

Guidelines for biological monitoring of workers in aluminium production facilities for urinary 1-hydroxypyrene (1-pyrenol)^{†‡}

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1. Introduction

Biological monitoring is getting increasing attention as an additional tool in the assessment of occupational exposure of workers. A specific metabolite of pyrene, 1-hydroxypyrene in urine is available as an indicator of uptake of polycyclic aromatic hydrocarbons (PAH). Over 300 reports in the scientific literature confirmed the potential of the methodology. Some excellent reviews have been published (Levin, 1995; Dor *et al.*, 1999; Bouchard

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& Viau, 1999; Jongeneelen, 2000).^{5,6,9,11}

At present, urinary 1-hydroxypyrene is widely used as a biological indicator to assess occupational exposure to PAH. It should be clearly pointed out that biomonitoring using metabolites in urine is a tool for assessment of exposure; it is an industrial hygiene tool. A number of previous studies in primary aluminium smelters show that the dermal exposure can be traced and quantified using this tool. Other studies show that the efficacy of respiratory protection can be tested.

This guideline presents recommendations for the application of urinary 1-hydroxypyrene in aluminium production.

2. Communication

If routine urine sampling is introduced in a certain aluminium plant, attention should be paid to communication. Success is highly dependent upon proper information regarding management and the plant workers. Important aspects of communication with the management are:

- To communicate intensively with the management of the plant and to explain what the additional value of the urine-test is.

- The biomonitoring test is not a medical evaluation, but it is a test for exposure assessment.

- Unexpected exposures (for instance detection of inappropriate use of respirators, extensive dermal exposure, unidentified ingestion by hand mouth shunt) can be detected.

- Workers will be requested to participate as volunteers; they will not be forced to participate.

Important aspects of communication with the involved workers of the plant are:

- To explain and inform the workers in group sessions why the urine testing is being introduced.

- To make clear that the urine-test is an exposure indicator, not a direct risk estimator. It is an industrial hygiene tool, not a tool for medical assessment.

- To organize a pre-investigation

meeting to inform about the PAH-biomonitoring and to instruct workers about urine sampling and the requested filling-in of the questionnaire.

- Workers are requested to participate on a voluntary basis; if someone objects he will not be forced to participate.

- Shortly after the study, results of the investigations will be communicated to the workers in meetings and leaflets with summary information provided.

3. Design of the biomonitoring survey

General

One possible approach is to sample urine randomly within the whole exposed workforce. However, this type of sampling requires a large number of samples. The preferred approach is to assess the exposure of job categories. This is already in many cases the common sampling strategy for exposure assessment of PAH with personal air sampling. Based on the results of the previous air sampling programs in the plant, workers of a primary aluminium production plant can be classified in similar exposure groups (SEG). Two types of technology are used, pre-bake aluminium production and the Söderberg type production. Examples of similar exposure groups in both types of plants are presented in Tables 1 and 2, respectively.

Sample size

For the assessment of the urinary 1-OHP level of a job category or a similar exposure group, it is recommended to collect at least 5 samples per group.

Sampling time

It is recommended to sample urine at the end-of-shift[§] at the end of the workweek[¶]. In the pilot phase of the introduction of biomonitoring a prior-to-shift sample at the beginning of

[§] end-of-shift = the last hour of the shift

[¶] end-of-workweek is after a minimum of 3 consecutive shifts.

Table 1 Similar exposure groups (SEG) for PAH exposure in a primary aluminium production plant with pre-bake type technology

Similar exposure group	Functions/work activities
Office-workers	Office work at the plant (white collar work)
Pot rooms workers	Transport of alumina/aluminium and anodes All other activities in pot rooms
Cathode plant workers	Transport/installation of pots Pot relining operations
Anode plant workers	Supervisors in anode plant
Paste production workers	All operations in paste plant
Bake oven workers	All operations in/near bake oven
Rodding shop workers	All operations in rodding shop
Cast house workers	All operations in cast house
Laboratory workers	All operations in laboratory Environmental measurements
Stack gas cleaning facility workers	All operations in stack gas cleaning facility
Various workers 1	Technical service operations in pot rooms and/or in cast house
Various workers 2	Technical service operations in anode and/or cathode plant
Various workers 3	All other technical service operations
Various workers 4	Transport/handling of cokes and anodes

Table 2 Similar exposure groups (SEG) for PAH exposure in Söderberg primary aluminium production plant

Similar exposure group	Functions/work activities
Office workers	Office work at the plant (white collar work)
Pot tender	
Spike puller	
Metal tapper	
Pot skimmer	
Pot measuring worker	
Pot service operator	
Gas manifold service	
Mech. Maintenance	
Electrician	
Gas cleaning worker	
Pot line operator	
Paste plant operator	

the workweek could additionally be sampled to study the possible accumulation of PAH in the highest exposed groups.

Metric of 1-OHP and descriptive statistics

Urine samples of workers are collected at the last shift of the workweek, end-of-shift. This will be the end-of-workweek value of 1-OHP. When also at the beginning of the workweek prior-to-shift urine samples are collected, two exposure metrics can be used:

- Increase of 1-OHP over the workweek: level end-of-workweek minus beginning of the workweek sample;

- End-of-week 1-OHP.

Descriptive statistical data of each group are calculated for both metrics using a log normal frequency distribution. The arithmetic mean (AM) and the 95% upper confidence limit of arithmetic mean (95%UCL of AM) are calculated. Data are reported as *n*, AM, 95% UCL of AM and range.

Local baseline of controls

A non-occupationally exposed group of referents will also be sampled to find the local baseline level (*N* = at least 10). Both smoking and non-smoking controls should be sampled. The descriptive statistical data of 1-OHP distributions are calculated for smoking and non-smoking controls.

Questionnaire

In order to be able to control for confounding by smoking and non-occupational exposure to PAH, each participating individual is interviewed using the questionnaire. Questions are about:

- Smoking habits,
- Other potential confounding exposures such as: medical use of coal tar containing products (*e.g.* coal tar ointment/shampoo) and the use of coal tar containing products at home (*e.g.* tar, creosote) during the week of measurement.

The questionnaire is shown in Fig. 1. When a worker indicates that he had

significant recent additional PAH-exposure from sources other than occupational exposure, the concurrent 1-OHP-data are regarded as *not valid* for the assessment of *occupational exposure* and these results are excluded from the data set.

Additional air sampling data

Urinary metabolite monitoring of PAH is complementary to personal air sampling of CTPV. In the pilot-phase simultaneous personal air sampling of CTPV and/or total PAH is very useful. Results of the personal air sampling can be used to get more insight in the relationship between inhalation of PAH and urinary 1-OHP of each SEG.

The airborne concentration of the two PAHs: pyrene and benzo(a)pyrene (determined in both the gaseous PAH and particulate PAH fraction of workroom air) will be used to calculate the ratio pyrene/benzo(a)pyrene. The ratio is needed when the ratio adjusted 1-OHP-levels of SEGs are calculated (see: criteria for interpretation of 1-OHP levels). Ratio adjusted 1-OHP-levels allow comparisons between similar exposure groups.

4. Sampling procedure, analysis and quality control

Urine sampling and storage

Urine samples should be collected in a clean 50 ml container. 20 ml of urine will allow a duplicate analysis. Urine is collected in a container without preservative. Samples should be stored immediately in the dark at -18°C . In this situation, the samples can be kept for a long period (at least a year). A 24 h carrier should be used to transport samples to the laboratory and samples should be properly packed to prevent thawing.

Recommended analytical method

Principle of the method. The total of free and conjugated 1-hydroxypyrene in urine is determined with high-pressure liquid chromatography (HPLC). After enzymatic hydrolysis to release the conjugated part of 1-hydroxypyrene the analyte is separated from the matrix and analyzed by HPLC with a fluorescence detector. References to the method include Jongeneelen FJ *et al.* (1987).⁸ Various adapted method descriptions are available: Boos *et al.* (1992);³ Simon *et al.* (1999).¹³ For a reference to the method evaluation see Angerer & Schaller (1991).²

The concentration 1-OHP is adjusted to creatinine excretion and expressed as $\mu\text{mol mol}^{-1}$ creatinine. Some prefer not to correct for creatinine and report concen-

Biological monitoring of 1-hydroxypyrene in urine.

Identification of worker:

Plant:

Site:

Name of worker:

ID-number:

Job title:

Tasks:

.....

Information of worker:

Age:

Gender: M / F

Smoking habits:
Do you smoke yes: no:

if answer is yes, average number of cigarettes/cigars/pipes per day:

Medical treatment with coal tar products in the last 7 days
(e.g.: coal tar ointment/shampoo, etc.) yes: no:

PAH exposure at home in the last 7 days before sampling:
(eg.: fencing treatment with creosote or carbolineum) yes: no:

Fig. 1 Questionnaire.

trations as $\mu\text{g L}^{-1}$. On average $1.0 \mu\text{g L}^{-1}$ of 1-OHP = $0.49 \mu\text{mol mol}^{-1}$ creatinine.

Accuracy of the method. Trueness: the recovery of the analyte in spiked urine samples > 80–90%.

Precision: the within-day variation is < 4%. The between-day variation is < 12.5%.

Detection limit: the detection limit of 1-hydroxypyrene in urine should be 0.5 nmol L^{-1} .

Quality control

Internal quality control. Internal quality control of determination of

1-hydroxypyrene and creatinine must be performed in order to prevent bias. The following laboratory procedures are recommended:

(i) Solvent blanks are processed in each batch of samples to monitor for interferences.

(ii) A bulk urine sample from several high exposed individuals should be prepared for internal quality control (IQC). The urine is divided into aliquots of 20 mL and stored at -18°C (IQC-samples). At every series of urine samples, two IQC samples are added to the sample series. When the IQC-samples are beyond the range of mean $\pm 1.96 \times$ standard deviation, the

variation is too high and an alert is given.

External quality control. External quality control is done in round robin testing. The “Intercomparison Program of Toxicological Analysis in Biological Materials” organized by the Department for Occupational, Social and Environmental Medicine of the University of Erlangen, Germany include 1-OHP as analyte (Lehnert *et al.*, 1999).¹⁰ The selected laboratory should comply with the demands of this German EQC-program and the results of 1-hydroxypyrene should be within the assigned tolerance range.

Pre-selected laboratories. The selected laboratory should be experienced and should meet the demands of an EQC-program or equivalent. A preliminary selection of laboratories with EQC for the analysis of 1-hydroxypyrene in urine is presented in Table 3.

5. Data and criteria for interpretation of 1-OHP levels

The concentration of 1-hydroxypyrene in an end-of-workweek urine sample reflects far more than 90% the exposure on the two shifts prior to sampling due to the half-life of elimination of approximately 18–20 h.

Background level without occupational exposure

A trace amount of 1-hydroxypyrene is detected in urine of non-occupational exposed controls. Dietary intake of PAH and smoking are the main source of environmental exposure to PAH and 1-hydroxypyrene in urine. Smokers have little higher levels of 1-OHP in urine. Levels of smoking workers and non-smoking workers should be reported separately. Levels of 1-OHP vary a little from country to country, probably due to variations in the environmental PAH background and/or dietary intake of PAH. The average level in non-smokers is 0.12 (0.0–0.30) and in smokers 0.25 (0.0–0.50). The 99-percentile in smoking and non-smoking non-occupational exposed controls is $0.49 \mu\text{mol mol}^{-1}$

Table 3 Preliminary list of analytical laboratories for 1-OHP testing

Name and address	Website	Country	Approved EQC ^a
ABL Laboratories, Assen, Netherlands	www.abl.nl	Netherlands	Yes
Finnish Institute Occupational Health, Oulu, Finland	www.occuphealth.fi	Finland	Yes
IPASUM, University of Erlangen, Germany	www.arbeitsmedizin.uni-erlangen.de/home.html	Germany	Yes
UCL -Louvain, Lab Toxicology Industrielle, Louvain, Belgium	www.md.ucl.ac.be/toxi/mbi.htm	Belgium	Yes
INSP Quebec -Tox lab Quebec, Canada	www.ctq.gc.ca	Canada	?

^a Within the tolerance limits.

creatinine (= 1.0 $\mu\text{g L}^{-1}$) (ACGIH¹, BEI documentation, 2003).

Health based recommendations for a limit value of 1-OHP

Individual studies have reported on the relation of 1-hydroxypyrene in urine and genotoxic endpoints. Buchet *et al.* (1995)⁴ reported in a study of cytogenetic endpoints in lymphocytes of a group of exposed coke oven and graphite anode workers, a no-genotoxic effect level of 1.4 $\mu\text{mol mol}^{-1}$ creatinine. At present this is the lowest published no-genotoxic-effect-level.

Other studies have reported on the relation of airborne PAH and 1-hydroxypyrene in urine at the end of workweek, in an end-of-shift sample. Some estimated the urinary concentration equal to the national OEL of PAH. Tjoe Ny *et al.* (1993)¹² proposed 4.9 $\mu\text{mol mol}^{-1}$ creatinine in end-of-workweek urine samples for Söderberg pot room workers.

A tentative limit value for several aluminium workers has been calculated with a correction corresponding for the known ratio pyrene/benzo(a)pyrene in several sectors of the aluminium industry, using the proposed biological exposure limit for coke ovens (Bouchard & Viau, 1999).⁵ The proposed tentative urinary limit values of 1-OHP were: Vertical pin Söderberg workers: 4.4, Anode workers: 8.0 and Pre-bake workers: 9.8 $\mu\text{mol mol}^{-1}$ creatinine, respectively.

Control based value of 1-OHP

Since the data for a health based limit value are very limited, certain benchmarks have been proposed for control purposes. The control based target value is not a health based limit value, but a target value that should be reachable according to presently modern production principles. Both UK-HSE and US-ACGIH suggested certain limits.

The BEI-committee of the US-ACGIH believes that at present a BEI is non-quantifiable and recommends that a value exceeding the level of 0.49 $\mu\text{mol mol}^{-1}$ creatinine (1.0 $\mu\text{g L}^{-1}$)

should be considered as a post-shift level indicating occupational exposure to PAH. This level is based on the observation in people without occupational or significant environmental exposure, but smokers included, that the OH-P level in at least 99% of the population is lower (BEI-draft OH-P, 2003).

The WATCH-committee of the UK-HSE (2000) concluded that it would be appropriate to establish a Benchmark BMGV as an end-of-shift urinary 1-hydroxypyrene concentration of 4.0 $\mu\text{mol mol}^{-1}$ creatinine. The UK's Benchmark Value BMGVs are established on the basis of the 90th percentile of biological monitoring values achieved in relevant industries using good practice. The 90th percentile for the HP results for all industries ($n = 218$) was 6.7 $\mu\text{mol mol}^{-1}$ creatinine. If data from only 11 workers in timber impregnation are excluded because their exposure is dominated by lighter PAHs the 90th percentile was 4 $\mu\text{mol mol}^{-1}$ creatinine. WATCH agreed on the following recommendations: 1-hydroxypyrene is a suitable marker for exposure to PAH and that a benchmark BMGV based on measurement of this metabolite in urine is appropriate. Based on 90th percentile data, a benchmark BMGV for 1-hydroxypyrene in end-of-shift urine samples could be 4 $\mu\text{mol mol}^{-1}$ (WATCH, 2000).

Target value of 1-OHP in aluminium production

A consensus on a broadly accepted biological limit value or target value of 1-OHP is not yet reached. It is recommended to select one of the four available levels of benchmark limits (see Table 4) as being the approach that fits the best to the current company EHS-policy and to test compliance to this level.

Other aspects of the interpretation of urinary 1-OHP data

Varying content of pyrene in the CTPV. The parent compound of 1-hydroxypyrene, pyrene, is a single

PAH in the PAH-mixture of CTPV. The PAH-mixture of the CTPV can vary substantially between different sites or job categories. In order to be able to make a comparison of exposure to the PAH-mixture between job categories or sites, it is necessary to introduce a correction for the proportion of pyrene and benzo(a)pyrene in the CTPV. A correction factor corresponding to the ratio of airborne pyrene/benzo(a)pyrene in the CTPV has been suggested to calculate the adjusted 1-OHP (Bouchard & Viau, 1999).⁵ This is a proper base to compare exposure of total PAH between SEG's. The adjusted 1-OHP for primary aluminium workers can be estimated according to:

$$1\text{-OHP}_{\text{adj}} = 1\text{-OHP}_{\text{actual}} \times [\text{pyr/bap}]_{\text{actual}}/2.5 \quad (1)$$

with $[\text{pyr/bap}]_{\text{actual}} = 5$ as a mean for primary aluminium production

Diluted urine. Highly diluted or concentrated urine samples may lead to erroneous results due to altered excretion mechanisms. Samples with a creatinine concentration beyond the range of 4–34 mmol L^{-1} are treated as not valid, and should be excluded from the data set before calculating the average exposure in the similar exposure groups.

Out-of-work exposures. The coincidental use of medicinal coal tar products (shampoo, ointment) or activities at home with coal tar products can lead to erroneous high 1-OHP-levels. Samples from individuals with out-of-work exposure should be excluded from the data set before calculating the average exposure in the similar exposure groups

Conversion factor of 1-hydroxypyrene.
 $1 \mu\text{mol L}^{-1}$ urine = $218 \mu\text{g L}^{-1}$ urine.
 $1 \mu\text{mol mol}^{-1}$ creatinine = $1.93 \mu\text{g g}^{-1}$ creatinine = $1.93 \mu\text{g L}^{-1}$ urine.

Table 4 Proposed target benchmark values for urinary 1-OHP in aluminium production

Level	Benchmark criteria	Urinary 1-hydroxypyrene ($\mu\text{mol mol}^{-1}$ creatinine) ^a
1	Level of exposure is not exceeding the level in not occupational exposed controls	95-percentile of non-occupational exposed local controls. If not available take 0.49 $\mu\text{mol mol}^{-1}$ creatinine (BEI-draft).
2	Level of exposure prevails genotoxic effects in workers	1.4 $\mu\text{mol mol}^{-1}$ creatinine
3	90-percentile in industries with good practices	4.0 $\mu\text{mol mol}^{-1}$ creatinine (UK BMGV draft)
4	Equal to OSHA -TLV level of 0.2 mg m^{-3} CTPV	4.6 ^b $\mu\text{mol mol}^{-1}$ creatinine for groups with a pyrene/bap-ratio in air samples of 5.

^a In end of workweek, end-of-shift urine samples. ^b This is equal to 2.3 $\mu\text{mol mol}^{-1}$ creatinine for SEG's with a pyrene/bap-ratio** of 2.5 or equal to 9.0 $\mu\text{mol mol}^{-1}$ creatinine for SEG's with a pyrene/bap-ratio of 10 and is very close to suggestion of 4.9 $\mu\text{mol mol}^{-1}$ creatinine in the Tjoe Ny-paper.

6. Appendix

Abbreviations

ACGIH	American Conference of Governmental Industrial Hygienists
Bap	Benzo(a)pyrene
BEI	Biological exposure index (USA)
BLV	Biological limit value
BMGV	Biological monitoring guidance value (UK)
CTPV	Coal tar pitch volatiles
PAH	Polycyclic aromatic hydrocarbons
Pyr	Pyrene
SEG	Similar exposure group
1-OHP	1-Hydroxypyrene = 1-pyrenol
HPLC	High pressure liquid chromatography
HSE	Health & Safety Executive (UK)
OEL	Occupational exposure limit
OSHA	Occupational Safety & Health Administration (USA)
TLV	Threshold limit value
IQC	Internal Quality Control
EQC	External Quality Control
WATCH	Advisory subcommittee of HSE

7. References

- 1 ACGIH, *BEI-documentation of PAH-draft*, ACGIH BEI-Committee, Cincinnati, Ohio, USA, 2003.
- 2 1-Hydroxypyrene, in *Analyses of hazardous substances in biological materials*, ed. Angerer & Schaller, DFG VCH Verlag, Weinheim, Germany, 1991, vol. 3, pp. 151–170.
- 3 K. S. Boos, J. Lintemann and A. Kettrup, Coupled Column HPLC method for the determination of 1-hydroxypyrene in urine of subjects exposed to PAH, *J. Chromatogr.*, 1992, **600**, 189–194.
- 4 J. P. Buchet, M. Ferreira, J. B. Burrión, T. Leroy, M. Kirsch-Volders, P. Van Hummelen, J. Jacques, L. Cupers, J. P. Delavignette and R. Lauwerys, Tumor markers in serum, polyamines nucleosides in urine and cytogenetic aberrations in lymphocytes of workers exposed to PAH, *Am. J. Ind. Med.*, 1995, **17**, 523–543.
- 5 M. Bouchard and C. Viau, Urinary 1-hydroxypyrene as a biomarker of exposure to polycyclic aromatic hydrocarbons: Biological monitoring strategies and methodology for determining biological exposure indices for various work environments, *Biomarkers*, 1999, **4**(3), 159–187.
- 6 F. Dor, W. Dab, P. Empereur-Bissonnet and D. Zmirou, Validity of biomarkers in environmental health studies: the case of PAHs and benzene, *Crit. Rev. Toxicol.*, 1999 Mar, **29**(2), 129–68 Review.
- 7 F. J. Jongeneelen, Biological exposure limit for occupational exposure to coal tar pitch volatiles at cokeovens, *Int. Arch. Occup. Environ. Health*, 1992, **63**, 511–516.
- 8 F. J. Jongeneelen, R. B. M. Anzion and P. T. Henderson, Determination of hydroxylated metabolites of polycyclic aromatic hydrocarbons in urine, *J. Chromatogr.*, 1987, **413**, 227–232.
- 9 F. J. Jongeneelen, Benchmark guideline for urinary 1-hydroxypyrene as biomarker of occupational exposure to PAH, *Annals Occup. Hyg.*, 2000 (in press).
- 10 G. Lehnert, K. H. Schaller and J. Angerer, Report on the status of the external quality-control programs for occupational medicine in biological materials in Germany, *Int. Arch. Occup. Environ. Health*, 1999, **72**, 60–64.
- 11 J. O. Levin, First international workshop on hydroxypyrene as a biomarker for PAH exposure in man-summary and conclusions, *Sci. Total Environ.*, 1995, **163**, 165–168.
- 12 Ny E. Tjoe, D. Heederik, H. Kromhout and F. Jongeneelen, The relation between PAH in air and in urine of workers in a Söderberg potroom, *Am. Ind. Hygiene Assoc. J.*, 1993, **54**, 277–284.
- 13 P. Simon, Automated column-switching high-performance liquid chromatography method for the determination of 1-hydroxypyrene in human urine, *J. Chromatogr., B*, 1999, **732**, 91–101.
- 14 WATCH, WATCH-subcommittee of Health & Safety Commission of UK, *CTPV and PAH*, Paper for meeting at HSE, London, Watch/21/2000, May 2000.

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