



Project Summary

Screening Methods for PAH Priority Pollutants in Wastewater

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A screening method using a simplified group test procedure for determining polynuclear aromatic hydrocarbons (PAH) priority pollutants in wastewaters was developed. This screening procedure, using total ultraviolet absorbance, is designed to serve as an indicator of pollutant levels and can be employed in conjunction with the more specific procedure, Method 610, which determines the levels of individual PAH priority pollutants. Aqueous sources used to validate this screening procedure were distilled water, effluents from a coking operation and a refinery, and the secondary effluent from a publicly owned treatment works (POTW).

Accuracy of the screening procedure was estimated by comparing the results obtained using this screening procedure and Method 610. The screening procedure exhibits a slight positive bias (+30%) in the four effluents tested.

This Project Summary was developed by EPA's Environmental Monitoring and Support Laboratory, Cincinnati, Ohio to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Under provisions of the Clean Water Act, EPA is required to promulgate guidelines establishing test procedures for the analysis of pollutants. The Clean Water Act Amendments of 1977 emphasize the control of toxic pollutants and declare the 65 "priority" pollutants and classes of pollutants to be toxic under Section 307(a). This full report is one of a series that investigates the analytical behavior of selected priority pollutants and

suggests a suitable test procedure for their measurements.

These 16 PAH's were: acenaphthene; benzo(g,h,i)perylene; fluorene; phenanthrene; dibenzo(a,h)anthracene; ideno(1,2,3-c,d)pyrene; acenaphthalene, anthracene, benzo(a)anthracene, benzo(a)pyrene; benzo(b)fluoranthene; benzo(k)fluoranthene; chrysene; fluoranthene; naphthalene; and pyrene.

The objective of this program was to develop a simplified group test procedure or "screening method" for determining PAH priority pollutants in aqueous samples. This method is designed to serve as an indicator of pollutant level which can be employed in conjunction with the more specific Method 610, previously developed by the EPA for determining 16 individual PAH priority pollutants in industrial wastewater.

The use of such methods will be especially useful for screening samples to determine whether the pollutant levels are relatively high or low.

The requirements which such screening methods are expected to meet are listed:

- (1) Quantification shall be positively correlated to that produced by the refined Section 304(h) Clean Water Act methods (as the sum of the individual pollutants).
- (2) The methods shall be able to stand alone as reliable indicators of pollutant level.
- (3) The cost shall be not more than one-half of that using the refined 304(h) gas chromatographic (GC) or combined gas chromatography/mass spectrometry (GC/MS) methods.
- (4) Response shall be class-specific, producing a very low frequency of false responses.

- (5) Responses need not be produced for individual pollutants. Quantification may be based on a unified response of the category.
- (6) Quantification shall produce a numerical value (rather than a range).
- (7) Detection limit shall be at least 1 $\mu\text{g/L}$.
- (8) Recovery shall be 70% or greater for the priority pollutants in the category.
- (9) The relative precision (as relative standard deviation) of replicate determinations shall be less than 50%.
- (10) Ruggedness shall be maximized.
- (11) The cost of the instrumentation required for the methods shall not exceed \$10,000.

The major objective of this program was to select, evaluate, and validate the method which best meets the requirements listed above for the priority pollutant PAH's. To accomplish this objective, a literature survey was conducted to formulate the most logical approach to meet these goals. Three candidate approaches (thin-layer chromatography, TLC), fluorescence and Ultra-Violet (UV) absorbance) were selected for laboratory evaluation.

Screening Methods Evaluation

The simplest method and one ultimately selected as the PAH screening method is the measurement of total UV absorbance. This method avoids the problem of different responses for individual PAH's at a specific wavelength measurement. For this method, a very inexpensive instrument is required, consisting of a deuterium lamp as a UV light source (~200-350 nm), a bandpass filter which blocks wavelengths below ~240 nm to remove contributions from the solvent, a cuvette to contain the isolated PAH extract, and a photodetector.

The approaches used for fluorescence measurements were similar to those used for absorption measurements (i.e., total fluorescence using broad bandpass filters). Advantages of fluorometry over UV spectrometry and higher selectivity (nonfluorescent potential interfering compounds are not detected). A disadvantage is that the sensitivity for naphthalene will be lower than for the PAH's because it absorbs and fluoresces at lower wavelengths. In addition to total fluorescence measurement, a spectrofluorometer can be used to obtain measurements at several specific wavelengths, to partially overcome the variability in response among the various PAH's.

TLC methods using very narrow channels on a silica gel TLC plate have been proposed. PAH's are eluted as a single band and the length of the band is related to the total PAH concentration. This procedure is actually the TLC equivalent of the total UV measurement method described. The method employs a single wavelength UV source as the visualization technique, and incorporates its own cleanup step, thus requiring only sample extraction and concentration. The variability in response between various PAH's is expected to be quite large, thus making quantification more difficult.

All three approaches were evaluated. The UV absorbance method was selected as the best of the three and the results obtained are described below.

Evaluation of UV Detection

The determination of total PAH concentration by monitoring UV absorbance was accomplished using two approaches. The first approach, the multiple wavelength method, involves monitoring the absorbance at two or more specified wavelengths and performing a weighted average calculation to determine total PAH concentration. This approach is useful only if two wavelengths are found such that each of the components of interest absorb strongly at one of the wavelengths but not at both wavelengths. This approach becomes difficult as the number of components of interest increases.

The second approach, called the total UV measurement approach, involves making a single UV absorbance measurement over a wide wavelength region, over which the average molar absorptivities of the components of interest are approximately equal.

In order to evaluate which of these UV methods would be most appropriate for the PAH's, the molar absorptivity of the PAH's over specified wavelength regions were calculated from literature data. The average molar absorptivities of the PAH's over narrow (10 nm) wavelength regions vary over two orders of magnitude. The molar absorptivities over certain wavelength regions (e.g., 240-320) vary by a factor of 15. Since the most responsive components are the high molecular weight components (e.g., dibenzo(a,h)-anthracene), the variation in response on a weight basis is substantially less.

In view of the high variation in response for PAH's over narrow wavelength ranges, and the greater complexity of the multiple wavelength approach, the total UV approach was selected for laboratory evaluation.

Two optical filters were evaluated for use in the total UV measurement. The first filter, Corning 7-54, is a bandpass filter transmitting UV radiation in the 240-380 nm region, whereas the second filter, Corning 9-54 is a cutoff filter transmitting UV radiation above 230 nm.

The apparent molar absorptivities and relative response (on a weight basis) for various PAH's using these two filters was obtained. Examination of the data reveals that a smaller compound-to-compound variation in response is obtained using the bandpass filter than using the cutoff filter. Sensitivity for the various compounds was somewhat better using the bandpass filter (except in the case of naphthalene which gave a slightly higher response using the cutoff filter). Consequently, the bandpass filter was chosen for use in all later experiments.

The linearity in response using the total UV approach was evaluated using a solution containing equimolar concentrations of all 16 priority pollutant PAH's in cyclohexane. The plot of total PAH concentration versus absorbance indicates that the response is nearly linear over the 4-120 μM range or approximately 0.2-24 $\mu\text{g/mL}$ (using an average molecular weight of 200 daltons).

The response data for individual PAH's as a function of concentration using the total UV approach was obtained. The response for many of the compounds is not linear over a wide range of concentrations. It is important to recognize that the concentrations of the individual components in this experiment are approximately 20 times greater than in the previous experiment where good linearity was obtained. This result implies that the total UV determination should be conducted within a rather narrow absorbance region (~0.05-0.35 absorbance units) so that nonlinear response does not bias the data. Dilution of sample extracts will be required to achieve this criterion in some cases.

The compound used for instrument calibration in the total UV method should have a molar absorptivity close to the average molar absorptivity of the 16 priority pollutant PAH's and should also exhibit good linearity so that a degree of bias introduced into the method can be minimized. The reference compound should also be readily available in pure form, inexpensive, and noncarcinogenic. Initially, anthracene was chosen as the reference compound for calibration purposes, since it appeared to best meet these requirements. However, examination of the response data led to the conclusion that fluoranthene would be a

better calibration standard, since it gave more nearly linear response over the desired absorbance range. Fluoranthene has the advantages that: 1) it is not a carcinogen; 2) it is readily available in pure form at low cost; and 3) its total UV response is near the median for the priority pollutant PAH's. Consequently, all further measurements using the total UV approach were made using fluoranthene as the calibration standard. The calibration curve was generally constructed over fluoranthene concentrations of 5×10^{-6} to 10^{-4} M or 1 to 20 $\mu\text{g}/\text{mL}$. Results were calculated as fluoranthene equivalents by comparing the UV absorbance for the sample extract to the fluoranthene calibration curve.

Evaluation of Column Cleanup Approaches

Silica gel and alumina adsorbents were evaluated in terms of ability to selectively remove interfering compounds. The results obtained using these two adsorbents are described below.

Silica Gel Column Cleanup

Silica gel columns were prepared using the procedure described in Method 610. Initial experiments involved eluting the ST INTF 1 and ST INTF 2 interference solutions, and collecting and analyzing the "PAH fraction." Concentrations of ST INTF 1 and ST INTF 2 were 100 $\mu\text{g}/\text{mL}$ of each compound. ST INTF 1 contains nonpolar compounds (e.g. benzene PCB's and ST INTF 2 contains polar components (e.g., nitrogen heterocyclics).

Because dioctyl phthalate (DOP) is a widespread contaminant in wastewater, this compound was examined separately at a concentration level of 10^{-3} M. PAH's were analyzed at a concentration level of 10^{-5} M per compound. All samples were applied to the silica gel column at a volume of 1.0 mL.

Three chromatographic fractions were obtained for the samples listed above (applied separately) as follows:

- Fraction 1 - 25 mL of pentane
- Fraction 2 - 25 mL of 40% CH_2Cl_2 in pentane
- Fraction 3 - 25 mL of CH_2Cl_2 .

The UV absorbance of each of these fractions at 230 nm was determined.

The UV absorbance data indicated that silica gel readily separates the polar compounds (ST INTF 2 and DOP) from the PAH's since UV absorbance was noted only in the methylene chloride fraction (Fraction 3) for these compounds. However, some of the nonpolar compounds were found to elute in the PAH fraction

(Fraction 2). All of the PAH's were found to elute in Fraction 2, as expected.

Additional experiments were conducted to determine which specific nonpolar compounds were eluting in the PAH fraction and to determine the elution volumes. If these compounds were eluting near the initial elution volume where PAH fraction collection began, then by addition of a larger amount of pentane these compounds could be separated from the PAH fraction.

Elution of the ST INTF 1 solution was performed with fraction collection at 10 mL, 10 mL, and 5 mL of pentane followed by 5 mL, 10 mL, and 10 mL, or 40% CH_2Cl_2 in pentane. These fractions were concentrated by Kuderna-Danish evaporation on a water bath at 50°C and analyzed by GC. Compound identification was based on retention time and recoveries were determined by comparing the peak areas obtained for the components in the fractions to the peak areas obtained for the ST INTF 1 stock solution.

Results of these analyses, indicate that trimethylbenzene and 2,4,2',4'-tetrachlorobiphenyl elute at the very end of the PAH fraction. Therefore, it is not possible to separate these compounds from PAH's using silica gel. Since PCB's absorb UV radiation and are frequently present in environmental samples, this finding represents a serious limitation of the silica gel cleanup procedure when UV absorbance is used as the determinative procedure for PAH's.

Alumina Column Cleanup

The selectivity of alumina adsorption chromatography columns for PAH's was evaluated. Elution volumes for ST INTF 1 compounds were determined using 10% CH_2Cl_2 in pentane as the eluent. Elution volumes for PAH's ST INTF 2, and DOP were determined using 100% CH_2Cl_2 as the eluent. The resulting fractions were analyzed by UV spectrometry as described earlier.

Results of these analyses indicated that the elution volume for ST INTF 1 compounds was from 5 to 15 mL using the 10% CH_2Cl_2 in pentane eluent while PAH's were completely retained on the column for the total elution volume of 50 mL. When using 100% CH_2Cl_2 as the eluent, PAH's showed two maxima. One maximum occurred at an elution volume between 15 and 25 mL and the second maximum between 35 and 45 mL. However, there was still some absorbance indicated for the tenth 5-mL fraction PAH's. DOP and ST INTF 2 compounds appeared to be totally retained on the

alumina column with an elution of 50 mL of CH_2Cl_2 .

Because PAH's were partially retained after eluting with 50 mL of CH_2Cl_2 , the above experiments were repeated using an elution volume of 60 mL of CH_2Cl_2 . Results of these experiments indicated that PAH's were eluted at a total elution volume to 55 mL and DOP and ST INTF 2 compounds were completely retained. Elution with 30 mL of diethyl ether was necessary to elute the DOP and ST INTF 2 compounds from alumina.

GC analysis was conducted on the later eluting PAH fractions to determine which compounds were being retained. Results for these analyses indicated the PAH's in the second maximum were primarily benzo(b)fluoranthene, indeno(1,2,3-cd)pyrene and dibenzo(a,h)anthracene with a small amount of benzo(a)pyrene.

On the basis of these results, alumina was selected as the absorbent of choice since both polar and nonpolar (e.g., PCB) components are separated from the PAH's using this approach.

Validation of Screening Method for PAH's

Based on the results presented above, a screening method for determining PAH's in wastewater was selected. This method, consists of the following steps:

- (1) Extraction of the sample (1 liter) with a single aliquot (150 mL) of CH_2Cl_2 . Only 100 mL of the CH_2Cl_2 is collected so as to reduce susceptibility to emulsion problems.
- (2) Drying (sodium sulfate) and Kuderna-Danish concentration of the extract.
- (3) Alumina cleanup.
- (4) Measurement of total UV absorbance relative to a fluoranthene calibration standard, using a filter photometer.

The total UV determinative procedure was judged to be superior to either the fluorescence or "channel" TLC methods because of great variability in response between the PAH compounds of interest for the latter methods. Bias (inaccuracy) of $\pm 1000\%$ or greater may be observed for the latter methods, depending on the specific PAH compounds present. By comparison, the total UV method bias should not exceed $\pm 100\%$, based on the response ratios determined for the priority pollutant PAH's.

The proposed method was initially applied to reagent water and two wastewaters, spiked at various levels with equimolar concentrations of all 16 PAH's. In all cases, reproducibility and recovery were excellent, and therefore, the proposed method was considered suitable for detailed validation.

Method validation consisted of: 1) determination of the method detection limit (MDL) for the screening method using the protocol; and 2) comparison of analytical results obtained for relevant water samples, unspiked at various levels, using the PAH screening method and the Method 610.

Method Detection Limit (MDL) Determination

The MDL for the PAH screening method was determined according to the EPA protocol. The spiked aliquots contained equimolar concentrations of all 16 priority pollutant PAH's. The process blank value (reagent water process blank) was subtracted from all the values prior to calculating the MDL, since this calculation procedure is recommended in the analytical method.

The MDL data for reagent water and three relevant water samples are presented in Table 1. The MDL values for the various water types are essentially the same (approximately 4 $\mu\text{g/L}$). These results seem reasonable, based on a reproducible process blank level of 2.5 $\mu\text{g/L}$ and the MDL of 4 $\mu\text{g/L}$, which is considered to be satisfactory compared to alternative methods, such as Method 610. Although Method 610 detects less than 4 $\mu\text{g/L}$ of most individual PAH's the detection limit in terms of total PAH will be higher (poorer) when a variety of PAH's are present in a given sample.

Comparison of Method 610 and PAH Screening Method

Aliquots of distilled water, POTW secondary effluent, a final effluent from a coking operation, and a refinery effluent were extracted and cleaned up according to the procedure outlined above. Three aliquots of each sample unspiked and spiked at each of two concentration levels were extracted. The extracts were analyzed by the PAH screening method and then exchanged into acetonitrile and analyzed by the high performance liquid chromatography approach described in Method 610. One extract of each sample (unspiked) was analyzed by GC/MS to confirm component identities.

The results of the Method 610 analyses for the three samples indicate that in most cases, with the exception of benzo(k) fluoranthene, the recovery was greater than 75% at both the high and low spike levels. The process blanks contained no detectable level of any of the 16 priority pollutant PAH compounds.

Table 1. Method Detection Limits for PAH Screening Method in Terms of Fluoranthene Equivalencies

Matrix	Spike Level ($\mu\text{g/L}$) ^a	Average Value ($\mu\text{g/L}$)	Standard Deviation ($\mu\text{g/L}$)	MDL ($\mu\text{g/L}$)	Percent Recovery
Reagent Water	5.5	5.8 ^b	1.1	3.5	105
Coking Effluent	5.5	4.5 ^b	1.2	3.1	82
Refinery Effluent	5.5	7.4 ^b	1.6	4.9	135

^a Spiked with an equimolar mixture of all 16 priority pollutant PAH's.

^b Blank corrected values.

Table 2. Comparison of Screening Method to Method 610 for Various Water Samples

Sample	PAH Spike Level ($\mu\text{g/L}$)	Average Percent Recovery ^a	
		Screening Method ^b	Method 610
Reagent Water	18.1	96 \pm 19	77 \pm 8
Reagent Water	3.6	99 \pm 8	76 \pm 6
POTW	36.2	76 \pm 3	90 \pm 9
POTW	3.6	92 \pm 12	75 \pm 6
Coking Effluent	36.2	78 \pm 4	87 \pm 3
Coking Effluent	3.6	97 \pm 15	61 \pm 6
Refinery Effluent	36.2	84 \pm 8	85 \pm 3
Refinery Effluent	3.6	135 \pm 32	43 \pm 12

^a Three replicates at each spike level.

^b In terms of fluoranthene equivalents.

The values obtained using the screening method are compared to the Method 610 results in Table 2 for all four water types. The values for the screening method are consistently higher than the Method 610 results. This positive bias results from the use of fluoranthene as the calibration standard. Since the molar absorptivity of fluoranthene is slightly less than the average molar absorptivity of the 16 PAH compounds, the absorbance of the PAH mixed standard used to spike the samples is greater than the fluoranthene standard. This factor is demonstrated by comparing the actual spike level (first column of Table 2) to the spike level in terms of "fluoranthene equivalents."

While this bias can be significantly reduced by selecting a different PAH spiking standard, this procedure would not be effective in a real-world situation (i.e., when the levels of individual PAH compounds are not known). In practice, the amount of bias will not be known and will vary from sample to sample, within the relative sensitivity factors (approximately \pm 100% relative to fluoranthene) of the various PAH compounds (last column of Table 10 in the full report). In certain cases where the major PAH species is known, the amount of bias can be minimized by calibrating with that particular PAH species.

The background level of the distilled water sample was approximately 2.5 $\mu\text{g/L}$ while the POTW, coking effluent,

and refinery effluent samples had slightly higher backgrounds due to the presence of PAH. The refinery effluent gave a background level of 11.8 $\mu\text{g/L}$. In practice, the screening method blank consistently gives a background level of approximately 2.0-2.5 $\mu\text{g/L}$. The method description suggests that results be corrected for method blank in order to reduce the degree of positive bias for the method. This amount of background may be due to contamination of the sample with trace amounts of UV absorbing material, such as phthalates, following the cleanup step.

GC/MS analysis of the wastewater extracts confirms that traces of phthalates (representing less than 5 $\mu\text{g/L}$ in the wastewater sample) are present in the extracts. Trace quantities of phenanthrene and naphthalene were identified in the refinery effluent and the POTW effluent. Methylthiobenzene was also identified in the refinery effluent. Napthalene, methyl-naphthalenes, dimethylnaphthalenes, phenanthrene, and pyrene were detected in the POTW secondary effluent.

The PAH screening method results and Method 610 results were subjected to linear regression analysis to determine the degree of correlation between the two methods. Screening method data were entered as the "Y" values and Method 610 were entered as the "X" values. The slope, intercept, correlation coefficient, and R-squared (residual) values were calculated for each water type as well as for the data set as a whole.

The slope values were approximately 1.3, with the exception of reagent water, which is indicative of a positive bias (~30%) for the screening method. The reasons for this bias are discussed above. The intercept values were approximately 3 µg/L, which is approximately equal to the MDL. This result indicates that the relative bias in the method is constant over the concentration range tested (i.e., there is not a large fixed bias inherent in the method). The correlation coefficients were somewhat better in wastewater than in distilled water, and are considered to be satisfactory in all cases. The R-squared values were always >95%, indicating that the data points fall relatively close to the regression line.

It is important to recognize that these correlation data are rigorously valid only for the particular spiking scheme shown. If other combinations of PAH's are present, a different slope will be obtained for the regression line, although the other parameters (e.g., correlation coefficient, intercept) will not be greatly affected. These results indicate that the PAH screening method is precise and positively correlated with Method 610, which is currently in use for the determination of priority pollutant PAH's in wastewater. An important consideration in the evaluation of these two methods is the analytical cost, since one of the primary objectives in the development of screening methods is to reduce the analytical costs associated with monitoring pollutants.

Summary

The PAH screening method requires considerably less time to conduct than does Method 610. The sample extraction step involves a single extraction step with a fixed proportion (2/3) of the extract being recovered. This process reduces the problems with emulsions substantially since the emulsive interface does not need to be recovered. In addition, the instrument calibration, sample analysis, and data reduction steps are accomplished much more rapidly for the screening method, since only a single numerical value (total PAH concentration) rather than 16 individual concentration values is obtained, and the lengthy chromatographic analysis is eliminated. In addition, the time required to prepare reference standard solutions is greatly reduced. Only a single standard (fluoranthene) is required.

The PAH screening method utilizes considerably less expensive equipment than does Method 610 (\$5,000 vs. \$25,000) and requires fewer materials and less trained personnel.

A potential use for the total UV approach, employed in the PAH screening method, is analysis of samples prior to GC/MS or high performance liquid chromatography determination. For example, the total UV approach could be used to screen sample extracts generated by Method 610 (prior to high performance liquid chromatography analysis) to selectively eliminate low level samples and thereby reduce analytical costs.

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The complete report, entitled "Screening Methods for PAH Priority Pollutants in Wastewater," (Order No. PB 84-132 992; Cost: \$11.50, subject to change) will be available only from:

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