

Assessment of Exposure to Polycyclic Aromatic Compounds (PACs) in Asphalt Workers: Measurement of Urinary PACs and their Metabolites with an ELISA Kit

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Abstract

Asphalt pavers are exposed to polycyclic aromatic compounds (PACs), several of which are carcinogens, by inhalation and dermal contact. However, data linking exposure to asphalt with specific health effects are limited. Measurement of PACs and PAC metabolites as biomarkers of exposure provide a useful tool in assessing health effects. An enzyme-linked immunosorbent assay (ELISA) kit developed for the determination of PACs in water was adapted for measuring PACs and their metabolites in urine and then applied to a pilot asphalt worker PAC exposure study. Currently, liquid-liquid extraction with gas chromatography/isotope dilution high-resolution mass spectrometry (GC/HRMS) is the preferred method to determine urinary PAC metabolites. Although sensitive and specific, GC/HRMS is time consuming and costly. PAC ELISA is promising as a more rapid and less costly routine method for determining worker exposure to PACs in asphalt emissions. The ELISA method measured from 14-720 ng/ml 1-hydroxypyrene equivalents with a lower limit of detection of 14 ng/ml urine. Measurements of PAC metabolite equivalents in urine from asphalt exposed and control concrete workers using the ELISA had a good correlation ($R=0.89$) to the sum of select GC/HRMS PAC urine metabolites (naphthalene, fluoranthene, phenanthrene, and pyrene) and the PAC ELISA results were indicative of potential asphalt exposure.

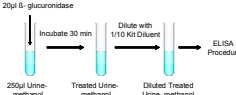


Sample Preparation

Sample Collection in the Field

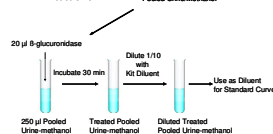
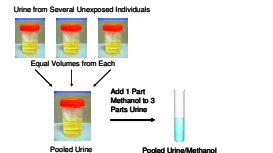


Sample Treatment in the Laboratory

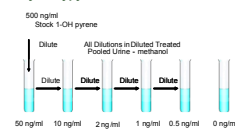


Calibration Curve

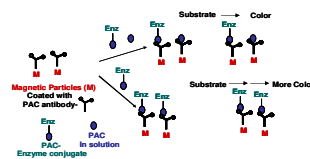
Urine Matrix



1-hydroxypyrene Standards for ELISA Procedure

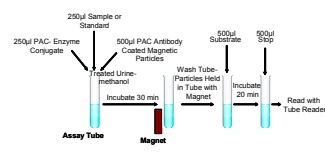


ELISA Principle



ELISA Principle: PAC in solution competes with PAC-enzyme conjugate for PAC antibody binding sites on the Magnetic Particles resulting in less conjugate binding to the magnetic particles. The binding of less conjugate results in less color being produced at higher concentrations of PAC in solution.

ELISA Procedure



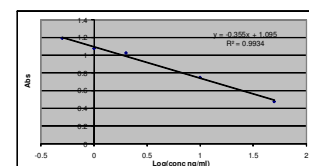
Magnetic Tube Rack



ELISA Tube Reader

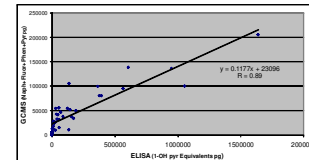
Results

Calibration Curve



Urine Methanol 1-hydroxypyrene Standard Curve: The absorbance read by the ELISA tube reader was plotted as a function of the log of concentration for the standard solutions of 1-hydroxypyrene in 1/10 urine. The plot was linear over the range of 0-50 ng/ml with a least detectable dose (LDD) of 14 ng/ml which results in a method range of 0-720 ng/ml and a method LDD of 14 ng/ml when dilution corrections are made.

Comparison of ELISA and GC-MS Results



The ELISA and GC-MS measurements were compared for worker urine samples. The total hydroxylated products of pyrene, phenanthrene, fluorene, and naphthalene as measured by GC-MS were compared with the ELISA measurements which are calibrated with 1-hydroxypyrene and the methods correlated well.

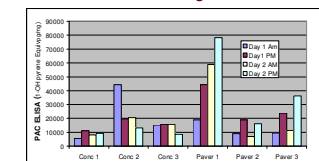
Some PACs and their Related Compounds Detected by ELISA

- Fluoranthene
- Pyrene
- Fluoranthene
- Chrysene
- Benzo (a) pyrene
- Anthracene

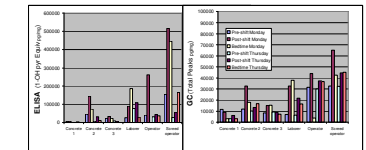
Hydroxylated PAC Metabolites Identified by GC/HRMS

- 1-Hydroxynaphthalene (1-NAP)
- 2-Hydroxynaphthalene (2-NAP)
- 2-Hydroxyfluorene (2-FLUOR)
- 3-Hydroxyfluorene (3-FLUOR)
- 1-Hydroxyphenanthrene (1-PHEN)
- 2-Hydroxyphenanthrene (2-PHEN)
- 3-Hydroxyphenanthrene (3-PHEN)
- 4-Hydroxyphenanthrene (4-PHEN)
- 1-Hydroxypyrene (1-HPY)
- 1-Hydroxybenzo (c) phenanthrene (1-BCP)
- 2-Hydroxybenzo (c) phenanthrene (2-BCP)
- 3-Hydroxybenzo (c) phenanthrene (3-BCP)
- 1-Hydroxy benzo (a) anthracene (1-BAA)
- 2-Hydroxychrysene (2-CHR)
- 3-Hydroxychrysene (3-CHR)
- 4-Hydroxychrysene (4-CHR)
- 6-Hydroxychrysene (6-CHR)
- 8-Hydroxychrysene (8-CHR)

Hydroxylated PACs in Worker Urine Pre and Post Working Shift



PAC ELISA 1-OH pyrene Equivalents in Worker Urine: Dilution and Creatinine Corrected. The concentration of hydroxylated PAC metabolites in worker urine was determined pre and post shift for concrete and asphalt paving workers. Only the asphalt paving workers showed a consistent increase in concentration of these metabolites after their working shift.



Comparison between ELISA and GC-MS: Although absolute numbers are different, similar trends are seen in between PAC levels as measured by ELISA and GC/HRMS.

Conclusions

The PAC ELISA technique was adapted to determine PAC metabolites in urine with a range of 0-720 ng/ml and an LDD of 14 ng/ml.

The PAC ELISA correlated with the total hydroxylated PAC concentration determined by GC-MS.

The PAC ELISA correlated with potential exposure to PAC in asphalt paving operations.

These preliminary results indicate that the ELISA PAC can provide a less expensive and more rapid means of determining exposure than GC-MS. The results from the more extensive second phase of the study will allow a more detailed determination of the correlation of the GC-MS and ELISA and correlation of ELISA measurements with exposure.

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