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1-Pyrenol: A Biomarker for Occupational Exposure to Polycyclic Aromatic Hydrocarbons

William P. Tolos,[A] Peter B. Shaw,[A] Larry K. Lowry,[A] Barbara A. MacKenzie,[A] Jou-Fang Deng,[B] and Harry L. Markel[B]

[A]Division of Biomedical and Behavioral Science and [B]Division of Surveillance, Hazard Evaluations and Field Studies, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, Ohio 45226

A biological monitoring method using the major urinary metabolite of pyrene, 1-pyrenol, has been successfully used to assess the exposure of aluminum reduction plant workers to coal tar pitch-derived polycyclic aromatic hydrocarbons (PAHs). The method used high-performance liquid chromatography with a fluorescence detector. The net mean change between workers pre- and postshift urinary 1-pyrenol concentrations was seventeenfold greater than the net mean change found in controls. The data strongly indicated that the net change in urinary 1-pyrenol concentration in workers was greater than found in controls. Evidence for an effect due to smoking in this context was negligible. The data show a strong positive correlation between environmental pyrene and all 17 environmental PAHs that were analyzed and urinary 1-pyrenol, verifying that pyrene was an appropriate choice to use as a marker for coal tar pitch-derived PAHs. **Tolos, W.P.; Shaw, P.B.; Lowry, L.K.; MacKenzie, B.A.; Deng, J-F.; Markel, H.L.: 1-Pyrenol: A Biomarker for Occupational Exposure to Polycyclic Aromatic Hydrocarbons. Appl. Occup. Environ. Hyg. 5:303–309; 1990.**

Introduction

Coal tar pitch fumes and dusts contain polycyclic aromatic hydrocarbons (PAHs), many of which are known or suspect carcinogens.[1–4] Occupational studies, especially among coke-oven workers, showed that long-term exposure to coal tar products is associated with an elevated rate of lung cancer.[5–7]

Historically, the quantitation of PAHs in environmental samples has been referred to as "benzene solubles" or "cyclohexane solubles"; however, the content of these extracts varies as does the recovery of the individual PAHs.[8] Two National Institute for Occupational Safety and Health (NIOSH) Health Hazard Evaluations investigating PAH exposures in coal liquefaction processes found no correlation between the benzene-soluble fraction and the total level of 29 PAHS analyzed individually.[8,9]

Biological monitoring is one type of monitoring that could be used to determine workers exposure to these hazardous compounds. Biological monitoring is an effective method for assessing human exposure because it indicates uptake of the chemicals by the worker regardless of the route of exposure.[10]

The current literature on biological monitoring methods for evaluating workers' exposure to PAHs is limited. The major difficulties in analyzing PAHs in biological specimens are that the PAH mixtures found in the work environment contain a great number of PAHs and that the mammalian system produces many different metabolites from each individual PAH. One approach to biological monitoring is to select a representative (marker) PAH and to develop methods to extract and quantify the metabolite of the marker in the urine of exposed workers. Recently, it was suggested that the major metabolite of pyrene (1-pyrenol) may be used as a marker compound for assessing workers exposure to coal tar pitch containing pyrene.[11–13]

This study utilized urinary 1-pyrenol (1-P) as a biomarker compound for monitoring workers for possible exposure to pyrene and PAHs derived from coal tar pitch fume and/or dust.

Biological monitoring for 1-P was conducted, along with industrial hygiene characterization of the work environment using personal breathing zone samples. The relationships between urinary 1-P and the 8-hour time-weighted average concentrations for coal tar pitch volatiles (CTPV) (as benzene solubles), pyrene, and the total level of 17 PAHs analyzed individually are reported.

Materials and Methods

Chemicals

The PAH metabolite, 1-pyrenol, was purchased from the NCI Chemical Carcinogen Repository, Kansas City, Missouri. Glass-distilled acetonitrile, methanol, and 2-propanol were obtained from Baxter Healthcare Corp. β-Glucuronidase, having sulfatase activity, was purchased

from Sigma Chemical Company. The Sep-Pak® C18 cartridges were obtained from Millipore Corp. Creatinine Baker Encore Kit reagents were purchased from Baker Instrument Corp. Orbo-43 sorbent tubes were obtained from Supelco, Inc. Zefluor® PTFE filters were purchased from Gelman Sciences, Inc.

1-Pyrenol Analysis

Urine samples were analyzed for 1-P by a high-performance liquid chromatographic method previously described.[11] Briefly, an aliquot (10 ml) of urine was buffered (pH = 5.5) then hydrolyzed enzymatically with β-glucuronidase/sulfatase (7000 Units) for 4 hours at 37.5°C in a water bath/shaker. A Sep-Pak C18 cartridge was used for clean up and isolation of 1-P.

The high-performance liquid chromatographic analysis was performed utilizing a Varian Vista model 5560 liquid chromatographic system equipped with a Perkin–Elmer LS-4 scanning fluorescence spectrometer. The separation was performed on a 25-cm × 4.6-mm SUPELCOSIL LC-18 column, using an acetonitrile–water gradient at a flow rate of 1.5 ml/min [40:60 (v/v) to 100:0 (v/v)/min]. The 1-P was quantitated selectively by measuring its response with the fluorescence detector (excitation at 244 nm, emission at 390 nm). The limit of detection for urinary 1-P was 0.45 nmol/L. The urinary 1-P limit of detection was defined as the mean of the blank plus 3 times the standard deviation of the blank. The concentration range was 1.2–850 nmol/L. The correlation coefficient for the standard curve was r = 0.991. The within day variability for urine samples spiked at 10 and 50 nmol/L of 1-P was 2.7 and 1.8 percent, respectively. The between day variability was 7.5 and 6.4 percent, respectively. Urinary creatinine determinations were performed by the Jaffee reaction using the Baker Encore Centrifugal Analyzer and were used to correct for the urine dilution. The results were expressed as the ratio of 1-P per mol creatinine (μmol 1-P/mol creatinine).

Environmental Analysis

Breathing zone air samples were collected on Teflon® filters (Zefluor, Gelman Sciences, Inc.) followed by Orbo-43 sorbent tubes (Supelco, Inc.) at a flow rate of 2.0 L/min for 8 hours. The particulates collected on the Teflon filters were analyzed for coal tar pitch volatiles (as benzene solubles) using NIOSH Method 5515.[14] The limit of detection for benzene solubles was 0.05 mg/sample. The volatiles collected in the Orbo-43 sorbent tubes were desorbed with benzene and analyzed for 17 individual PAHs by gas chromatography using NIOSH Method 5515.[14] Analyses were performed by DataChem, Salt Lake City, Utah.

Experimental

Work Process

Anodes for use in aluminum reduction are produced in the plant where sampling was conducted. The anode is derived from liquid pitch and coke which is pressed and heated into shape. The resulting block, referred to as a green carbon block, is transported (by overhead crane) to the anode prebake area. One layer of five green carbon blocks is placed into each oven pit and covered with a layer of coke by the crane operator and the packer–puller. A second layer of blocks is placed on top of the first and also covered with coke. The oven pits are lined with brick and ceramic fiber and are heated by natural gas to 1200°C for a total of 32–48 hours. The blocks are then allowed to cool for 26 days before removing them from the ovens. The anodes are packed and unloaded in the ovens during the first and second shift, while the fires are tended during all three shifts.

Biological Monitoring Subjects

Participating in the 1-P study were 28 workers (26 males and 2 females), all of whom worked in the anode bake area. The anode bake area workers were classified as crane operators, packer–pullers, gas furnace operators, vacuum truck operators, mechanics, equipment operators, and brick layers. Two nonoccupationally exposed people (nonsmokers) in a building nearby served as on-site controls.

All subjects were given a short questionnaire to classify workers by exposure groups and smoking status. Questions were also asked regarding other factors that might influence urinary 1-P levels such as dietary habits and personal hygiene. The latter data were not analyzed further.

TABLE I. Workers Urinary 1-Pyrenol Mean Concentrations[A]

Job Title	Day	Preshift	Postshift	Change
Crane Operator n = 9	1	0.64 ± 0.14	2.87 ± 0.21	2.22 ± 1.01
Crane Operator n = 9	3	0.72 ± 0.12	2.72 ± 0.68	2.00 ± 0.62
Packer-Puller n = 9	1	0.57 ± 0.23	2.41 ± 0.52	1.84 ± 0.42
Packer-Puller n = 9	3	0.77 ± 0.12	3.01 ± 0.80	2.20 ± 0.72
Equip. Operator n = 1	1	0.68	2.50	1.82
Equip. Operator n = 2	3	0.56	2.55	1.99
Gas Furnace Operator n = 3	1	0.43 ± 0.29	3.60[B]	3.17[B]
Gas Furnace Operator n = 3	3	0.57 ± 0.36	3.10 ± 0.71	2.32 ± 0.60
Mechanic n = 1	1	0.76	2.00	1.24
Mechanic n = 1	3	0.65	2.30	1.65
Brick Layer n = 4	1	0.56 ± 0.25	1.93 ± 0.21	1.38 ± 0.39
Brick Layer n = 4	3	0.68 ± 0.13	2.05 ± 0.17	1.37 ± 0.19
Controls[C] n = 23	1	0.44 ± 0.40	0.55 ± 0.33	0.11 ± 0.23
Controls[C] n = 23	3	0.47 ± 0.33	0.61 ± 0.37	0.14 ± 0.21

[A]The units for urinary 1-pyrenol are μmol/mol creatinine. Results are expressed as mean ± one standard deviation.
[B]Only one urine specimen collected.
[C]Worksite controls were included.

Pre- and postshift urine samples were collected in polyethylene bottles from shift workers. Second- and third-shift workers were sampled on Monday (day 1) and on Wednesday (day 3). First-shift workers were sampled on Tuesday (day 1) and on Thursday (day 3). All samples were kept frozen at −20°C until analyzed for 1-P.

Environmental Samples

Personal breathing zone environment samples were collected for 18 of the 28 workers who participated in the biological monitoring test. The 18 workers consisted of 7 packer–pullers, 9 crane operators, and 2 equipment operators. Sampling was done on Monday for second-shift workers and on Tuesday for first-shift workers.

Results and Discussion

Biological Data

Only two on-site controls (nonsmokers) participated in the study. In order to have an adequate number of controls to compare with exposed workers, controls from a previously published study were used.[11] Urine samples from 10 nonsmokers and 11 smokers, not occupationally exposed to PAHs, were determined for 1-P. Smokers were defined as a person who smoked ten or more cigarettes per day; whereas, nonsmokers were people who did not smoke any cigarettes.

The urinary 1-P concentration means for days 1 and 3 for the two on-site controls were 0.33 and 0.34 μmol/mol creatinine, respectively. These data were similar to data from the previous study. The nonsmoker controls ($n = 10$) had a mean urinary 1-P concentration of 0.27 ± 0.29 μmol/mol creatinine (± one standard deviation). Controls who smoked showed a mean 1-P concentration of 0.77 ± 0.33 (± one standard deviation). Since the on-site and previous referents had similar urinary 1-P concentrations, the control data were combined.

Table I shows urinary 1-P concentrations in workers according to job title and day of workweek. These data could have been analyzed by observing only postshift concentrations of urinary 1-P or the net change in urinary 1-P from preshift to postshift concentrations. Comparing only postshift concentrations is a plausible approach only if there are no differences in preshift concentrations between workers and controls. Since the data for controls did not appear to be normally distributed ($p = 0.0003$ for the Shapiro-Wilk statistic) and the distributions for controls and workers appeared different (as judged by stem and leaf plots and histograms), neither a t-test nor the Wilcoxon rank-sum test would be appropriate to compare workers and controls.[15] Consequently, the median test was used to compare the preshift levels of urinary 1-P for workers and controls on day 1.[16] The test yielded a chi-square value of 7.097, indicating that the workers did have higher preshift levels than the controls as shown in Table II. Therefore, dealing with only postshift concentrations would be invalid because they could be affected by preshift concentrations.

TABLE II. Data Used in Median Test of Preshift Concentrations of Urinary 1-Pyrenol on Day 1

	Preshift Urinary 1-Pyrenol Concentrations		
	≤ Median	> Median	Total
Worker	8	17	25
Control	15	6	21
Total	23	23	46

The median was 0.535 μmol 1-pyrenol/mol creatinine. The test yielded $X^2 = 7.097$ (df = 1, $p = 0.008$).

Changes between pre- and postshift 1-P concentrations were compared. For day 1, the net mean change between pre- and postshift concentrations of urinary 1-P for controls was 0.106 ± 0.050 μmol/mol creatinine and for workers was 1.944 ± 0.155 μmol/mol creatinine. These results indicated an effect due to worker status (i.e., worker versus control).

Since the data for workers did not appear to be normally distributed (Shapiro-Wilk statistic yielded $p = 0.0044$) and the distributions for workers and controls appeared dissimilar, the median test was again applied to see if there was a difference between net change in urinary 1-P in workers and controls (Table III). The differences between pre- and postshift urinary 1-P concentrations were classified as "low" if the net change in concentration was less than or equal to the median for day 1. The subjects were categorized as having a "high" increase if the increase was above the median increase (the median increase on day 1 for controls and workers was 1.135 μmol 1-P/mol creatinine). Data from only the first day were used because measurements on succeeding days were on the same subjects and such observations would not have been independent of those on the first day. The Pearson chi-square statistic was 38.64, indicating a considerably higher level of net increases in urinary 1-P of workers than in the controls.

TABLE III. Data Used in Median Test of Net Change in Urinary 1-Pyrenol for Workers and Controls on Day 1

	Urinary 1-Pyrenol Net Change		
	≤ Median	> Median	Total
Workers	2	23	25
Controls	21	0	21
Total	23	23	46

The median was 1.135 μmol 1-pyrenol/mol creatinine. The test yielded $X^2 = 38.64$ (df = 1, $p < 0.00001$).

When smokers' and nonsmokers' net change in urinary 1-pyrenol was compared using the median test, the chi-square statistic was 0.000 (Table IV), indicating no significant effect due to smoking. These results indicated that the effect of smoking on net change in urinary 1-P concentration in this study was negligible when compared to the effect of worker status.

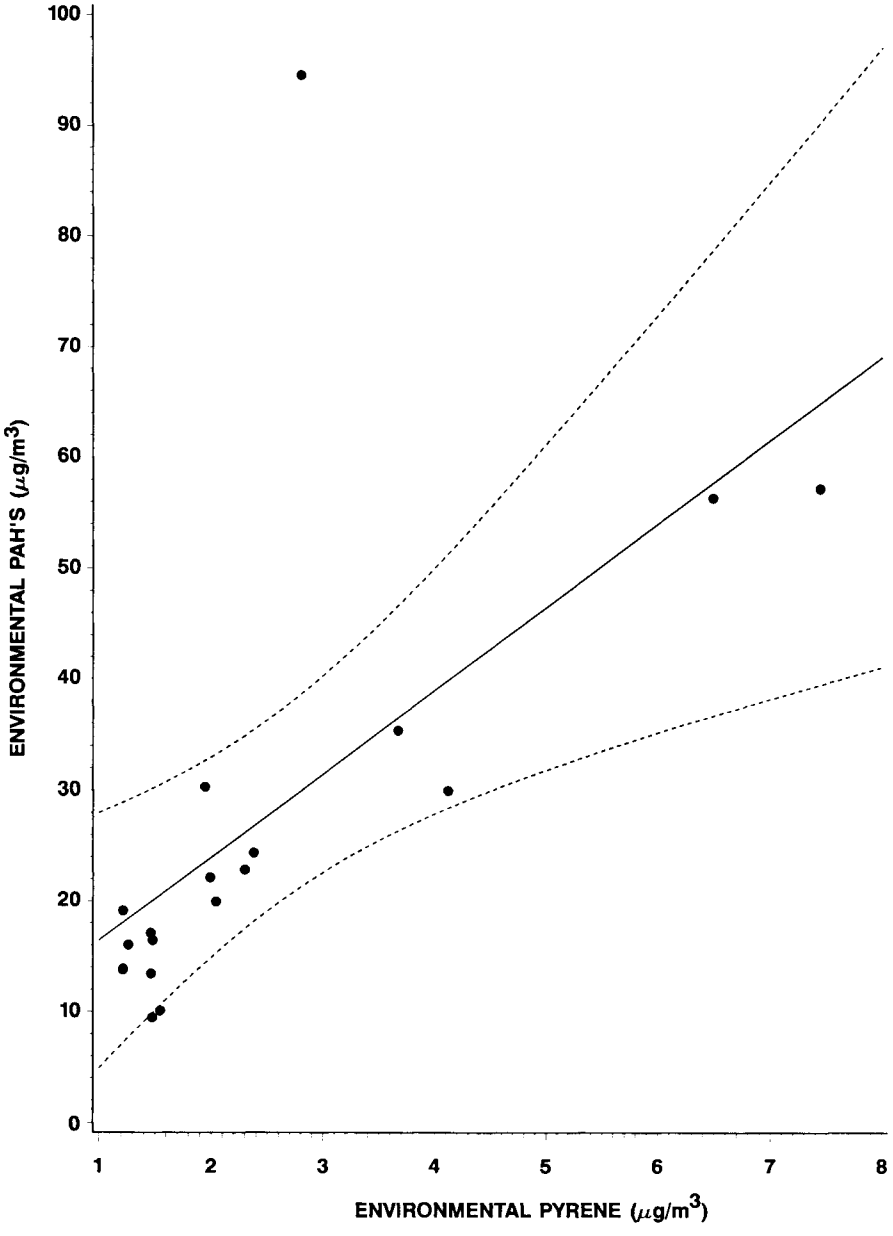

FIGURE 1. Total environmental PAHs versus environmental pyrene. The solid line is the linear regression line and the broken lines are the 95% confidence limits for the predicted means.

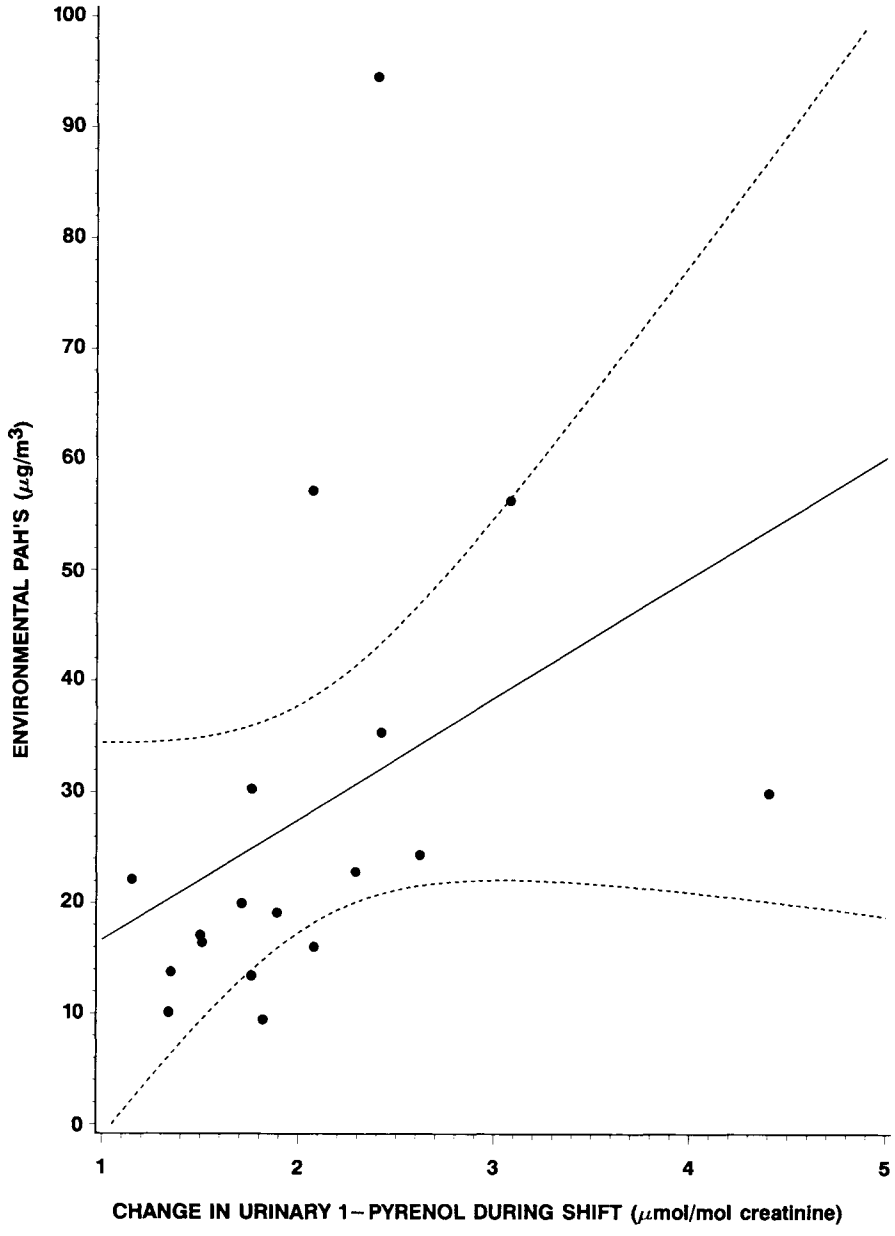

FIGURE 2. Total environmental PAHs versus net change in urinary 1-pyrenol. Linear regression line and 95% confidence limits for predicted means are shown.

TABLE IV. Data Used in Median Test of Net Change in Urinary 1-Pyrenol for Smokers and Nonsmokers on Day 1*

	Urinary 1-Pyrenol Net Change		
	≤ Median	< Median	Total
Nonsmokers	14	14	28
Smokers	9	9	18
Total	23	23	46

*The intent of this table is to show that smoking has a negligible impact on levels of urinary 1-pyrenol when workers are exposed to pyrene.

The Median was 1.135 μmol 1-pyrenol/mol creatinine. The test yielded $X^2 = 0.000$ (df = 1).

The Wilcoxon-signed rank test was used to check for possible 1-P buildup during the first shift and the second shift. Table I shows the combined data for both shifts. The preshift concentration of urinary 1-P on day 1 was compared with the preshift level on day 3. Using $H_0:\mu_1 = \mu_2$ versus $H_1:\mu_1 < \mu_2$, there was no evidence of buildup for the first shift ($p = 0.4722$). However, for the second shift, there was considerable evidence of buildup ($p = 0.00585$), i.e., the test rejected $H_0:\mu_1 = \mu_2$ in favor of $H_1:\mu_1 < \mu_2$. One explanation for this occurrence is that the second-shift workers had two days off prior to their day 1 preshift sampling, which would give them a lower 1-P base level than the first-shift workers who were exposed the day prior to their day 1 preshift sampling.

Environmental Data

Table V shows the biological and environmental data for 18 workers sampled on day 1. The breathing zone time-weighted average (TWA) concentrations for coal tar pitch volatiles (benzene solubles) are also shown in Table V. No pattern relating the workers' breathing zone benzene solubles to either the workers' urinary 1-P concentrations or their breathing zone pyrene or PAHs concentrations is evident.

Environmental pyrene was next evaluated for its use as a marker compound. The Spearman's correlation coefficient between the total pyrene and the total PAH concentrations for the 18 workers was $r = 0.82$ ($p = 0.0001$) (Figure 1). This result indicates that pyrene is a good compound to use as a marker compound for PAH exposure derived from coal tar pitch in this plant. The mean (± one standard deviation) percent pyrene in the environmental samples for the 18 workers was 9.96 (± 3.17). The percent pyrene for an individual was the sum of volatile pyrene + particulate pyrene divided by the sum of total volatile PAHs + total particulate PAHs multiplied by 100.

The Spearman's correlation coefficient was used in the above case and in two other cases below because it does not depend, in significance tests, on the assumption of bivariate normality, as does Pearson's correlation coefficient. None of the variables (total environmental pyrene, total environmental PAHs, or change in urinary 1-pyrenol) were normally distributed (the Shapiro–Wilk test rejected normality with $p \leq 0.0085$ in all cases), hence none of the pairs could have had a bivariate normal distribution.

Relationship Between Biological and Environmental Data for Day 1

In order to determine what relationship existed between urinary 1-P and environmental variables, a number of correlations were examined. The relationship between the net change in urinary 1-P and the total PAHs (Figure 2) was

TABLE V. Day 1 Biological and Environmental Data for Workers

Job Title	Workers' Shift	Urinary 1-P[A] "Pre"	Urinary 1-P[A] "Post"	CTPV[B] (mg/m³)[C]	Pyrene (μg/m³)	17 PAHs (μg/m³)
Packer-Puller	1	0.54	2.3	0.4	1.46	13.5
	1	0.88	3.3	0.2	3.67	35.4
	1	0.40	1.9	ND[D]	1.46	17.1
	1	0.83	2.9	0.3	7.44	57.2
Crane Operator	1	0.69	2.4	ND	2.04	20.0
	1	0.74	2.5	ND	1.94	30.3
	1	0.65	2.0	0.2	1.21	13.8
	1	0.78	3.4	0.4	2.38	24.4
	1	0.52	3.6	ND	6.49	56.4
Equipment Operator	1	0.68	2.5	0.2	1.48	9.5
V.T. Operator	1	1.10	3.5	0.6	2.80	94.6
Packer-Puller	2	0.66	2.0	0.2	1.54	10.1
	2	0.21	2.5	ND	2.30	22.8
	2	0.49	2.0	ND	1.48	16.5
Crane Operator	2	0.60	5.0	ND	4.12	30.0
	2	0.85	2.0	ND	1.99	22.2
	2	0.41	2.3	ND	1.22	19.1
	2	0.52	2.6	ND	1.26	16.1

[A]Urinary 1-P units are μmol 1-P/mol creatinine.
[B]Coal tar pitch volatiles (benzene solubles) LOD ~ 0.05 mg/sample.
[C]OSHA permissible exposure limit is 0.20 mg/m³.
[D]Nondetectable.

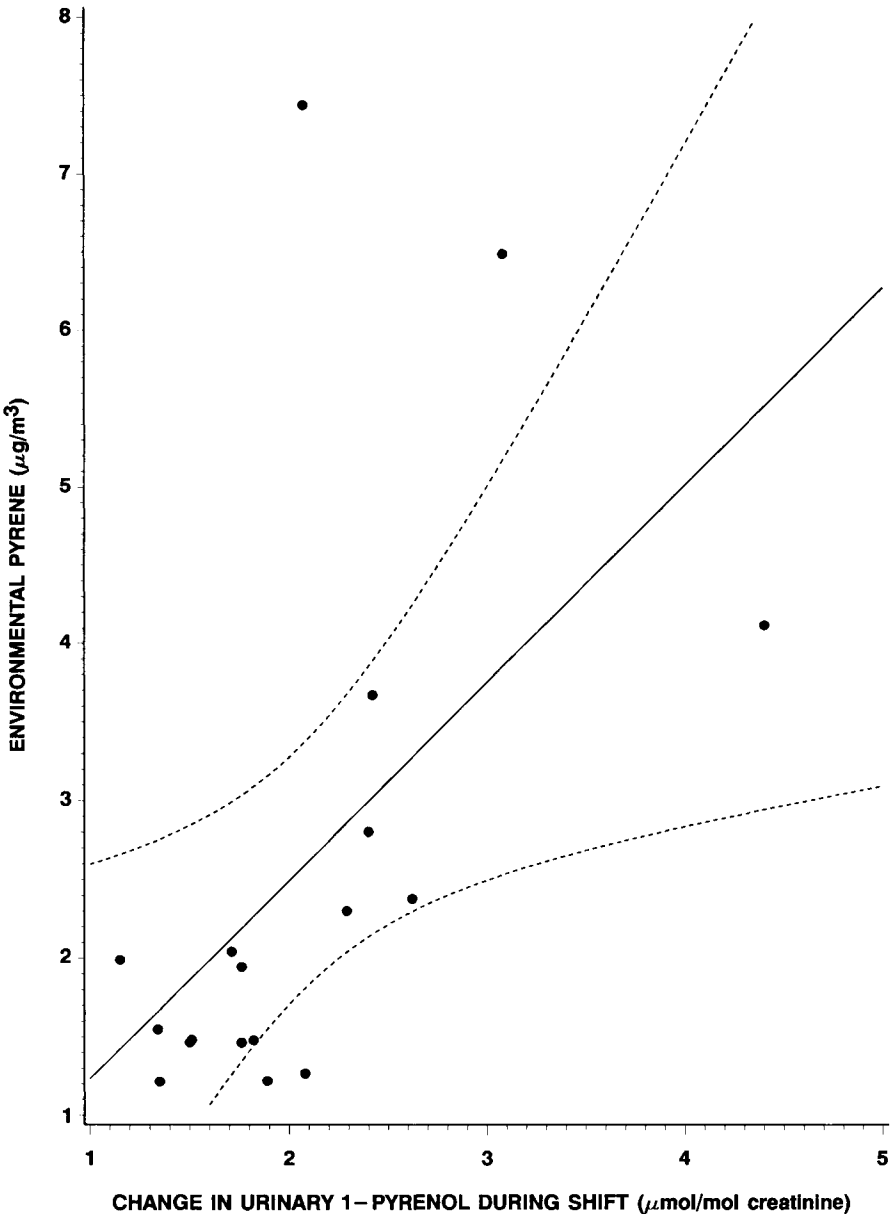

FIGURE 3. Environmental pyrene versus net change in urinary 1-pyrenol. Includes linear regression line and 95% confidence limits for predicted means.

significant and produced a Spearman's correlation coefficient r = 0.62 (p = 0.006). The Spearman correlation coefficient between the change in urinary 1-P levels and the environmental pyrene for 18 workers was r = 0.61 (p = 0.0068) (Figure 3). Thus, a strong relationship was observed between the concentrations of urinary 1-P in workers and environmental PAHs.

Conclusions and Recommendations

This study has provided evidence that 1-P in urine, a metabolite of pyrene, can be used as a biomarker for occupational pyrene exposure. The results show that the liquid chromatographic method can distinguish occupationally exposed workers from nonoccupationally exposed subjects. The impact of smoking relative to that of worker's status was negligible in this study. These observations complement results recently reported on pyrene exposure of road workers laying asphalt.[17,18]

It is recommended that the pre- *and* postshift levels in urinary 1-pyrenol be used for evaluating exposure to environmental pyrene or PAHs containing pyrene.

The environmental data show a strong positive correlation between pyrene and the total polycyclic aromatic hydrocarbons that were analyzed, verifying that pyrene in this study is an appropriate choice to use as an environmental PAH marker. However, it is recommended that the work environment be tested first for pyrene content which is required for using pyrene as a marker for assessment of exposure to total PAH. The fact that benzene solubles were not detected in several samples suggests they may

not be adequately sensitive for ascertaining overall PAH concentration.

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References

1. National Institute for Occupational Safety and Health: Petroleum Refinery Workers Exposure to PAHs at Fluid Catalytic Crackers, Coker, and Asphalt Processing Units. DHHS (NIOSH), Pub. No. 83-111. NIOSH, Cincinnati, OH (1983).
2. National Institute for Occupational Safety and Health: Criteria for a Recommended Standard—Occupational Exposure to Coal Tar Products. DHEW (NIOSH) Pub. No. 78-107. NIOSH, Cincinnati, OH (1977).
3. Bingham, E.; Trosset, R.P.; Warshawsky, D.: Carcinogenic Potential of Petroleum Hydrocarbons. J. Environ. Pathol. Toxicol. 3:483 (1979).
4. Niemeier, R.W.; Thayer, P.S.; Menzies, K.T.; et al: A Comparison of the Skin Carcinogenicity of Condensed Roofing Asphalt and Coal Tar Pitch Fumes. In: Proceedings of the Third NCI/EPA/NIOSH Collaborative Workshop: Progress Joint Environmental and Occupational Cancer Studies, pp. 629–670. National Cancer Institute, Rockville, MD (1984).
5. Mazumdar, S.; Redmond, C.; Sollecito, W.; Sussman, N.: An Epidemiological Study of Exposure to Coal Tar Pitch Volatiles Among Coke Oven Workers. JAPCA 25(4):382 (1975).
6. Menck, H.R.; Henderson, E.E.: Occupational Differences in Rates of Lung Cancer. J. Occup. Med. 18(12):797 (1976).
7. Kawai, M.; Amanoto, H.; Harada, K.: Epidemiologic Study of Occupational Lung Cancer. Arch. Environ. Health 14:859 (1967).
8. National Institute for Occupational Safety and Health: A Study of Coal Liquefaction Processes: Industrial Hygiene Comprehensive Survey. Final Report, Cresap Test Facility, Cresap, West Virginia. DHEW Pub. Contract No. 210-78-0101. NIOSH, Cincinnati, OH (1980).
9. National Institute for Occupational Safety and Health: A Study of Coal Liquefaction Processes: Industrial Hygiene Comprehensive Survey. Final Report, Solvent Refined Coal Pilot Plant, Fort Lewis, Washington. DHEW Pub. Contract No. 210-78-0101. NIOSH, Cincinnati, OH (1980).
10. Lauwerys, R.R.: Industrial Chemical Exposure Guidelines for Biological Monitoring, pp. 1–7. Biomedical Publications, Davis, CA (1983).
11. Tolos, W.P.; Lowry, L.K.; MacKenzie, B.A.: 1-Pyrenol in Urine: A Biological Monitoring Method to Assess Exposure to Polynuclear Aromatic Hydrocarbons Containing Pyrene. In: Eleventh Symposium on Polynuclear Aromatic Hydrocarbons. Gordon and Breach Publication, New York (1989).
12. Jongeneelen, F.J.; Bos, R.P.; Anzion, R.B.; et al.: Biological Monitoring of Polycyclic Aromatic Hydrocarbons. Metabolites in Urine. Scand. J. Work Environ. Health 12:137 (1986).
13. Keimig, S.D.; Morgan, D.P.: Establishing the Dose–Detection Thresholds of Index Polycyclic Aromatic Hydrocarbon Compounds. In: Seventh International Symposium on Polynuclear Aromatic Hydrocarbon. Battelle Press, Columbus, OH (1983).
14. National Institute for Occupational Safety and Health: NIOSH Manual for Analytical Methods, 3rd ed. DHHS (NIOSH) Pub. No. 84-100. NIOSH, Cincinnati, OH (1984).
15. Hollander, M.; Wolfe, D.A.: Nonparametric Statistical Methods, pp. 67–74. John Wiley & Sons, New York (1973).
16. Conover, W.J.: Practical Nonparametric Statistics, 2nd ed. John Wiley & Sons, New York (1980).
17. Jongeneelen, F.J.; Anzion, R.B.M.; Scheepers, P.T.J.; et al.: 1-Hydroxypyrene in Urine as a Biological Indicator of Exposure to Polycyclic Aromatic Hydrocarbons in Several Work Environments. Ann. Occup. Hyg. 32(1):35 (1988).
18. Jongeneelen, F.J.; Scheepers, P.T.J.; Groenendijk, A.; et al.: Airborne Concentrations, Skin Contamination, and Urinary Metabolite Excretion of Polycyclic Aromatic Hydrocarbons Among Paving Workers Exposed to Coal Tar Derived Road Tars. Am. Ind. Hyg. Assoc. J. 49(12):600 (1988).

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