# HYDROXY-PAHS IN HUMAN URINE SAMPLES

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**Introduction**

Environmental pollution through polycyclic aromatic hydrocarbons (PAHs), which are formed during incomplete combustion processes, has been of concern for many years. Not only because of their wide distribution and persistence in the environment but also because of their diverse toxic potentials in the human body.[1]

Since combustion processes occur naturally as well as anthropogenic, these compounds are distributed ubiquitously, although a special source of PAH intake is posed by cigarette smoke. The consumption of cigarettes leads to a higher PAH intake into the human body. After ingestion, PAHs are rapidly metabolised through different enzymatic transformation processes to hydroxylated derivatives and excreted primarily in the urine, but also in feces.[1] In order to assess human exposure to environmental and occupational PAHs, methods have been developed to determine the level of OH-PAHs in human urine, using 1-hydroxy-pyrene (1-PYR) as biological indicator, since pyrene is present in all PAH-mixtures. Therefore, it is not only an indicator of pyrene-uptake, but has also been regarded as an indirect indicator for all PAHs.[2] A more sophisticated approach has been developed by the US Center for Disease Control and Prevention (CDC Environmental Health, Atlanta), where a number of 25 OH-PAHs are analysed individually.[3] This method has been adapted and validated by our laboratory in order to determine the different OH-PAHs listed in table 1 in human urine samples. It has been applied for biomonitoring of a population living around an industrial area in Southern Europe.

| No. | Parent PAH | Metabolite/Analyte | Abbreviation |
|---|---|---|---|
| **1** | Naphthalene | 1-hydroxynaphthalene | 1-NAP |
| **2** | Naphthalene | 2-hydroxynaphthalene | 2-NAP |
| **3** | Fluorene | 9-hydroxyfluorene | 9-FLUO |
| **4** | Fluorene | 2-hydroxyfluorene | 2-FLUO |
| **5** | Fluorene | 3-hydroxyfluorene | 3-FLUO |
| **6** | Phenanthrene | 1-hydroxyphenanthrene | 1-PHE |
| **7** | Phenanthrene | 2-hydroxyphenanthrene | 2-PHE |
| **8** | Phenanthrene | 3-hydroxyphenanthrene | 3-PHE |
| **9** | Phenanthrene | 4-hydroxyphenanthrene | 4-PHE |
| **10** | Pyrene | 1-hydroxypyrene | 1-PYR |

*Table 1: List of analysed PAH-compounds (adapted from CDC[3])*

**Materials and methods**

For validation purposes, anonymous urine samples have been collected from the laboratory staff and pooled in order to generate two different pools with respect to the different contamination levels for smokers (7 individuals) and non-smokers (12 individuals). These pool samples have been taken for internal QA/QC and have been analysed in order to create the necessary baseline data for validation and control charts. Series of 41 samples (smokers) and 35 samples for non-smokers have been analysed at the Eurofins GfA Lab Service in Hamburg.

The analytical method consisted of the partly adapted laboratory procedure manual developed by the CDC[3]. 1 ml of sample is treated with β-glucuronidase/arylsulfatase for enzymatic hydrolysis, then extracted against pentane and derivatised by N-methyl-N-(trimethylsilyl)-trifluoroacetamid (MSTFA) for gaschromatographic analysis. The following measurement was performed on a HRGC-HRMS system using a 60m SLB5ms 0.25 mm i.d. / 0.25 µm $d_f$ for gaschromatographic separation and a Waters Autospec HRMS at mass resolution $R \geq 10.000$ for detection. Quantification was performed using the isotope dilution method, with a $^{13}C$-labelled standard for each native compound. As recovery standard $^{13}C_{12}$-labelled PCB #105 was used.

Due to light sensitivity and high volatility of some of the compounds, careful handling (light exclosure, gentle evaporation etc.) is mandatory.

Results are reported both in ng/l as well as adjusted for urinary creatinine in ng/g creatinine, in order to adjust analyte concentrations for the effects of fluid balance (variability of urinary output). Creatinine content was determined by an external partner laboratory being accredited according to DIN EN ISO 15189:2007.
QA/QC measures consisted e.g. in monitoring the quantification standard recovery rates (criterion range 40-130%; except for 1-NAP and 2-NAP, where recoveries for the isotope-labelled standard as low as 20% were accepted), as well as batch blanks. The limit of quantification was established using averaged blank values plus 5-fold standard deviation. For calibration including the derivatisation step for the standard solutions, an initial multipoint calibration curve was established for reference purposes, and daily single-point calibrations were used with the initial calibration as reference criterion.

**Results and discussion:**
Results from the analysis of pool samples (smokers and non-smokers) are presented in table 2a, as well as reference results from the US NHANES studies.[3] Results adjusted for creatinine content are shown in table 2b.
Comparing the results from both pools it is easy to state that the findings for some of the compounds are as expected significantly higher in the smokers than in the non-smokers pool (e.g. 1-NAP and 2-NAP), whereas some of them (e.g. 1-PHEN and 2-PHEN) are well within the same range or even lower (4-PHEN). Also it can be seen that all results, even those from the non-smokers, where samples came from donators with presumably no specific source of PAH intake like cigarette smoke, are well above the LOQ. Therefore it is obvious to assume that exposition to PAHs through the environment is still an issue.

| | **Urine pool Smoker** (avg. of 7 individuals) | **Urine pool Non-smoker** (avg. of 12 individuals) | **Reference** (NHANES 2004) (avg. of ca. 2750 individuals) | **LOQ** | **Typical lab blank** |
|---|---|---|---|---|---|
| | ***ng/l*** | ***ng/l*** | ***ng/l*** | ***ng/sample*** | ***ng/sample*** |
| **1-NAP** | 11606 | 1798 | 2050 | 0,07 | 0,024 |
| **2-NAP** | 14136 | 4583 | 2470 | 0,10 | 0,038 |
| **9-FLUO** | 1015 | 1017 | 219 | 0,04 | < 0,0077 |
| **3-FLUO** | 998 | 178 | 134 | 0,01 | < 0,0043 |
| **2-FLUO** | 1720 | 469 | 318 | 0,01 | < 0,0039 |
| **4-PHEN** | 40 | 56 | 42 | 0,03 | < 0,0061 |
| **3-PHEN** | 427 | 243 | 105 | 0,01 | < 0,0034 |
| **1-PHEN** | 390 | 334 | 140 | 0,03 | < 0,0057 |
| **2-PHEN** | 199 | 162 | 54 | 0,01 | < 0,0043 |
| **1-PYR** | 264 | 125 | 50 | 0,02 | < 0,0049 |

*Table 2a: Results for OH-PAH content in smokers and non-smokers pool samples*

| | **Urine pool Smoker** (avg. of 7 individuals) | **Urine pool Non-smoker** (avg. of 12 indivduals) | **Reference** (NHANES 2004) |
|---|---|---|---|
| | ***ng/g Creatinine*** | ***ng/g Creatinine*** | ***ng/g Creatinine*** |
| **1-NAP** | 8290 | 1160 | 1910 |
| **2-NAP** | 10097 | 2957 | 2310 |
| **9-FLUO** | 725 | 656 | 205 |
| **3-FLUO** | 713 | 115 | 125 |
| **2-FLUO** | 1229 | 303 | 298 |
| **4-PHEN** | 28 | 36 | 39 |
| **3-PHEN** | 305 | 157 | 98 |
| **1-PHEN** | 279 | 216 | 132 |
| **2-PHEN** | 142 | 105 | 51 |
| **1-PYR** | 188 | 81 | 46 |

*Table 2b: Results for OH-PAH content in smokers and non-smokers pool adjusted for creatinine content*

Comparing pool and NHANES results, it can be seen that the concentrations found in the NHANES study are generally lower, even compared to the non-smokers pool. This must be due to generally different water contents of the urine samples, since results calculated to creatinine basis do not show such a distinct difference, which illustrates the importance for creatinine correction. Nevertheless, it is important to keep in mind, that there are probably differences in sampling as well as regionally different contamination levels and patterns, since the NHANES data reflect contamination levels of the US population, which makes it even more difficult to compare the results.

Regarding method quality and validation, our methodological work consisted mainly of establishing the laboratory background blank (see table 2a), repeatability, trueness and recovery rates of the $^{13}$C-labelled standards. Figure 1 shows the results of monitoring the recovery rates of the $^{13}$C-labelled quantification standards.

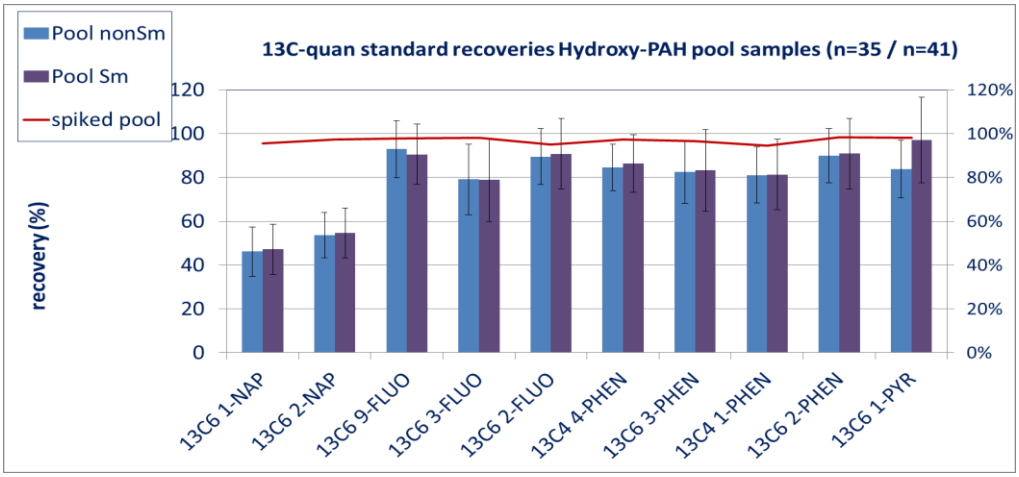


*Figure 1: Recovery rates for $^{13}$C-labelled standards compared to recovery rates for native compounds (red line)*

Recovery rates for most of the standards are between 80 – 100%, with reasonable relative standard deviations of 15 – 25%. The only exceptions are posed by 1-NAP and 2-NAP, where the average recovery rate is only between 40 – 60%, and therefore lies underneath the set quality criterion of 50%. This is probably due to high volatility of these compounds. But since the relative standard deviation of both of these compounds is between 15 – 25% as well, a good reproducibility can be concluded. Therefore a lower quality criterion of 20 – 130% is acceptable. Low standard deviations also indicate the good repeatability of this method.

For evaluating the trueness of these results the non-smoker pool sample has been spiked with native OH-PAHs and analysed (standard addition). The reference value has been calculated by adding the results of the non-smoker pool (see table 2a) and the concentration spiked to the samples. Spike levels and results are presented in table 3.

| | Average pool non-smoker [ng/l] | Spike [ng/l] | Reference value [ng/l] | Average [ng/l] | Median [ng/l] | Min [ng/l] | Max [ng/l] | SD [ng/l] | rel. SD [%] | Recovery **[%]** |
|---|---|---|---|---|---|---|---|---|---|---|
| 1-NAP | 1798 | 3125 | 4923 | 4734 | 4743 | 4662 | 4789 | 58 | 1,2 | **96,2** |
| 2-NAP | 4583 | 3125 | 7708 | 7536 | 7543 | 7401 | 7659 | 106 | 1,4 | **97,8** |
| 9-FLUO | 1017 | 781 | 1798 | 1776 | 1772 | 1754 | 1808 | 24 | 1,3 | **98,8** |
| 3-FLUO | 178 | 781 | 959 | 938 | 938 | 905 | 969 | 30 | 3,2 | **97,7** |
| 2-FLUO | 469 | 781 | 1250 | 1197 | 1199 | 1160 | 1229 | 33 | 2,8 | **95,7** |
| 4-PHEN | 56 | 781 | 837 | 815 | 815 | 782 | 847 | 27 | 3,4 | **97,3** |
| 3-PHEN | 243 | 781 | 1024 | 991 | 1007 | 930 | 1018 | 41 | 4,1 | **96,7** |
| 1-PHEN | 334 | 781 | 1115 | 1052 | 1062 | 999 | 1085 | 37 | 3,6 | **94,3** |
| 2-PHEN | 162 | 781 | 943 | 931 | 938 | 889 | 962 | 33 | 3,6 | **98,8** |
| 1-PYR | 125 | 781 | 906 | 889 | 884 | 842 | 948 | 50 | 5,6 | **98,1** |

*Table 3: Recovery rates for native OH-PAHs (trueness) for spiked pool samples (n = 4)*

Recovery rates for the native compounds are shown in table 3 as well as in figure 1 (red line). Two major conclusions can be drawn:

1. Low relative standard deviations underline the stability of this method. Furthermore high native recovery rates confirm the trueness of the analysed results.
2. Figure 1 shows that low recovery rates for the $^{13}$C-labelled standard have no effect on the determination of the correct native content, since they are still consistently at 95% or higher.

The described method developed in our laboratory has the potential to be applied for various exposure situations of populations. The method can easily be extended for the measurement of additional Hydroxy-PAHs.

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**References:**

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