Pilot Study for Relative Bioavailability Study of PAH in Coal Tar Pitch of Clay Target Fragments

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Abstract

An *in vivo* bioavailability study is being performed to determine the relative bioavailability of PAHs in clay pigeon target fragments at military range sites. These fragments, which are mixed with native soil, are composed of PAHs in a site-aged coal tar pitch/limestone matrix, which is expected to reduce the bioavailability of PAHs compared to that seen in animal studies using pure benzo(a)pyrene (BaP) in solvents added to rodent chow. The Pilot Study includes development of the analytical method and execution of the first phase of the *in vivo* study. A high resolution mass spectrometry method has been developed to detect low level PAH metabolites in mouse urine to a reporting limit of 25 pg/mL or lower based on a 4 mL sample. The method and method validation procedures are described. The goals of the *in vivo* pilot study with mice are to (a) test the methods of preparation and homogeneity of the test articles, which include dried and sieved (250 micron) site soil, site soil extracts, pure BaP in solvents, and pure clay pigeon fragments mixed into rodent chow, (b) determine the fraction of soil in rodent chow consumed by mice over 14 days, (c) test the urine analysis analytical method with *in vivo* samples, (d) compare PAH metabolite concentrations at two time points (7 days and 14 days) to assist in optimization of the Final Study design, (e) determine the adequacy of urine volume from pooling urine from four mice per sample, and (f) obtain preliminary indications of the relative bioavailability of the three selected PAHs: BaP, benz(a)anthracene, and chrysene.

Test Diets: Preparation and Validation

- Soil samples and fragments analyzed for PAH concentrations and test articles were prepared for soil, fragments, and extracts
- Test diets prepared based on PAH concentrations in test article soil samples
- Test diets analyzed to determine actual PAH concentrations
- Concentrations of extract diet matched the concentrations target soil diets
- Target BaP concentration = 25 ppm; actual soil = 26.7 ppm; actual extract = 24.3 ppm

In-Life Conclusions

- Female B6C3F1 mice (4/cage) fed test diets for 14 days while daily urine was collected
- Animals best tolerated soil in the diet at 5% of diet composition by mass
- Body weight decreases were seen at 10 and 20%
- Target fragments and extracts of soil and target fragments were associated with significant reduced diet consumption and body weight gain

Analytical Method

Target analytes

- 3-OH-Chrysene
- 3-OH-Benzo[a]pyrene
- 9-OH-Benzo[a]pyrene
- 3-OH-Benz[a]anthracene

Quantification standards

- ^{13}C6-3-OH-Chrysene
- ^{13}C6-3-OH-Benz[a]anthracene
- D11-3-OH-Benzo[a]pyrene

De-conjugation controls

- 1-Naphthyl b-D-glucuronide sodium salt
- Pyrene-1-Sulfate potassium salt
- ^{13}C6-OH-1-Naphthalene
- ^{13}C6-1-OH-Pyrene

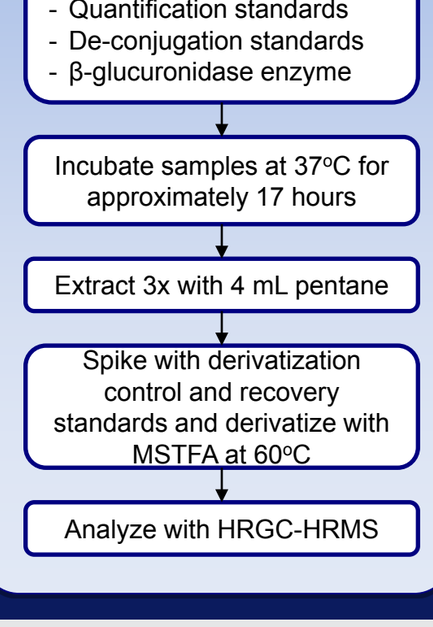
Derivatization control

- ^{13}C12-4'-OH-3,',4,5'-TetraCB

Recovery standard

- ^{13}C12-PCB 81

Analysis flow chart



Method Performance Results

Analyte	Mean % rec. (n=5)	%RSD
3-OH-Chrysene	101	1.9
3-OH-Benz[a]anthracene	77.4	1.5
9-OH-Benzo[a]pyrene	89.7	4.9
3-OH-Benzo[a]pyrene	98.5	4.0

Accuracy

Accuracy, expressed as mean % recovery from five replicates of spiked synthetic urine, ranged from 77% to 101%. Accuracy values from human urine determined from a matrix spike/matrix spike duplicate (MS/MSD) ranged from 84% to132%.

Precision

Precision determined from replicate (n=5) analysis of spiked synthetic urine. Percent relative standards deviation values of less than 5% were observed.

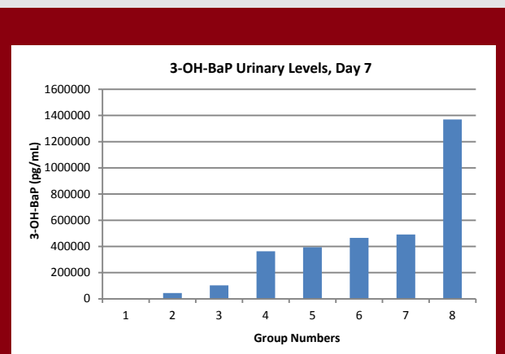
Method Detection Limits (MDLs)

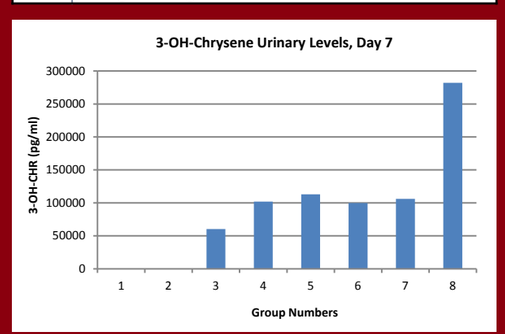
- MDL values ranged from 13 to 24 pg/mL for a 4 mL urine sample.
- MDL values were generated following the protocol as specified in Federal Register 40 CFR Part 136, Appendix B, rev. 1.11, no iteration.

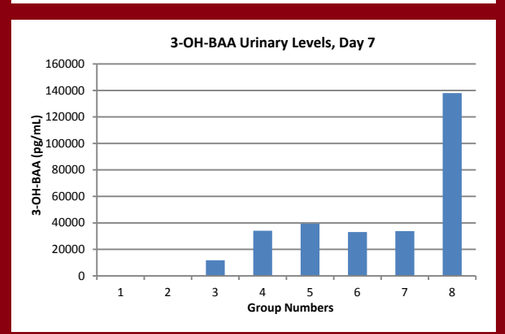
Conclusions

- Sample collection, test article preparation, diet preparation, and in-life portions of the protocol have been optimized to provide usable urine samples for analysis.
- The HRGC-HRMS method developed for this study was capable of measuring OH-PAHs at low picograms per milliliter levels.
- Unique aspects of the method include the use of isotope labeled surrogates for accurate quantification, de-conjugation control standards to monitor efficiency of enzymatic de-conjugation, standards to confirm efficiency of the derivatization and recovery standard to ensure sample injection, enable surrogate recovery quantification, and demonstrate good recovery of standards through the procedure for each sample.
- Laboratory blanks were low to allow detection of PAH metabolites at background levels and in experimental control samples.
- OH-PAHs, particularly 3-OH-Benzo[a]pyrene and 9-OH-Benzo[a]pyrene are prone to loss/degradation during extraction. This degradation was overcome by the use of an antioxidant.

Group Numbers	
1	Control Soil
2	BaP Positive Control
3	5% Site Soil
4	10% Site Soil
5	20% Site Soil
6	Site Soil Extract
7	10% Pulverized Target Fragment
8	Target Fragment Extract







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