 1987) recommend sampling at the beginning and end of the working week if the half life is longer than 5 h because of possible accumulation, which is the case for the workers in the target plant. We agree with this suggestion, however it must take into account the field data and it should be borne in mind that the excretion maximum often occurs several hours after the postshift, especially in case of skin exposure, as predicted from experimental data with human volunteers (Viau and Vyskocil, 1995). The elapsed time between the postshift and the maximum is about 3 h for the target plant, the same value obtained in the aluminum plant (Vu Duc and Lafontaine, 1999). In these two studies 16 excretion maximum values were observed at the postshift and 29 after the postshift (arithmetic mean: 3 h, range 0.5–10.75 h, SD 2.7 h).

Consequently, it seems judicious within a framework of a simple biomonitoring strategy to add a third sampling 3 h after the end of the working week, more particularly for workers with apparent cutaneous exposure.

**Relationship between pyrene and PyOH**

Several approaches can be employed to estimate the relationship between inhaled pyrene and excreted urinary PyOH. Generally, hygienists use the air and urine concentrations. In our study, the correlation was poor ($r = 0.37$) when inhaled pyrene was represented by average atmospheric concentration and excreted PyOH by its increase over the shift (Fig. 6). The coefficient was only slightly greater if the postshift values were taken into consideration ($r = 0.39$) and notably greater for increase between preshift and maximum of excretion ($r = 0.48$).

The determining of this relationship from the amount of pyrene inhaled during the shift and the corresponding amount of PyOH excreted appears more pertinent. With the same ventilation rate allocated to each worker, the coefficient of correlation was relatively weak ($r = 0.51$) but improved greatly with corrected rates ($r = 0.74$). This clearly demonstrates the importance of correcting particularly when the physical activity of the workers is different. It increases even more ($r = 0.81$) if worker C, who is likely to be the most exposed to dermal absorption, is excluded.

**Routes of exposure**

At the present time, nobody contests the significance of the percutaneous penetration of PAHs. This is not only due to direct contact with the polluting matter (the nude, still hot, targets in our study) but also to the deposition of the ambient atmospheric particles on the skin and through contaminated clothes. However, there is still no agreement on the quantitative distribution between the dermal and respiratory routes.

In fact, this kind of data is very limited. Of the few authors who have carried out these determinations, most conclude that skin contamination is the main determinant of PyOH internal dose. Their
argument is logically based on the excreted PyOH/
inhaled pyrene ratio which greatly exceeds unity.

The cutaneous part is calculated either from the
difference between the total excreted PyOH and the
part due to inhalatory pyrene (Quinlan et al., 1995;
Heikkilä et al., 1995), or by direct estimation from
exposure pads stuck to the skin of workers (Van
Rooij et al., 1993c). All the results are in agreement:
on average, 70% or more of urinary excreted PyOH
correspond to dermal absorption. However, this
rate could be overestimated because the possible
deposits on the inner walls of the cassette as well as
losses during sampling (Bonnet et al., 2000) are not
taken into account.

The determination of the respective parts of cu-
taneous and respiratory absorption was not the aim
of our study. It would appear however that the
inhalatory track is generally the main entrance
route of pyrene for the exposed people of the target
factory, except when PyOH excretion maximum
occurs several hours after postshift: worker C
every day) or workers A, B and E (one day,
depending on maintenance interventions). Indeed
the excreted PyOH/inhaled pyrene ratio is below
unity, and consequently greatly below the values
obtained in the above mentioned studies. Moreover
a good relation between inhaled pyrene and
excreted PyOH has been found.

The same conclusion has been drawn for workers
exposed during cathode relining in the aluminum
electrolysis plant: the excreted PyOH/inhaled pyrene
ratio is generally below 0.3 and the reduction in the
amount inhaled (about 95%) by wearing efficient
respiratory protection leads to a significant re-
duction in the amount excreted PyOH (about
75%) (data to be published). Another study
(Breznicki et al., 1997) was carried out with a more
appropriate experimental approach (comparison
between exposure without protection and single
dermal exposure: inhalation of PAH free air through
a facial mask). The estimated dermal absorption
value was about 20% of the total absorbed dose.
More recently, the effect of dust protective masks
has been studied in an electrode plant (Bentsen
et al., 1998). The reduction of PyOH urinary excretion
due to this simple protection was estimated at
about 41%.

Considering the differences between the
approaches used by the authors, the kind of working
areas with various emitting sources, the working
and hygienic habits and other confounding individ-
ual factors, the determination of the respective
parts of dermal and respiratory routes cannot be
compared easily. Other experiments should be car-
ried out in order to eliminate these divergences: ex-
periments in the field with workers protected
differently, but also controlled dermal exposure
with volunteers (Viau and Vyskočil, 1995). In the
meantime, the cutaneous protection at workplaces
must be considered along with elementary hygienic
habits.

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