



## Project Summary

# EPA Method Study 20 Method 610 — PNA's

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The U.S. Environmental Protection Agency (USEPA) sponsored an interlaboratory study in which 16 laboratories participated, to provide precision and accuracy statements for the proposed EPA Method 610 for the 16 selected polynuclear aromatic hydrocarbons (PNA's) comprising Category 9 of the priority pollutant which may be present in municipal and industrial aqueous discharges. The specific PNA's are as follows:

Napthalene	Benzo(a) anthracene
Acenaphthylene	Chrysene
Acenaphthene	Benzo(b)fluoranthene
Fluorene	Benzo(k)fluoranthene
Phenanthrene	Benzo(a)pyrene
Anthracene	Dibenzo(a,h)anthracene
Fluoranthene	Benzo(g,h,i)perylene
Pyrene	Indeno(1,2,3-cd)pyrene

Method 610 involves extraction of the pollutants with methylene chloride followed by silica gel cleanup and subsequent high performance liquid chromatography (HPLC) analysis utilizing fluorescence and ultraviolet (UV) detection.

The study design was based on Youden's non-replicate design for collaborative tests of analytical methods. Three Youden pair ampules of the test compounds were spiked into six types of test waters and then analyzed. The test waters were distilled water, tap water, a surface water, and three different industrial wastewater effluents. The resulting data were analyzed statistically using USEPA's computer program entitled, Interlaboratory Method Validation Study (IMVS).

Mean recovery of the PNA's based upon inserting analyte concentrations into the regression equations ranged from 43-110 percent. Overall precision was in the range of 16-91 percent and

single-analyst precision ranged from 11-50 percent.

A statistically significant effect due to water type was established for six of the 16 water types. However, because distilled water had consistently lower recoveries than the wastewaters, and the distilled waters were the first samples to be analyzed, the statistical effect was judged to be due to the analytical learning process and therefore of no practical importance.

*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

EPA first promulgated guidelines establishing test procedures for the analysis of pollutants in 1973 following the passage of the Federal Water Pollution Control Act in 1972 by Congress. Pursuant to the amendment and publication of these guidelines EPA entered into a Settlement Agreement—the Consent Decree—requiring it to study and, if necessary to regulate, 65 "priority" pollutants and classes of pollutants of known or suspected toxicity to the biota. Subsequently, Congress passed the Clean Water Act of 1977 mandating the control of toxic pollutants discharged into ambient waters by industry.

In order to facilitate the implementation of the Clean Water Act, EPA selected 129 specific toxic pollutants, 113 organic and 16 inorganic, for initial study. The organic pollutants were divided into 12 categories based on their chemical structure. Analytical methods were

developed for these 12 categories by EPA through in-house and contracted research.

Method 610 was developed in the Battelle-Columbus Laboratories under a contract with the Physical and Chemical Methods Branch, Environmental Monitoring and Support Laboratory—Cincinnati. The interim Method 610 is described in the Federal Register, Vol. 44, No. 233, December 3, 1979. The method requires extraction of the pollutants from the aqueous sample with methylene chloride. The extract is then subjected to silica gel chromatographic cleanup. The PNA fraction is concentrated using Kuderna-Danish evaporation, exchanged to acetonitrile and analyzed by HPLC with UV absorption and fluorescence detection.

## Procedure

The study was patterned after Youden's nonreplicate design for collaborative evaluation of precision and accuracy for analytical methods in which samples are analyzed in pairs, each member of a pair having a slightly different concentration of the constituent of interest. The analyst was directed to do a single analysis and to report one value for each sample, as for a normal routine sample. Samples of three Youden pairs used in this study contained low, medium, and high concentrations of the Category 9 compounds which were spiked into each of six different water types and then analyzed.

Prior to the formal interlaboratory method study, participants were familiarized with both the study design and the procedure by analyzing one trial Youden pair sample followed by attendance at a prestudy conference. After resolving various method interpretations and analytical problems at the prestudy conference, participating laboratories were supplied with the test materials required by the study design and instructed to begin the analyses.

The test waters were:

- a. Distilled water
- b. A municipal drinking water
- c. A surface water, for example, a river, vulnerable to synthetic chemical contamination
- d. Three industrial wastewaters from industries that were potential candidates to be regulated for priority pollutants.

Analyses were conducted on distilled water to evaluate the proficiency of the analyst in using the method on a sample free of interferences. Municipal drinking and surface waters were included as test waters since these water types are subject to contamination. Hence, it was considered important to obtain informa-

tion about the performance of Method 610 in such matrices as well as those found in industrial wastewater effluents.

Statistical analyses of the data were performed using the IMVS computer program developed at Battelle-Columbus Laboratories and which is a revised version of the EPA COLST program. The program is designed to output the raw data in tabular form and to compile summary statistics including:

- Number of data points
- True value
- Mean recovery
- Accuracy as percent relative error
- Overall standard deviation
- Overall percent relative standard deviation
- Single-analyst standard deviation
- Single-analyst percent relative standard deviation.

The overall standard deviations indicate the dispersion expected among values generated from multiple laboratories. The single-analyst standard deviations indicate the dispersion expected among replicate determinations within a single laboratory.

## Results and Discussion

The data collected during this interlaboratory study were analyzed statistically to establish the relationship between precision and mean recovery, and between accuracy and the true concentration. These relationships are summarized by the linear regression equations presented in Table 1.

The results of the regression analyses indicate apparent linear relationships between (1) overall standard deviation and mean recovery; (2) single-analyst precision and mean recovery; and (3) true concentration and mean recovery.

The percent recoveries of the PNA compounds ranged from 43-110 percent. The overall relative standard deviations ranged from 16 to 91 percent and single-analyst relative standard deviations ranged from 11 to 48 percent.

One of the questions of interest in this study was whether water types affected the precision and accuracy of the method. An analysis of variance procedure (ANOVA) was used to test for the effect of water type on precision and accuracy. Based on the results of this analysis, a statistically significant effect due to water type was established for the following PNA's:

acenaphthalene	dibenzo(a,h)anthracene
anthracene	benzo(g,h,i)perylene
benzo(k)fluoranthene	indeno(1,2,3-cd)pyrene

Mean recoveries of these six compounds from distilled water were no better, and in many cases were poorer than for the wastewater samples. One would anticipate that the data would be somewhat better for distilled water, since there is little likelihood of interferences or matrix affects. Since the distilled water data were in all cases collected prior to wastewater data, the analysts were more experienced in utilizing the method when they analyzed the wastewater samples. This may have resulted in a learning curve effect which improved the data for wastewater as compared to distilled water. Therefore, the observed statistical effect was judged to be due to the analytical learning process and was of no practical importance.

For the other 10 PNA's, there were no effects of statistical significance due to water types among mean recoveries, overall precisions or single-analyst precisions.

Several operational problems were reported by the laboratories while conducting Method 610 analyses, the most relevant of which were:

- HPLC column performance was found to be somewhat variable. Some laboratories had to examine two or three commercially available reverse phase columns, HC - ODS Sil-X, 250 mm x 2.6 mm ID, before one was found that would adequately separate benzo(g,h,i)perylene and dibenzo(a,h)anthracene. The other PNA compounds were generally well resolved on this type of column.
- Fluorescence detector response for the various compounds was quite different for the several types of detectors used. Two laboratories used filter type excitation, rather than a grating monochromator, which produces a much higher relative response for anthracene causing it to interfere with fluoranthene. Mercury vapor lamps were found to give a low output at 280 nm, resulting in low response for all compounds. Use of a phosphor coated lamp improved response somewhat. In general, fluorescence detectors employing deuterium lamps and grating monochromators for excitation gave consistent results.

## Conclusions and Recommendations

Generally, use of Method 610 by experienced analysts should enable industries to meet the requirements of the National Pollutant Discharge Elimination System for discharging the subject

pollutants into the environment.

It is recommended that the following precautions be observed by the analyst using Method 610 to help ensure reliability of the resultant data.

- Some of the compounds are light sensitive and thus exposure to light should be kept at a minimum. All sample extracts should be stored in the dark prior to analysis. The elution profile of the column should be checked to ensure that elution of the PNA's in the proper order is occurring.
- The HPLC system performance is important since a large number of compounds must be separated. The equilibration time (at 40% acetonitrile/60% water) between runs should be at least 25 minutes and should be consistent from run to run. Solvents for HPLC must be filtered through a submicron filter and then degassed, either by heating or by a helium purge, to prevent bubble formation.
- The sensitivity of the detectors should be checked daily.

**Table 1.** Regression Equations for Accuracy and Precision of Method 610 by Compound and Water Type

Water Type	Naphthalene	Acenaphthylene	Acenaphthene	Fluorene
Applicable Conc. Range	(10.00 - 375.00)	(10.00 - 425.00)	(10.00 - 260.00)	(10.00 - 463.00)
Distilled Water				
Single-Analyst Precision	SR = 0.39X - 0.18	SR = 0.36X + 0.29	SR = 0.39X + 0.76	SR = 0.44X - 1.12
Overall Precision	S = 0.41X + 0.74	S = 0.42X + 0.52	S = 0.53X + 1.32	S = 0.63X - 0.65
Accuracy	X = 0.57C - 0.70	X = 0.69C - 1.89	X = 0.52C + 0.54	X = 0.56C - 0.52
Tap Water				
Single-Analyst Precision	SR = 0.36X + 0.24	SR = 0.38X - 0.01	SR = 0.29X + 0.27	SR = 0.25X + 1.16
Overall Precision	S = 0.39X + 0.73	S = 0.44X - 0.03	S = 0.47X + 0.45	S = 0.50X - 0.16
Accuracy	X = 0.60C - 0.62	X = 0.71C - 2.58	X = 0.51C - 1.55	X = 0.59C - 1.30
Surface Water				
Single-Analyst Precision	SR = 0.24X + 1.94	SR = 0.27X + 0.30	SR = 0.17X + 1.48	SR = 0.40X - 0.93
Overall Precision	S = 0.41X + 1.07	S = 0.30X + 0.08	S = 0.48X + 0.23	S = 0.52X - 0.74
Accuracy	X = 0.60C - 0.82	X = 0.74C - 2.07	X = 0.53C - 0.59	X = 0.57C - 0.25
Wastewater (C-94)				
Single-Analyst Precision	SR = 0.19X + 1.34	SR = 0.19X + 1.02	SR = 0.35X - 0.79	SR = 0.25X + 1.60
Overall Precision	S = 0.36X + 0.26	S = 0.32X - 0.01	S = 0.50X - 0.21	S = 0.52X - 1.26
Accuracy	X = 0.62C + 0.72	X = 0.83C - 1.16	X = 0.59C - 0.46	X = 0.60C - 0.03
Wastewater (C-95)				
Single-Analyst Precision	SR = 0.23X - 0.48	SR = 0.32X - 0.81	SR = 0.24X + 0.33	SR = 0.21X + 2.56
Overall Precision	S = 0.32X - 1.09	S = 0.36X - 0.13	S = 0.47X + 0.08	S = 0.47X - 0.44
Accuracy	X = 0.58C + 1.04	X = 0.75C - 0.80	X = 0.57C + 0.30	X = 0.53C + 0.73
Wastewater (C-96)				
Single-Analyst Precision	SR = 0.31X + 0.26	SR = 0.17X + 0.57	SR = 0.28X + 0.34	SR = 0.35X + 0.10
Overall Precision	S = 0.41X - 0.15	S = 0.23X + 1.09	S = 0.43X - 0.54	S = 0.49X - 0.39
Accuracy	X = 0.65C - 0.76	X = 0.83C - 1.89	X = 0.62C + 0.12	X = 0.54C + 0.36

X = Mean Recovery

C = True Value for the Concentration

Table 1. (continued)

Water Type	Phenanthrene	Anthracene	Fluoranthene	Pyrene
Applicable Conc. Range	(5.00 - 280.00)	(10.00 - 400.00)	(0.30 - 15.00)	(2.00 - 90.00)
Distilled Water				
Single-Analyst Precision	$SR = 0.28X + 0.05$	$SR = 0.23X + 1.16$	$SR = 0.22X + 0.06$	$SR = 0.25X + 0.14$
Overall Precision	$S = 0.47X - 0.25$	$S = 0.41X + 0.45$	$S = 0.32X + 0.03$	$S = 0.42X - 0.00$
Accuracy	$X = 0.72C - 0.95$	$X = 0.63C - 1.26$	$X = 0.68C + 0.07$	$X = 0.69C - 0.12$
Tap Water				
Single-Analyst Precision	$SR = 0.26X + 0.10$	$SR = 0.22X + 0.61$	$SR = 0.23X + 0.01$	$SR = 0.25X + 0.02$
Overall Precision	$S = 0.35X - 0.16$	$S = 0.41X + 0.10$	$S = 0.32X + 0.01$	$S = 0.39X + 0.09$
Accuracy	$X = 0.71C - 0.71$	$X = 0.63C - 2.05$	$X = 0.71C - 0.03$	$X = 0.68C + 0.09$
Surface Water				
Single-Analyst Precision	$SR = 0.23X - 0.34$	$SR = 0.19X + 0.22$	$SR = 0.27X - 0.04$	$SR = 0.22X - 0.10$
Overall Precision	$S = 0.37X - 0.62$	$S = 0.34X - 0.69$	$S = 0.44X - 0.01$	$S = 0.30X - 0.12$
Accuracy	$X = 0.70C - 0.26$	$X = 0.64C - 0.45$	$X = 0.59C + 0.05$	$X = 0.74C - 0.08$
Wastewater (C-94)				
Single-Analyst Precision	$SR = 0.11X + 0.74$	$SR = 0.19X + 0.99$	$SR = 0.12X + 0.03$	$SR = 0.17X + 0.15$
Overall Precision	$S = 0.26X - 0.22$	$S = 0.39X - 0.41$	$S = 0.35X - 0.01$	$S = 0.26X - 0.02$
Accuracy	$X = 0.79C - 0.61$	$X = 0.69C - 0.26$	$X = 0.75C - 0.00$	$X = 0.71C + 0.02$
Wastewater (C-95)				
Single-Analyst Precision	$SR = 0.15X - 0.03$	$SR = 0.19X + 0.10$	$SR = 0.17X - 0.01$	$SR = 0.27X - 0.04$
Overall Precision	$S = 0.28X - 0.03$	$S = 0.33X - 0.31$	$S = 0.29X + 0.02$	$S = 0.34X - 0.19$
Accuracy	$X = 0.73C - 0.48$	$X = 0.64C - 0.34$	$X = 0.70C + 0.02$	$X = 0.66C + 0.25$
Wastewater (C-96)				
Single-Analyst Precision	$SR = 0.35X - 0.50$	$SR = 0.24X - 0.29$	$SR = 0.40X - 0.06$	$SR = 0.20X - 0.00$
Overall Precision	$S = 0.38X - 0.28$	$S = 0.35X - 0.91$	$S = 0.38X + 0.03$	$S = 0.25X + 0.14$
Accuracy	$X = 0.70C - 0.47$	$X = 0.66C + 0.08$	$X = 0.75C + 0.01$	$X = 0.77C + 0.01$

X = Mean Recovery

C = True Value for the Concentration

Table 1. (Continued)

Water Type	Benzo(a)Anthracene	Chrysene	Benzo(b)Fluoranthene	Benzo(k)Fluoranthene
Applicable Conc. Range	(0.50 - 16.00)	(2.00 - 60.00)	(0.20 - 11.00)	(0.12 - 6.00)
Distilled Water				
Single-Analyst Precision	$SR = 0.28X + 0.04$	$SR = 0.32X - 0.18$	$SR = 0.21X + 0.01$	$SR = 0.44X - 0.01$
Overall Precision	$S = 0.34X + 0.02$	$S = 0.56X - 0.22$	$S = 0.38X - 0.00$	$S = 0.69X + 0.01$
Accuracy	$X = 0.73C + 0.05$	$X = 0.77C - 0.18$	$X = 0.78C + 0.01$	$X = 0.59C + 0.00$
Tap Water				
Single-Analyst Precision	$SR = 0.23X + 0.13$	$SR = 0.40X - 0.37$	$SR = 0.24X - 0.00$	$SR = 0.48X + 0.06$
Overall Precision	$S = 0.37X + 0.05$	$S = 0.55X - 0.10$	$S = 0.32X - 0.01$	$S = 0.91X - 0.01$
Accuracy	$X = 0.77C + 0.05$	$X = 0.82C - 0.09$	$X = 0.83C + 0.00$	$X = 0.98C - 0.03$
Surface Water				
Single-Analyst Precision	$SR = 0.18X - 0.01$	$SR = 0.39X - 0.51$	$SR = 0.26X - 0.01$	$SR = 0.19X + 0.16$
Overall Precision	$S = 0.34X - 0.05$	$S = 0.50X - 0.20$	$S = 0.48X - 0.03$	$S = 0.76X + 0.01$
Accuracy	$X = 0.76C - 0.02$	$X = 0.77C + 0.39$	$X = 0.73C + 0.01$	$X = 1.02C + 0.04$
Wastewater (C-94)				
Single-Analyst Precision	$SR = 0.24X + 0.03$	$SR = 0.29X - 0.06$	$SR = 0.21X - 0.00$	$SR = 0.18X - 0.01$
Overall Precision	$S = 0.32X + 0.06$	$S = 0.44X - 0.09$	$S = 0.39X - 0.02$	$S = 0.47X + 0.01$
Accuracy	$X = 0.73C + 0.12$	$X = 0.97C - 0.28$	$X = 0.80C - 0.01$	$X = 0.61C + 0.03$
Wastewater (C-95)				
Single-Analyst Precision	$SR = 0.28X - 0.04$	$SR = 0.25X + 0.42$	$SR = 0.28X - 0.01$	$SR = 0.46X - 0.07$
Overall Precision	$S = 0.43X - 0.04$	$S = 0.48X + 0.10$	$S = 0.42X - 0.02$	$S = 0.68X - 0.01$
Accuracy	$X = 0.69C + 0.03$	$X = 1.22C - 0.58$	$X = 0.90C - 0.00$	$X = 1.09C + 0.03$
Wastewater (C-96)				
Single-Analyst Precision	$SR = 0.18X + 0.00$	$SR = 0.24X + 0.02$	$SR = 0.26X - 0.01$	$SR = 0.22X - 0.00$
Overall Precision	$S = 0.32X + 0.04$	$S = 0.45X + 0.14$	$S = 0.37X - 0.01$	$S = 0.69X - 0.03$
Accuracy	$X = 0.76C + 0.00$	$X = 1.01C - 0.07$	$X = 0.90C + 0.00$	$X = 0.99C - 0.05$

X = Mean Recovery

C = True Value for the Concentration

**Table 1.** (Continued)

Water Type	Benzo(a)Pyrene	Dibenzo(a,h)Anthracene	Benzo(g,h,i)Perylene	Indeno(1,2,3-cd)Pyrene
Applicable Conc. Range	(0.20 - 15.00)	(0.50 - 24.00)	(1.00 - 50.00)	(0.75 - 22.00)
Distilled Water				
Single-Analyst Precision	SR = 0.38X - 0.01	SR = 0.24X + 0.02	SR = 0.25X + 0.04	SR = 0.29X + 0.02
Overall Precision	S = 0.53X - 0.01	S = 0.45X + 0.03	S = 0.58X + 0.10	S = 0.42X + 0.01
Accuracy	X = 0.56C + 0.01	X = 0.41C + 0.11	X = 0.44C + 0.30	X = 0.54C + 0.06
Tap Water				
Single-Analyst Precision	SR = 0.29X - 0.01	SR = 0.42X - 0.01	SR = 0.24X - 0.06	SR = 0.33X - 0.04
Overall Precision	S = 0.53X - 0.00	S = 0.44X + 0.04	S = 0.29X + 0.00	S = 0.38X + 0.02
Accuracy	X = 0.54C - 0.02	X = 0.68C + 0.09	X = 0.71C - 0.07	X = 0.70C - 0.05
Surface Water				
Single-Analyst Precision	SR = 0.24X - 0.01	SR = 0.34X + 0.04	SR = 0.40X - 0.16	SR = 0.27X - 0.04
Overall Precision	S = 0.47X - 0.00	S = 0.49X - 0.02	S = 0.60X - 0.12	S = 0.42X - 0.06
Accuracy	X = 0.65C + 0.01	X = 0.71C - 0.03	X = 0.67C + 0.05	X = 0.60C + 0.02
Wastewater (C-94)				
Single-Analyst Precision	SR = 0.30X - 0.01	SR = 0.24X + 0.00	SR = 0.25X - 0.04	SR = 0.25X - 0.06
Overall Precision	S = 0.44X - 0.01	S = 0.35X + 0.00	S = 0.36X - 0.08	S = 0.42X - 0.04
Accuracy	X = 0.67C + 0.02	X = 0.71C - 0.05	X = 0.72C - 0.05	X = 0.67C + 0.01
Wastewater (C-95)				
Single-Analyst Precision	SR = 0.31X + 0.01	SR = 0.25X + 0.12	SR = 0.27X + 0.01	SR = 0.39X - 0.01
Overall Precision	S = 0.40X - 0.00	S = 0.39X - 0.00	S = 0.48X - 0.17	S = 0.50X + 0.04
Accuracy	X = 0.72C - 0.01	X = 0.77C + 0.02	X = 0.71C + 0.14	X = 0.95C - 0.05
Wastewater (C-96)				
Single-Analyst Precision	SR = 0.20X - 0.00	SR = 0.36X - 0.07	SR = 0.34X - 0.17	SR = 0.37X - 0.07
Overall Precision	S = 0.41X - 0.02	S = 0.45X + 0.08	S = 0.42X - 0.04	S = 0.44X - 0.05
Accuracy	X = 0.70C + 0.01	X = 0.71C + 0.16	X = 0.69C + 0.20	X = 0.83C - 0.11

X = Mean Recovery

C = True Value for the Concentration

Glenn Kinzer, Ralph Riggin, Thomas Bishop, Michelle A. Birts, and Paul Strup are with Battelle-Columbus Laboratories, Columbus, OH 43201.

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The complete report, entitled "EPA Method Study 20, Method 610—PNA's," (Order No. PB 84-211 614; Cost: \$14.50, subject to change) will be available only from:

National Technical Information Service  
5285 Port Royal Road  
Springfield, VA 22161  
Telephone: 703-487-4650

The EPA Project Officers can be contacted at:  
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