PETROLEUM COKE:
A 96-HOUR STATIC-RENEWAL ACUTE TOXICITY TEST WITH THE FATHEAD MINNOW (*Pimephales promelas*)

AMENDED FINAL REPORT

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 472A-113

OECD GUIDELINE 203
and
U.S. EPA OPPTS NUMBER 850.1075

AUTHORS:

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STUDY INITIATION DATE: April 22, 2004

STUDY COMPLETION DATE: June 22, 2006

AMENDED REPORT DATE: April 10, 2007

SUBMITTED TO:

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: American Petroleum Institute

TITLE: Petroleum Coke: A 96-Hour Static-Renewal Acute Toxicity Test with the Fathead Minnow (*Pimephales promelas*)

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This study was conducted in compliance with OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17) (1); and TSCA Good Laboratory Practice Standards (40 CFR Part 792) (2), with the following exceptions:

The characterization of the test and reference substances, and the stability of the substances under conditions of storage at the test site, were not determined in compliance with Good Laboratory Practice Standards.

Periodic analyses of well water for potential contaminants were performed using a certified laboratory and standard U.S. EPA analytical methods, but not under Good Laboratory Practice Standards.

STUDY DIRECTOR:

[redacted]

Wildlife International, Ltd.

10 April 2007
Date

SPONSOR:
American Petroleum Institute, by:

[redacted]

4/26/2007
Date

QUALITY ASSURANCE STATEMENT

This study was examined for compliance with OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98)17) (1); and TSCA Good Laboratory Practice Standards (40 CFR Part 792) (2). The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

		DATE REPORTED TO:	
ACTIVITY:	DATE CONDUCTED:	STUDY DIRECTOR:	MANAGEMENT:
Protocol	April 23, 2004	April 23, 2004	May 3, 2004
Test Substance Preparation	March 24, 2005	March 24, 2005	March 29, 2005
Analytical Sampling, Observations and Temperature Measurements	April 1, 2005	April 1, 2005	April 4, 2005
Matrix Fortification	April 1, 2005	April 1, 2005	April 5, 2005
Analytical Data and Draft Report	June 21, 2005	June 21, 2005	June 22, 2005
Biological Data and Draft Report	June 21 – 22, 2005	June 22, 2005	June 23, 2005
Final Report	June 19, 2006	June 19, 2006	June 22, 2006
Amended Report	April 9, 2007	April 9, 2007	April 9, 2007

All inspections were study-based unless otherwise noted.

[redacted]

4/10/2007
Date

AMENDED REPORT APPROVAL

SPONSOR: American Petroleum Institute

TITLE: Petroleum Coke: A 96-Hour Static-Renewal Acute Toxicity Test with the Fathead Minnow (*Pimephales promelas*)

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 472A-113

This report was reviewed by the individuals involved in the conduct and management of the study, and was found to be an accurate reflection of the methods used, data collected and results of the study.

STUDY DIRECTOR:

[redacted]

10 April 2007
Date

PRINCIPAL INVESTIGATORS:

[redacted]

4-10-07
Date

April 10, 2007
Date

WILDLIFE INTERNATIONAL, LTD. MANAGEMENT:

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Date

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SUMMARY

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TITLE: Petroleum Coke: A 96-Hour Static-Renewal Acute Toxicity Test with the Fathead Minnow (*Pimephales promelas*)

WILDLIFE INTERNATIONAL, LTD. PROJECT NUMBER: 472A-113

TEST SUBSTANCE: Petroleum Coke

GUIDELINES: OECD Guideline for Testing of Chemicals, 203: *Fish, Acute Toxicity Test*; U.S. EPA Series 850 – Ecological Effects Test Guidelines OPPTS Number 850.1075: *Fish Acute Toxicity Test, Freshwater and Marine*

TEST DATES:	Study Initiation:	April 22, 2004
	Experimental Start (OECD):	March 24, 2005
	Experimental Start (EPA):	March 28, 2005
	Biological Termination:	April 1, 2005
	Experimental Termination:	April 2, 2005

LENGTH OF EXPOSURE: 96 Hours

TEST ORGANISMS: Fathead Minnow (*Pimephales promelas*)

SOURCE OF TEST ORGANISMS: Wildlife International, Ltd. Culture
Easton, Maryland 21601

AGE OF TEST ORGANISMS: Juveniles

MEASUREMENTS OF 10 NEGATIVE CONTROL FISH:	Mean Wet Weight:	0.20 g	Range: 0.14 – 0.30 g
	Mean Total Length:	2.9 cm	Range: 2.6 – 3.2 cm

TEST CONCENTRATIONS:

Nominal WAF Loading Rate
Negative Control
1000 mg/L

RESULTS: Based on nominal WAF loading rate:

LL50:	>1000 mg/L
No-Mortality Level:	1000 mg/L
NOELR:	1000 mg/L

INTRODUCTION

Wildlife International, Ltd. conducted a 96-hour static-renewal acute toxicity test to determine the effects of a water accommodated fraction of petroleum coke on the fathead minnow, *Pimephales promelas*, for the American Petroleum Institute at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland. Petroleum coke is defined as the product formed by subjecting the heavy tar-like residue remaining following oil refining to high temperatures and pressures. It consists of primarily elemental carbon with considerably smaller amounts of hydrocarbons, sulfur and trace amounts of heavy metals. The in-life phase of the definitive test was conducted from March 28, 2005 to April 1, 2005. Raw data generated by Wildlife International, Ltd. and a copy of the final report are filed under Project Number 472A-113 in archives located on the Wildlife International, Ltd. site.

OBJECTIVE

The objective of this study was to determine the acute effects of a water accommodated fraction of petroleum coke on the fathead minnow (*Pimephales promelas*) during a 96-hour exposure period under static-renewal conditions in a sealed exposure system.

EXPERIMENTAL DESIGN

Fathead minnows were exposed to a single water accommodated fraction (WAF) of the test substance and a negative (dilution water) control for 96 hours. The nominal WAF loading rate was 1000 mg/L. For this test, the term loading rate means the total amount of test substance added to the dilution water volume (mg/L) to achieve the respective WAF solution. Because petroleum coke is a multi-component substance not fully soluble in water, WAFs are an acceptable means of creating exposure solutions for ecotoxicity tests (3). The WAF loading rate was selected in consultation with the Sponsor, and was based upon the results of an exploratory range finding toxicity test (Appendix 1). Three replicate test chambers were maintained in each treatment and control group, with 10 fish in each test chamber, for a total of 30 fathead minnows per test concentration. The fish were transferred to newly prepared WAF solutions and control water at approximately 24-hour intervals. Water samples were collected at test initiation, prior to renewal of solutions at 24 hours, and at test termination for analysis for selected constituents of petroleum coke. Because petroleum coke is a complex mixture of elemental carbon and low levels of hydrocarbons and metals, several polyaromatic hydrocarbons and metals were selected to be monitored in the test solutions during the test. The components selected for measurement were those that are either of ecological concern or were known to occur in petroleum coke in amounts that might be measured in a WAF solution. Those constituents of interest included the following:

PAH	Metals and Sulfur
Acenaphthene	Nickel
Acenaphthylene	Vanadium
Anthracene	Iron
Benzo(a)anthracene	Copper
Benzo(a)pyrene	Selenium
Benzo(b)fluoranthene	Arsenic
Benzo(g,h,i)perylene	Sulfur
Benzo(k)fluoranthene	
Chrysene	
Dibenzo(a,e)pyrene	
Dibenz(a,h)anthracene	
Fluoranthene	
Fluorene	
Indeno(1,2,3-cd)pyrene	
Naphthalene	
Phenanthrene	
Pyrene	
1-Methylnaphthalene	
2-Methylnaphthalene	

Fathead minnows were impartially assigned to exposure chambers at test initiation. Observations of mortality and other clinical signs were made approximately 3, 24, 48, 72 and 96 hours after test initiation. The cumulative percent mortality observed in the treatment group was used to determine whether the 24, 48, 72 and 96-hour LL50 values were greater or less than the 1000 mg/L WAF loading rate. The no-mortality level and the no-observed-effect-loading rate (NOELR) were determined by visually interpreting the mortality and observation data.

MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, "Petroleum Coke: A 96-Hour Static-Renewal Acute Toxicity Test with the Fathead Minnow (*Pimephales promelas*)" (Appendix 2). The protocol was based on procedures outlined in the OECD Guideline for

Testing of Chemicals, 203: *Fish, Acute Toxicity Test* (4); and U.S. EPA Series 850 – Ecological Effects Test Guidelines OPPTS Number 850.1075: *Fish Acute Toxicity Test, Freshwater and Marine* (5).

Test Substance

The test substance was green petroleum coke (CAS Number 64741-79-3). The test substance was received from Experimental Pathology Laboratories, Herndon, VA for API on October 7, 2003. It was assigned Wildlife International, Ltd. identification number 6485 upon receipt and was stored under ambient conditions. The test substance, black pellets, was identified as 2 mm particle size Petroleum Coke (aka Milled Pellets).

The identity, strength, purity, composition (Appendix 4), storage stability, and method of synthesis, fabrication and/or derivation (Appendix 3) of each batch of the test substance and the maintenance of these records were the responsibility of the Sponsor.

Reference Substances

Purified polyaromatic hydrocarbons (PAH) were made up of components received from three manufacturers. The following reference standards were received from AccuStandard Inc. and were stored under ambient conditions:

Component	Test Substance Number	Lot/Batch	CAS Number	Date Received	Expiration Date	Description
Benzo(a)pyrene	6705	052803MT-AC	50-32-8	6/07/04	6/03/07	green powder
Anthracene	6706	A33783	120-12-7	6/07/04	6/03/07	white powder
Benz(a)anthracene	6707	19587	56-55-3	6/07/04	6/03/07	colorless plates
Acenaphthylene	6708	011504MS-AC	208-96-8	6/07/04	6/03/07	yellow powder
Acenaphthene	6709	01915EQ	83-32-9	6/07/04	6/03/07	white crystal
Benzo(b)fluoranthene	6710	020402AG-AC	205-99-2	6/07/04	6/03/07	white flakes
Benzo(g,h,i)perylene	6711	122 500MT-AC	191-24-2	6/07/04	6/03/07	green powder
Benzo(k)fluoranthene	6712	112603AG-AC	207-08-9	6/08/04	6/03/07	yellow powder
Chrysene	6713	13103	218-01-9	6/08/04	6/03/07	white powder
Dibenz(a,h)anthracene	6714	13246	53-70-3	6/08/04	6/03/07	green powder
Fluoranthene	6715	19762	206-44-0	6/08/04	6/03/07	white powder
Fluorene	6716	19675	86-73-7	6/08/04	6/03/07	white powder
Indeno(1,2,3-cd)pyrene	6717	19641	193-39-5	6/08/04	6/03/07	yellow powder
Naphthalene	6718	167A-A	91-20-3	6/08/04	6/03/07	white flakes
Phenanthrene	6719	090903AG-AC-1	85-01-8	6/08/04	6/03/07	white powder
Pyrene	6720	09617LR	129-00-0	6/08/04	6/03/07	green crystal

The following reference standard was received from Cambridge-Isotope Labs and was stored under ambient conditions:

Component	Test Substance Number	Lot/Batch	CAS Number	Date Received	Expiration Date	Description
Dibenzo(a,e)pyrene	6518	I1-7628	192-65-4	10/22/03	Not given	Solids

The following reference standards were received from ChemService and were stored under ambient conditions:

Component	Test Substance Number	Lot/Batch	CAS Number	Date Received	Expiration Date	Description
2-Methylnaphthalene	6765	310-43C	91-57-6	8/03/04	9/01/08	Solid
1-Methylnaphthalene	6766	325-31A	90-12-0	8/03/04	5/01/09	Liquid

Analytical standards for each of the seven metal and sulfur elements of interest were received from Spex Industries (Metuchen, N.J. 08840) and were stored under ambient conditions. All of the materials were 1,000 mg/L Spex CertiPrep® plasma standards in 2% HNO_3, with the exception of the sulfur standard which was a 10,000 mg/L preparation in water. The following tabulation summarizes pertinent data for each analytical standard:

Component	Test Substance Number	Lot/Batch	CAS Number	Date Received	Expiration Date	Description
Arsenic (As)	6543	10-06AS	7440-38-2	11/06/03	11/15/05	Clear liquid
Copper (Cu)	6544	9-183CU	7440-50-8	11/06/03	11/15/05	Blue liquid
Iron (Fe)	6545	9-184FE	7439-89-6	11/06/03	11/15/05	Clear Liquid
Nickel (Ni)	6546	10-29NI	7440-02-0	11/06/03	11/15/05	Blue liquid
Selenium (Se)	6547	10-31SE	7782-49-2	11/06/03	11/15/05	Clear Liquid
Sulfur (S)	6890	S9-51S	7704-34-9	10/18/04	10/15/05	Liquid
Vanadium (V)	6549	10-88V	7440-62-2	11/06/03	11/15/05	Yellow Liquid

Test Organism

The fathead minnow, *Pimephales promelas*, was selected as the test species for this study. Fathead minnows are representative of an important group of aquatic vertebrates, and were selected for use in the test based upon past history of use in the laboratory. Fathead minnows used in the test were obtained from cultures maintained by Wildlife International, Ltd., Easton, Maryland.

Identification of the species was verified by the supplier of the original culture (Osage Catfisheries, Inc., Osage Beach, Missouri).

All fish used in the test were from the same source and year class, and the length of the longest fish measured was no more than twice the length of the shortest. The average total length of 10 negative control fish measured at the end of the test was 2.9 cm, with a range of 2.6 to 3.2 cm. The average wet weight (blotted dry) of 10 negative control fish measured at the end of the test was 0.20 grams, with a range of 0.14 to 0.30 grams. Loading was defined as the total wet weight of fish per liter of test water and was 0.50 g fish/L.

The fish were held for at least 14 days prior to the test in water from the same source and at approximately the same temperature as used during the test. During the 14-day holding period preceding the test, water temperatures ranged from 23.3 to 23.7ºC, measured with a hand-held liquid-in-glass thermometer. The pH of the water ranged from 8.1 to 8.4, measured with a Fisher Scientific Accumet Model 915 pH meter. Dissolved oxygen ranged from 7.4 to 8.0 mg/L (≥84% of saturation), measured with a Yellow Springs Instruments Model 51B dissolved oxygen meter. During the holding period, the fish were fed daily a commercially-prepared diet supplied by Zeigler Brothers, Inc., Gardners, Pennsylvania. The fish were not fed for at least two days prior to the test or during the test.

During the 14-day period prior to the test the fish showed no signs of disease or stress. At test initiation, the fathead minnows were collected from the culture tank and impartially distributed one and two at a time to the test chambers until each contained 10 fish.

Dilution Water

The water used for culturing and testing was freshwater obtained from a well approximately 40 meters deep located on the Wildlife International, Ltd. site. The well water is characterized as moderately-hard water. The specific conductance, hardness, alkalinity and pH of the well water during the four-week period immediately preceding the test are presented in Appendix 6.

The well water was passed through a sand filter to remove particles greater than approximately 25 µm, and pumped into a 37,800-L storage tank where the water was aerated with spray nozzles. Prior to use, the water again was filtered (0.45 µm) to remove fine particles. The results of periodic analyses performed to measure the concentrations of selected organic and inorganic constituents in the well water are presented in Appendix 7.

Test Apparatus

Test chambers consisted of 1 gallon (3.8 L) glass jars with Teflon®-lined lids. Each test chamber was completely filled with test solution to minimize headspace. The depth of test solution in a representative test chamber was 25.4 cm. Each test chamber was labeled with the project number, WAF loading rate and replicate. Test chambers were impartially positioned in a temperature-controlled environmental chamber set to maintain the desired test temperature throughout the test period.

Preparation of Test Concentrations

The test and control solutions were prepared prior to test initiation on Day 0. New solutions were prepared daily in separate test chambers, and all organisms were transferred from old to new solutions at 24-hour each renewal period. The test solution was prepared in the form of a water accommodated fraction (WAF) at a nominal loading rate of 1000 mg/L. The WAF was prepared by mixing the test substance into 12 L of dilution water (well water) in each of two 13.2 L Pyrex® aspirator bottles. The solutions were stirred with Teflon®-coated stir bars on magnetic stir plates for approximately 24 hours. Care was taken to maintain a vortex depth of approximately 30% of the test solution height. The duration of the mixing period was established during an exploratory WAF equilibration test (Appendix 8). Following the mixing period, the WAF solutions were allowed to settle for approximately one hour before the solutions were decanted into the test chambers through a spigot and tubing placed approximately 2-3 cm from the bottom of the aspirator bottle. Approximately 2 L of solution from each WAF bottle was combined to fill each test chamber.

Analytical Sampling

Samples were collected from each control and treatment group at the beginning of the test, prior to renewal of solutions at approximately 24 hours, and at test termination to measure concentrations of soluble components of the test substance. Samples were decanted from the WAF preparation vessel at the beginning of the test, and were pooled from solution collected at mid-depth from each replicate test chamber prior to the 24-hour renewal and at test termination. At each sampling interval, one set of samples was collected for analysis of selected organic compounds, and a second set of samples was collected for analysis of selected inorganic elements. All samples were stored until analyzed at the end of the test. Samples collected for organic analyses were preserved by refrigerated storage with zero headspace. Samples collected for inorganic analyses were preserved by the addition of sufficient nitric acid (HNO_3) to achieve a final acid concentration of 2%.

Analytical Method by HPLC

The method used for the analysis of the test samples for organic compounds was based upon methodology developed by Wildlife International, Ltd. (6). The analytical method consisted of diluting the samples in freshwater, as necessary, and analyzing by direct injection high performance liquid chromatography (HPLC) with either UV detection at 220 nm or fluorescence detection at 340 nm to 425 nm.

Concentrations of each PAH compound in the fortified samples were determined using an Agilent Model 1100 High Performance Liquid Chromatograph, equipped with either an Agilent Series 1100 Variable Wavelength Detector or a Jasco Model FP-1520 Fluorescence Detector. Chromatographic separations were achieved using a YMC Pack ODS-AM column (150 mm x 4.6 mm, 3 µm particle size). A method flow chart is provided in Appendix 9.1 and instrumental parameters for the analysis of PAH components are summarized in Appendix 9.2.

Five calibration standards of PAH, ranging in concentration from 5.00 to 50.0 µg/L, were prepared prior to the test using a stock solution of PAH analytical standards in methanol (Appendix 9.3). The calibration standards were analyzed with each sample set. Linear regression equations were generated using the peak area responses versus the respective concentrations of the calibration standards. The concentration of PAH in the samples was determined by substituting the peak area responses of the samples into the applicable linear regression equation.

The method limit of quantitation (LOQ) was defined as 5.00 µg/L, calculated as the product of the concentration of the lowest calibration standard (5.00 µg/L) and the dilution factor of the matrix blank samples (1.00). One matrix blank sample was analyzed with each sample set to determine possible interferences. No interferences were observed at or above the LOQ during the sample analyses.

Samples of freshwater were fortified at 10.0, 40.0 and 100 µg/L using a stock solution of PAH analytical standard in methanol (Appendix 9.3), and were analyzed concurrently with the test samples. The measured concentrations for the matrix fortification samples ranged from 92.9 to 105% of fortified concentrations (Tables 1 through 19).

Representative calibration curves are presented in Appendices 9.4 through 9.22. Representative chromatograms of low and high-level calibration standards are presented in Appendices 9.23 and 9.24, respectively. Representative chromatograms of a freshwater matrix blank sample and a matrix fortification sample are presented in Appendices 9.25 and 9.26, respectively. A representative chromatogram of a test sample is presented in Appendix 9.27.

Analytical Method by ICP-AES

The analytical method used for the analysis of the inorganic elements, As, Se, Fe, Ni, Se, V and S, in the test samples was based upon methodology developed by Wildlife International, Ltd. (7). The analytical method consisted of acidifying the samples 2% by volume with concentrated nitric acid and direct injection into the ICP-AES system. Concentrations of As, Cu, Fe, Ni, Se, S and V in the samples were determined using a Perkin-Elmer Optima 3000 DV ICP-AES configured in axial view mode and equipped with a Cetac U-5000AT$^+$ Ultrasonic Nebulizer (sample introduction). Simultaneous measurements were made for six of the seven elements (As, Cu, Fe, Ni, Se and V). For sulfur, a single element method was employed due to the need for higher concentration-level calibration standards. A method flowchart is provided in Appendix 10.1 and instrumental parameters for the analysis of the seven elements are summarized in Appendix 10.2.

Multi-element calibration standards were analyzed with the test samples. Preparations of stock and calibration standard solutions are detailed in Appendix 10.3. The calibration standard series was injected at the beginning and end of each analytical run. In addition, a standard was injected following a maximum of five sample analyses. For a given injection of a sample (including standards), the ICP-AES instrument integrated the steady-state emission signal at designated emission wavelengths for a method-specified period (read time). The net integrated intensity was then automatically corrected by subtraction of the mean corrected intensity of the calibration blank (determined at sequence initiation). The measurement cycle was automatically repeated two additional times during the sample injection (read replicates). The mean of the three measurements produced a mean corrected intensity for each monitored element in the sample. Linear regression equations for each monitored element were generated using mean corrected intensities versus the respective concentrations of the element in the calibration standards. Representative calibration curves for As, Cu, Fe, Ni, Se, S and V are presented in Appendices 10.4 – 10.10. The concentrations of each of the seven elements in the test samples were calculated by substituting their mean corrected intensities into the applicable linear regression equation, and applying the appropriate dilution and unit conversion factors. Representative emission spectra of low- and high-level calibration standards are presented in Appendices 10.11 – 10.13. An example calculation for a study sample is provided in Appendix 10.14.

A matrix blank was analyzed for each component concurrent with the study samples to determine possible interferences. No interferences were observed at or above the limit of quantitation (LOQ) during the sample analyses (Appendices 10.15 – 10.17). A sample of freshwater was fortified at 2X (S) or 2.5X (As, Cu, Fe, Ni, Se and V) the method LOQ for each element and analyzed for each component concurrent with the study samples using the combined stock and the sulfur reference standard. Results are presented in Tables 20 through 26. Emission spectra of freshwater matrix blank and matrix fortification samples are presented in Appendices 10.15 – 10.17. The measured concentrations for the matrix fortification samples ranged from 95.3 to 108% of fortified concentrations (Tables 20 through 26). Representative emission spectra of a WAF test sample are presented in Appendices 10.18 – 10.20.

Environmental Conditions

Ambient room light was used to illuminate the test systems. Fluorescent light bulbs that emit wavelengths similar to natural sunlight (Colortone® 50) were controlled with an automatic timer to provide a photoperiod of 16 hours of light and 8 hours of darkness. A 30-minute transition period of low light intensity was provided at the beginning and end of the 16-hour light period to avoid sudden changes in light intensity. Light intensity at test initiation was 299 lux over the water surface of one control replicate.

The target test temperature during the study was 22 ± 2ºC. Temperature was monitored continuously during the entire test in a container of water placed adjacent to the test chambers using a Fulscope ER/C Recorder. The recorder measurements were verified prior to test initiation with a liquid-in-glass thermometer. Temperature, dissolved oxygen and pH were measured in the newly prepared WAF solutions at the beginning of the test and at each 24-hour renewal, and in each test chamber prior to each renewal and at test termination. Hardness, alkalinity, specific conductance and total organic carbon (TOC) were measured in a sample of dilution water collected at test initiation.

Light intensity was measured using a SPER Scientific Ltd. Model 840006C light meter. Manual temperature measurements were made using a liquid-in-glass thermometer. Measurements of pH were made using a Thermo Orion Model 525Aplus pH meter, and dissolved oxygen was measured using a Thermo Orion Model 850Aplus dissolved oxygen meter. Specific conductance was measured using a Yellow Springs Instrument Model 33 Salinity-Conductivity-Temperature meter. Hardness and alkalinity measurements were made by titration based on procedures in *Standard Methods for the Examination of Water and Wastewater* (8). TOC was measured using a SHIMADZU Model TOC-5000 total organic carbon analyzer.

Observations

Observations were made periodically to determine the number of mortalities. The numbers of individuals exhibiting signs of toxicity or abnormal behavior also were evaluated. Observations were made approximately 3, 24, 48, 72 and 96 hours after test initiation.

Conditions for the Validity of the Test

The following criteria used to judge the validity of the test were met, except as noted:

1. mortality of the fish in the negative control group did not exceed 10% by the end of the test, and
2. the dissolved oxygen concentration was at least 60% of the air-saturation value throughout the test in the newly prepared test solutions, but decreased to a minimum of 35% of saturation in the 24-hour old test solutions. However, this had no adverse effect on the test organisms.

Data Analyses

The cumulative percent mortality observed in the treatment group was used to determine whether the 24, 48, 72 and 96-hour LL50 values were greater or less than the 1000 mg/L WAF loading rate. The no-mortality level and the no-observed-effect-loading rate (NOELR) were determined by visually interpreting the mortality and observation data.

RESULTS AND DISCUSSION

Measurement of Test Concentrations

Results of analyses to measure polyaromatic hydrocarbons and metals in the WAF and control solutions are presented in Tables 1 – 26 and in the analytical chemistry reports (Appendices 8 and 9). All measurements of PAHs and metals in control and WAF solutions were below detection limits for the methods. Therefore, estimates of the LL50, no-mortality and NOELR values are based on the nominal loading rate of 1000 mg/L used in the test.

Observations and Measurements

In the test chambers, the negative control solutions appeared clear and colorless. The 1000 mg/L solutions appeared clear and colorless at test initiation, and clear and colorless with test material visible on the bottom of the test chambers at test termination.

Measurements of temperature, dissolved oxygen and pH of the test solutions are presented in Table 27. Water temperatures were within the 22 ± 2°C range established for the test. Measurements of water pH ranged from 8.1 to 8.7. Dissolved oxygen concentrations were ≥8.5 mg/L (≥98% of saturation) at the beginning of each renewal period. By the end of each 24-hour period, dissolved oxygen concentrations in the test chambers decreased to a minimum of 3.0 mg/L (35% of saturation). Based on the survival and observation data, the low dissolved oxygen concentrations had no adverse effect on the test organisms. Measurements of hardness, alkalinity, specific conductance and TOC in the dilution water at test initiation were typical of Wildlife International, Ltd. well water (Table 28).

Daily observations of mortality and other clinical signs of toxicity observed during the test are presented in Table 29. No mortalities occurred during the test. All fathead minnows in the negative control group and in the 1000 mg/L treatment group appeared normal throughout the exposure period. The 24, 48, 72 and 96-hour LL50 values were estimated to be greater than the single WAF loading rate of 1000 mg/L (Table 30). The no-mortality level and the NOELR were both 1000 mg/L.

CONCLUSIONS

Fathead minnows (*Pimephales promelas*) were exposed under static-renewal conditions for 96 hours to a single water accommodated fraction (WAF) of petroleum coke at a nominal WAF loading rate of 1000 mg/L in a sealed exposure system. The 96-hour LL50 value was determined to be >1000 mg/L, the single WAF loading rate tested. The no-mortality level and the NOELR were both 1000 mg/L.

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1 **OECD.** 1998. *OECD Principles of Good Laboratory Practice* ENV/MC/CHEM (98) 17.

2 **Title 40 of the Code of Federal Regulations, Part 792**. 1989. *Toxic Substances Control Act (TSCA) Good Laboratory Practice Standards*.

3 **Organization for Economic Cooperation and Development.** 2000. *Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures.* ENV/JM/MONO (2000) 6. OECD Environmental Health and Safety Publication Series on Testing and Assessment No. 23. OECD, Paris, September 2000.

4 **Organisation for Economic Cooperation and Development**. 1992. *Fish, Acute Toxicity Test*. OECD Guideline for Testing of Chemicals. Guideline 203. Paris.

5 **U.S. Environmental Protection Agency.** 1996. Series 850 – Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1075: *Fish Acute Toxicity Test, Freshwater and Marine*.

6 **Timothy Z. Kendall and Willard B. Nixon.** 2005. Analytical Method Verification for the Determination of Water Soluble Components of Petroleum Coke in Freshwater Using Gas Chromatography (GC) or High Performance Liquid Chromatography (HPLC). Wildlife International, Ltd. Project Number 472C-104. Unpublished.

7 **Raymond L. Van Hoven and Willard B. Nixon.** 2005. Analytical Method Verification for the Determination of Water Soluble Components of Petroleum Coke in Freshwater Using Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). Wildlife International, Ltd. Project Number 472C-105. Unpublished.

8 **APHA, AWWA, WPCF.** 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th Edition.

Table 1

Measured Concentrations of Naphthalene Analyzed by HPLC/UV

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (μg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (μg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	9.97	99.7
40.0	MAS-2	--	40.6	102
100	MAS-3	--	93.3	93.3
			Mean =	98.3
			Standard Deviation =	4.51
			CV =	4.59%

[1] The limit of quantitation (LOQ) was 5.00 μg/L, calculated as the product of the lowest standard concentration (5.00 μg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 2

Measured Concentrations of Acenaphthylene Analyzed by HPLC/UV

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (µg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (µg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	10.1	101
40.0	MAS-2	--	40.2	100
100	MAS-3	--	98.1	98.1
			Mean =	99.7
			Standard Deviation =	1.47
			CV =	1.48%

[1] The limit of quantitation (LOQ) was 5.00 µg/L, calculated as the product of the lowest standard concentration (5.00 µg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 3

Measured Concentrations of 1-Methylnaphthalene Analyzed by HPLC/UV

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (µg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (µg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	9.93	99.3
40.0	MAS-2	--	40.7	102
100	MAS-3	--	93.9	93.9
			Mean =	98.4
			Standard Deviation =	4.12
			CV =	4.19%

[1] The limit of quantitation (LOQ) was 5.00 µg/L, calculated as the product of the lowest standard concentration (5.00 µg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 4

Measured Concentrations of 2-Methylnaphthalene Analyzed by HPLC/UV

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (μg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (μg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	9.92	99.2
40.0	MAS-2	--	41.0	103
100	MAS-3	--	92.9	92.9
			Mean =	98.4
			Standard Deviation =	5.10
			CV =	5.18%

[1] The limit of quantitation (LOQ) was 5.00 μg/L, calculated as the product of the lowest standard concentration (5.00 μg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 5

Measured Concentrations of Fluorene Analyzed by HPLC/UV

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (μg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (μg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	10.5	105
40.0	MAS-2	--	40.8	102
100	MAS-3	--	98.5	98.5
			Mean =	102
			Standard Deviation =	3.25
			CV =	3.19%

[1] The limit of quantitation (LOQ) was 5.00 μg/L, calculated as the product of the lowest standard concentration (5.00 μg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 6

Measured Concentrations of Acenaphthene Analyzed by HPLC/UV

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (μg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (μg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	9.82	98.2
40.0	MAS-2	--	40.3	101
100	MAS-3	--	97.7	97.7
			Mean =	99.0
			Standard Deviation =	1.78
			CV =	1.80%

[1] The limit of quantitation (LOQ) was 5.00 μg/L, calculated as the product of the lowest standard concentration (5.00 μg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 7

Measured Concentrations of Phenanthrene Analyzed by HPLC/UV

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (μg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (μg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	10.0	100
40.0	MAS-2	--	40.3	101
100	MAS-3	--	98.5	98.5
			Mean =	99.8
			Standard Deviation =	1.26
			CV =	1.26%

[1] The limit of quantitation (LOQ) was 5.00 μg/L, calculated as the product of the lowest standard concentration (5.00 μg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 8

Measured Concentrations of Anthracene Analyzed by HPLC with Fluorescence Detection

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (μg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (μg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	9.95	99.5
40.0	MAS-2	--	40.6	101
100	MAS-3	--	97.7	97.7
			Mean =	99.4
			Standard Deviation =	1.65
			CV =	1.66%

[1] The limit of quantitation (LOQ) was 5.00 μg/L, calculated as the product of the lowest standard concentration (5.00 μg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 9

Measured Concentrations of Fluoranthene Analyzed by HPLC with Fluorescence Detection

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (μg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (μg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	10.0	100
40.0	MAS-2	--	40.6	101
100	MAS-3	--	96.2	96.2
			Mean =	99.1
			Standard Deviation =	2.53
			CV =	2.56%

[1] The limit of quantitation (LOQ) was 5.00 μg/L, calculated as the product of the lowest standard concentration (5.00 μg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 10

Measured Concentrations of Pyrene Analyzed by HPLC with Fluorescence Detection

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (μg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (μg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	9.93	99.3
40.0	MAS-2	--	40.4	101
100	MAS-3	--	94.7	94.7
			Mean =	98.3
			Standard Deviation =	3.26
			CV =	3.32%

[1] The limit of quantitation (LOQ) was 5.00 μg/L, calculated as the product of the lowest standard concentration (5.00 μg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 11

Measured Concentrations of Chrysene Analyzed by HPLC/UV

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (μg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (μg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	9.80	98.0
40.0	MAS-2	--	40.2	100
100	MAS-3	--	104	104
			Mean =	101
			Standard Deviation =	3.06
			CV =	3.02%

[1] The limit of quantitation (LOQ) was 5.00 μg/L, calculated as the product of the lowest standard concentration (5.00 μg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 12

Measured Concentrations of Benzo(a)anthracene Analyzed by HPLC
with Fluorescence Detection

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (μg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (μg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	10.1	101
40.0	MAS-2	--	40.7	102
100	MAS-3	--	98.8	98.8
			Mean =	101
			Standard Deviation =	1.64
			CV =	1.62%

[1] The limit of quantitation (LOQ) was 5.00 μg/L, calculated as the product of the lowest standard concentration (5.00 μg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 13

Measured Concentrations of Benzo(b)fluoranthene Analyzed by HPLC
with Fluorescence Detection

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (µg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (µg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	10.1	101
40.0	MAS-2	--	41.1	103
100	MAS-3	--	100	100
			Mean =	101
			Standard Deviation =	1.53
			CV =	1.51%

[1] The limit of quantitation (LOQ) was 5.00 µg/L, calculated as the product of the lowest standard concentration (5.00 µg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 14

Measured Concentrations of Benzo(k)fluoranthene Analyzed by HPLC
with Fluorescence Detection

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (µg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (µg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	9.79	97.9
40.0	MAS-2	--	40.1	100
100	MAS-3	--	103	103
			Mean =	100
			Standard Deviation =	2.56
			CV =	2.56%

[1] The limit of quantitation (LOQ) was 5.00 µg/L, calculated as the product of the lowest standard concentration (5.00 µg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 15

Measured Concentrations of Benzo(a)pyrene Analyzed by HPLC
with Fluorescence Detection

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (μg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (μg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	9.87	98.7
40.0	MAS-2	--	40.2	100
100	MAS-3	--	102	102
			Mean =	100
			Standard Deviation =	1.66
			CV =	1.66%

[1] The limit of quantitation (LOQ) was 5.00 μg/L, calculated as the product of the lowest standard concentration (5.00 μg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 16

Measured Concentrations of Dibenz(a,h)anthracene Analyzed by HPLC
with Fluorescence Detection

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (μg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (μg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	9.77	97.7
40.0	MAS-2	--	40.4	101
100	MAS-3	--	104	104
			Mean =	101
			Standard Deviation =	3.15
			CV =	3.12%

[1] The limit of quantitation (LOQ) was 5.00 μg/L, calculated as the product of the lowest standard concentration (5.00 μg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 17

Measured Concentrations of Indeno(1,2,3-cd)pyrene Analyzed by HPLC/UV

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (μg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (μg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	9.84	98.4
40.0	MAS-2	--	40.6	101
100	MAS-3	--	103	103
			Mean =	101
			Standard Deviation =	2.31
			CV =	2.28%

[1] The limit of quantitation (LOQ) was 5.00 μg/L, calculated as the product of the lowest standard concentration (5.00 μg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 18

Measured Concentrations of Benzo(g,h,i)perylene Analyzed by HPLC
with Fluorescence Detection

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (μg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (μg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	9.72	97.2
40.0	MAS-2	--	40.4	101
100	MAS-3	--	99.4	99.4
			Mean =	99.2
			Standard Deviation =	1.91
			CV =	1.92%

[1] The limit of quantitation (LOQ) was 5.00 μg/L, calculated as the product of the lowest standard concentration (5.00 μg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 19

Measured Concentrations of Dibenzo(a,e)pyrene Analyzed by HPLC
with Fluorescence Detection

Nominal Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (mg/L)[1]	Percent of Nominal[2]
Negative Control	1	0	< LOQ	--
1000	2	0	< LOQ	--
Negative Control	5	24	< LOQ	--
1000	6	24	< LOQ	--
Negative Control	9	96	< LOQ	--
1000	10	96	< LOQ	--

Matrix Blank and Fortification Samples

Nominal Concentration (μg/L)	Sample Identification (472A-113-)	Sampling Interval (Hour)	Measured Concentration (μg/L)[1]	Percent of Nominal[2]
0.0	MAB-1	--	< LOQ	--
10.0	MAS-1	--	9.82	98.2
40.0	MAS-2	--	40.6	101
100	MAS-3	--	104	104
			Mean =	101
			Standard Deviation =	2.90
			CV =	2.87%

[1] The limit of quantitation (LOQ) was 5.00 μg/L, calculated as the product of the lowest standard concentration (5.00 μg/L) and the dilution factor of the matrix blank samples (1.00).

[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

Table 20

Measured Concentrations of Arsenic Analyzed by ICP-AES

Nominal Petroleum Coke Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hours)	Measured Arsenic Concentration (µg/L) [1,2]
Negative Control	3	0	< LOQ
1000	4	0	< LOQ
Negative Control	7	24	< LOQ
1000	8	24	< LOQ
Negative Control	11	96	< LOQ
1000	12	96	< LOQ

Matrix Blank and Fortification Samples

Nominal Arsenic Concentration (µg/L)	Sample Identification (472A-113-)	Measured Arsenic Concentration (µg/L) [1]	Percent of Nominal [1]
0.0	MAB-1	< 20.0	--
50.0	MAS-1	53.9	108

[1] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

[2] The limit of quantitation (LOQ) for these analyses was set at 20 µg/L.

Table 21

Measured Concentrations of Copper Analyzed by ICP-AES

Nominal Petroleum Coke Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hours)	Measured Copper Concentration (µg/L) [1,2]
Negative Control	3	0	< LOQ
1000	4	0	< LOQ
Negative Control	7	24	< LOQ
1000	8	24	< LOQ
Negative Control	11	96	< LOQ
1000	12	96	< LOQ

Matrix Blank and Fortification Samples

Nominal Copper Concentration (µg/L)	Sample Identification (472A-113-)	Measured Copper Concentration (µg/L) [1]	Percent of Nominal [1]
0.0	MAB-1	< LOQ	--
50.0	MAS-1	53.1	106

[1] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

[2] The limit of quantitation (LOQ) for these analyses was set at 20 µg/L.

Table 22

Measured Concentrations of Iron Analyzed by ICP-AES

Nominal Petroleum Coke Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hours)	Measured Iron Concentration (µg/L) [1,2]
Negative Control	3	0	< LOQ
1000	4	0	< LOQ
Negative Control	7	24	< LOQ
1000	8	24	< LOQ
Negative Control	11	96	< LOQ
1000	12	96	< LOQ

Matrix Blank and Fortification Samples

Nominal Iron Concentration (µg/L)	Sample Identification (472A-113-)	Measured Iron Concentration (µg/L) [1]	Percent of Nominal [1]
0.0	MAB-1	< LOQ	--
25.0	MAS-1	23.8	95.3

[1] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

[2] The limit of quantitation (LOQ) for these analyses was set at 10 µg/L.

Table 23

Measured Concentrations of Nickel Analyzed by ICP-AES

Nominal Petroleum Coke Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hours)	Measured Nickel Concentration (µg/L) [1,2]
Negative Control	3	0	< LOQ
1000	4	0	< LOQ
Negative Control	7	24	< LOQ
1000	8	24	< LOQ
Negative Control	11	96	< LOQ
1000	12	96	< LOQ

Matrix Blank and Fortification Samples

Nominal Nickel Concentration (µg/L)	Sample Identification (472A-113-)	Measured Nickel Concentration (µg/L) [1]	Percent of Nominal [1]
0.0	MAB-1	< LOQ	--
25.0	MAS-1	25.3	101

[1] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

[2] The limit of quantitation (LOQ) for these analyses was set at 10 µg/L.

Table 24

Measured Concentrations of Selenium Analyzed by ICP-AES

Nominal Petroleum Coke Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hours)	Measured Selenium Concentration (µg/L) [1,2]
Negative Control	3	0	< LOQ
1000	4	0	< LOQ
Negative Control	7	24	< LOQ
1000	8	24	< LOQ
Negative Control	11	96	< LOQ
1000	12	96	< LOQ

Matrix Blank and Fortification Samples

Nominal Selenium Concentration (µg/L)	Sample Identification (472A-113-)	Measured Selenium Concentration (µg/L) [1]	Percent of Nominal [1]
0.0	MAB-1	< LOQ	--
500	MAS-1	536	107

[1] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

[2] The limit of quantitation (LOQ) for these analyses was set at 200 µg/L.

Table 25

Measured Concentrations of Vanadium Analyzed by ICP-AES

Nominal Petroleum Coke Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hours)	Measured Vanadium Concentration (µg/L) [1,2]
Negative Control	3	0	< LOQ
1000	4	0	< LOQ
Negative Control	7	24	< LOQ
1000	8	24	< LOQ
Negative Control	11	96	< LOQ
1000	12	96	< LOQ

Matrix Blank and Fortification Samples

Nominal Vanadium Concentration (µg/L)	Sample Identification (472A-113-)	Measured Vanadium Concentration (µg/L) [1]	Percent of Nominal [1]
0.0	MAB-1	< LOQ	--
1.00	MAS-1	1.02	102

[1] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

[2] The limit of quantitation (LOQ) for these analyses was set at 0.40 µg/L.

Table 26

Measured Concentrations of Sulfur Analyzed by ICP-AES

Nominal Petroleum Coke Concentration (mg/L)	Sample Identification (472A-113-)	Sampling Interval (Hours)	Measured Sulfur Concentration (mg/L) [1,2]
Negative Control	3	0	< LOQ
1000	4	0	< LOQ
Negative Control	7	24	< LOQ
1000	8	24	< LOQ
Negative Control	11	96	< LOQ
1000	12	96	< LOQ

Matrix Blank and Fortification Samples

Nominal Sulfur Concentration (mg/L)	Sample Identification (472A-113-)	Measured Sulfur Concentration (mg/L) [1]	Percent of Nominal [1]
0.0	MAB-1	< LOQ	--
20.0	MAS-1	20.2	101

[1] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

[2] The limit of quantitation (LOQ) for these analyses was set at 10 mg/L.

Table 27

Temperature, Dissolved Oxygen and pH of Water in the Test Chambers

					24 Hours						48 Hours		
		0 Hour [1]			Prior to Renewal			Renewal [1]			Prior to Renewal		
Nominal WAF Loading Rate (mg/L)	Replicate	Temp.[2] (ºC)	DO[3] (mg/L)	pH	Temp.[2] (ºC)	DO[3] (mg/L)	pH	Temp.[2] (ºC)	DO[3] (mg/L)	pH	Temp.[2] (ºC)	DO[3] (mg/L)	pH
Negative Control	A	22.5	8.5	8.6	22.3	3.9	8.2	21.5	8.6	8.6	22.1	4.6	8.4
	B	--	--	--	22.3	3.7	8.2	--	--	--	22.1	3.5	8.3
	C	--	--	--	22.3	3.0	8.1	--	--	--	22.1	3.2	8.1
1000	A	22.4	8.5	8.6	22.4	3.6	8.3	21.6	8.6	8.6	22.2	3.0	8.3
	B	--	--	--	22.3	3.3	8.3	--	--	--	22.2	3.7	8.3
	C	--	--	--	22.3	3.0	8.3	--	--	--	22.2	3.2	8.3

[1] Measurements at 0 hours and at each 24-hour renewal were taken from the WAF solutions, rather from individual replicates, in order to begin each renewal period with test chambers completely filled with no headspace.

[2] Manual temperature measurements. Temperature measured continuously during the test ranged from 22.0 to 22.5ºC, measured to the nearest 0.5ºC.

[3] A dissolved oxygen concentration of 5.2 mg/L represents 60% saturation at 22ºC in freshwater.

Table 27 (Continued)

Temperature, Dissolved Oxygen and pH of Water in the Test Chambers

Nominal WAF Loading Rate (mg/L)	Replicate	48 Hours			72 Hours						96 Hours		
		Renewal [1]			Prior to Renewal			Renewal [1]					
		Temp.[2] (ºC)	DO[3] (mg/L)	pH	Temp.[2] (ºC)	DO[3] (mg/L)	pH	Temp.[2] (ºC)	DO[3] (mg/L)	pH	Temp.[2] (ºC)	DO[3] (mg/L)	pH
Negative Control	A	21.8	8.5	8.7	22.2	5.2	8.3	22.5	8.6	8.6	22.2	4.4	8.2
	B	--	--	--	22.1	4.5	8.2	--	--	--	22.2	3.4	8.1
	C	--	--	--	22.1	3.4	8.2	--	--	--	22.2	3.1	8.1
1000	A	22.0	8.6	8.7	22.2	3.6	8.2	22.6	8.6	8.6	22.2	3.9	8.2
	B	--	--	--	22.2	4.3	8.3	--	--	--	22.2	3.7	8.1
	C	--	--	--	22.2	3.6	8.3	--	--	--	22.2	3.4	8.2

[1] Measurements at 0 hours and at each 24-hour renewal were taken from the WAF solutions, rather from individual replicates, in order to begin each renewal period with test chambers completely filled with no headspace.

[2] Manual temperature measurements. Temperature measured continuously during the test ranged from 22.0 to 22.5ºC, measured to the nearest 0.5ºC.

[3] A dissolved oxygen concentration of 5.2 mg/L represents 60% saturation at 22ºC in freshwater.

Table 28

Specific Conductance, Hardness, Alkalinity and Total Organic Carbon
Measured in the Dilution Water at Test Initiation

Parameter	Day 0
Specific Conductance (µmhos/cm)	290
Hardness (mg/L as $CaCO_3$)	128
Alkalinity (mg/L as $CaCO_3$)	178
Total Organic Carbon (mg C/L)	1.1

Table 29

Cumulative Mortality and Observations

Nominal WAF Loading Rate (mg/L)	Replicate	No. Exposed	3 Hours		24 Hours[3]		48 Hours[3]		72 Hours[3]		96 Hours		Cumulative Percent Mortality
			No. Dead[1]	Obs.[2]	No. Dead	Obs.	No. Dead	Obs.	No. Dead	Obs.	No. Dead	Obs.	
Negative Control	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	
	C	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	
1000	A	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0
	B	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	
	C	10	0	10 AN	0	10 AN	0	10 AN	0	10 AN	0	10 AN	

[1] Cumulative number of dead fish.
[2] Observations: AN = appear normal.
[3] Test solutions were renewed at approximately 24-hour intervals.

Table 30

Estimates of the LL50 Values

Time	LL50 [1] (mg/L)	95% Confidence Interval (mg/L)	Statistical Method
24 Hours	>1000	-- [2]	NA [2]
48 Hours	>1000	-- [2]	NA [2]
72 Hours	>1000	-- [2]	NA [2]
96 Hours	>1000	-- [2]	NA [2]

[1] Based on the single WAF loading rate tested.

[2] There were no mortalities in the treatment group. Therefore, LL50 values and 95% confidence limits could not be statistically calculated and were determined by visual interpretation of the data.

Appendix 1

Exploratory Non-GLP Rangefinding Toxicity Test with Petroleum Coke

Wildlife International, Ltd.
ECOTOXICOLOGY & ANALYTICAL TESTING SERVICES

EXPLORATORY NON-GLP RANGEFINDING TOXICITY TEST WITH PETROLEUM COKE

96-Hour Static-Renewal Acute Toxicity Rangefinding Test with the Fathead Minnow

Project Number 472A-113

Introduction

An exploratory non-GLP rangefinding test was conducted from December 7 to 11, 2004 in the Wildlife International, Ltd. aquatic toxicology laboratory. The test was conducted under static-renewal conditions for 96 hours, with organisms transferred to newly prepared test solutions at 24-hour intervals.

Methods and Materials

Test solutions were prepared as water accomodated fractions (WAF) at nominal loading rates of 10, 100 and 1000 mg test substance/L. An untreated control group was maintained concurrently. For each WAF, a calculated amount of test substance was mixed with 12 L of Wildlife International, Ltd. well water in a 13.2 L Pyrex aspirator bottle with tubulation. The solution was stirred for approximately 24 hours on a magnetic stir plate, with a vortex maintained at approximately 30% of the solution height. After mixing, each solution was allowed to settle for approximately one hour, and the solution was decanted into one test chamber per concentration. Test chambers were one gallon glass jars with Teflon-lined caps, filled completely with test solution to minimize headspace. New test solutions were prepared at approximately 24-hour intervals using the same procedures.

Five juvenile fathead minnows (*Pimephales promelas*) were placed in one test chamber per treatment and control group at initiation of the test. The test chambers were placed in a temperature-controlled environmental chamber set to maintain the target temperature of 20 ± 2°C. All organisms were observed for mortality and signs of toxicity at 24, 48, 72 and 96 hours of exposure. Dissolved oxygen concentrations in the test chambers were measured in the new solutions at initiation, and in the 24-hour old solutions at 24, 48, 72 and 96 hours.

Results

The results of the rangefinding test are included in the attached table. All fish in the control and treatment groups were normal in appearance and behavior throughout the 96-hour exposure period. Based on the nominal WAF concentrations, the 96-hour LL50 value for the rangefinding test was estimated to be greater than 1000 mg/L, the highest loading rate tested. No effects were seen at the highest loading rate used in the test (no observed effect level = 1000 mg/L loading rate). Dissolved oxygen concentrations remained ≥86 % of saturation.

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PETROLEUM COKE

RESULTS OF AN EXPLORATORY NON-GLP RANGEFINDING TEST WITH THE FATHEAD MINNOW

STUDY:	Petroleum Coke: A 96-Hour Static-Renewal Acute Toxicity Test with the Fathead Minnow (*Pimephales promelas*)
SPONSOR:	American Petroleum Institute
PROJECT NO.:	472A-113

Nominal Loading Rate[1] (mg/L)	Number Dead in 24 Hours / Cumulative Number Dead / Number Originally Exposed (Observations[2])				Cumulative Percent Mortality
	24 Hours[3]	48 Hours[3]	72 Hours[3]	96 Hours	
Negative Control	0 / 0 / 5 (5 AN)	0 / 0 / 5 (5 AN)	0 / 0 / 5 (5 AN)	0 / 0 / 5 (5 AN)	0
10	0 / 0 / 5 (5 AN)	0 / 0 / 5 (5 AN)	0 / 0 / 5 (5 AN)	0 / 0 / 5 (5 AN)	0
100	0 / 0 / 5 (5 AN)	0 / 0 / 5 (5 AN)	0 / 0 / 5 (5 AN)	0 / 0 / 5 (5 AN)	0
1000	0 / 0 / 5 (5 AN)	0 / 0 / 5 (5 AN)	0 / 0 / 5 (5 AN)	0 / 0 / 5 (5 AN)	0

---

[1] Test solutions were prepared as WAFs by mixing test material into well water for 24 hours and allowing the material to settle for one hour before decanting the solution into test chambers. Test chambers were glass jars filled completely with no headspace, with Teflon-lined caps.

[2] Observations: AN = appear normal.

[3] Test solutions were renewed at 24-hour intervals.

Appendix 2

Protocol, Amendments and Deviations

PROTOCOL

PETROLEUM COKE: A 96-HOUR STATIC-RENEWAL ACUTE TOXICITY TEST WITH THE FATHEAD MINNOW (*Pimephales promelas*)

OECD Guideline 203

and

U.S. EPA OPPTS Number 850.1075

Submitted to

American Petroleum Institute
1220 L Street, N.W.
Washington, DC 20005

Wildlife International, Ltd.

8598 Commerce Drive
Easton, Maryland 21601
(410) 822-8600

March 30, 2004

PETROLEUM COKE: A 96-HOUR STATIC-RENEWAL ACUTE TOXICITY TEST WITH THE FATHEAD MINNOW (*Pimephales promelas*)

SPONSOR:	American Petroleum Institute 1220 L Street, N.W. Washington, DC 20005
SPONSOR'S REPRESENTATIVE:	[redacted]
SPONSOR'S TECHNICAL STUDY MONITOR:	[redacted]
TESTING FACILITY:	Wildlife International, Ltd. 8598 Commerce Drive Easton, Maryland 21601
STUDY DIRECTOR:	[redacted] Wildlife International, Ltd.
LABORATORY MANAGEMENT:	[redacted]

FOR LABORATORY USE ONLY

Proposed Dates: To Be Amended.

Experimental Start Date: ____________ Experimental Termination Date: ____________

Project No.: 472A-113

Test Concentrations: ____________

Test Substance No.: 6485 Reference Substance No. (if applicable): ____________

PROTOCOL APPROVAL

[redacted]

22 April 2004
DATE

22 April 2004
DATE

01 April, 2004
DATE

PROTOCOL NO.: 472/033004/FAT/SUB472

INTRODUCTION

Wildlife International, Ltd. will conduct a static-renewal acute toxicity test to determine the effects of water soluble components of petroleum coke on the fathead minnow (*Pimephales promelas*) for the Sponsor at the Wildlife International, Ltd. aquatic toxicology facility in Easton, Maryland. Petroleum coke is defined as the product formed by subjecting the heavy tar-like residue remaining following oil refining to high temperatures and pressures. It consists of primarily elemental carbon with considerably smaller amounts of hydrocarbons, sulfur and trace amounts of heavy metals. The study will be performed based on guidelines provided in OECD Guideline for Testing of Chemicals, 203: *Fish, Acute Toxicity Test* (1) and the U.S. EPA Series 850 - Ecological Effects Test Guidelines OPPTS Number 850.1075: *Fish Acute Toxicity Test, Freshwater and Marine* (2). Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site, or at an alternative location to be specified in the final report.

OBJECTIVE

The objective of this study is to determine the acute effects of water soluble components of petroleum coke a test substance on the fathead minnow (*Pimephales promelas*), during a 96-hour exposure period under static-renewal test conditions.

EXPERIMENTAL DESIGN

Fathead minnows will be exposed to a geometric series of at least five water accommodated fractions (WAF) of the test substance and a negative (dilution water) control for 96 hours. Two replicate test chambers will be maintained for each treatment and control group, with 10 fathead minnows in each test chamber for a total of 20 fathead minnows per treatment level. WAF solutions and control water will be renewed at approximately 24-hour intervals. At each renewal period, fresh WAF solutions will be prepared and the fish will be transferred from the old solutions to the new solutions.

WAF loading rates will be selected in consultation with the Sponsor, and will be based upon information such as the results of exploratory range-finding toxicity data, known toxicity data, physical/chemical properties of the test substance or other relevant information. For this test, the term loading rate means the total amount of test substance added to the dilution water volume (mg/L) to achieve the respective WAF solution. Generally, each test substance loading rate in the definitive test will be at least 60% of the next higher loading rate unless information concerning the concentration-effect curve indicates that a different dilution factor would be more appropriate. Water samples will be collected at specified intervals for analysis of the selected constituents of petroleum coke.

To control bias, fathead minnows will be indiscriminately assigned to exposure chambers at test initiation. No other potential sources of bias are expected to affect the results of the study. Observations of mortality and other clinical signs will be made throughout the 96-hour test period. Cumulative percent mortality observed in the treatment groups will be used to calculate, when possible, LL50 values at 24, 48, 72, and 96 hours. The no-mortality level and the no-observed-effect level (NOEL) will be determined by visually interpreting the mortality and clinical observation data.

MATERIALS AND METHODS

Test Substance

The test substance is green coke (CAS Number 64741-79-3) sieved to approximately 2 mm particle size. Information on the characterization of test, control or reference substances is required by OECD Principles of Good Laboratory Practice (3) and TSCA Good Laboratory Practice Standards (4). The Sponsor is responsible for providing Wildlife International, Ltd. written verification that the test substance has or has not been characterized according to GLPs prior to its use in the study. If written verification of GLP test substance characterization is not provided to Wildlife International, Ltd., it will be noted in the compliance statement of the final report.

The Sponsor is responsible for all information related to the test substance and agrees to accept any unused test substance and/or test substance containers remaining at the end of the study

Preparation of Water Accommodated Fraction Solutions

The test substance will be mixed directly with dilution water on a weight:volume basis. Each WAF will be prepared individually in a 13.2 L Pyrex® aspirator bottle with tubulation by mixing an amount of the test substance in approximately 12 L of dilution water using a Teflon®-coated stir bar on a magnetic stir plate. Care will be taken to maintain a vortex depth of approximately 30% of the test solution height. The length of the mixing time in the definitive test will be determined based on results of the WAF equilibration trial. Following the mixing period, the WAF solutions will then be allowed to settle for approximately 1 hour and the test solution will be decanted off the top (or bottom, as appropriate).

Justification for Route of Exposure

The test substance will be administered to the test organism in water in the form of water accommodated fractions. The route of exposure is justified because it is the primary exposure pathway of aquatic organisms to chemicals.

Test Organism

The fathead minnow (*Pimephales promelas*) has been selected as the test species for this study. Fathead minnows are representative of an important group of aquatic vertebrates, and have been selected for use in the test based upon past use history in the laboratory. Fish will be from the same source and year class, and the length of the longest fish measured will be no more than twice that of the shortest. Fish will weigh between 0.1 and 3.0 grams (wet weight blotted dry), and the total weight in each test chamber will not exceed 0.8 grams fish per liter of solution. The recommended total length of the fish is 2.0 ± 1.0 cm. Total lengths and wet weights of the individual fish in one negative control replicate will be measured at the end of the test and will be considered representative of the total length and weight of all fish used in the study. Fish will be obtained from in-house cultures or from a commercial supplier or hatchery. The identity of the species will be verified by the supplier, or by Wildlife International, Ltd. personnel using appropriate taxonomic keys, such as Eddy (5).

Fathead minnows will be held for at least 14 days prior to the test in water from the same source and at approximately the same temperature as used during the test. Changes in water temperature will not exceed 3°C in any 72-hour period during holding and dissolved oxygen will be > 80% of saturation during holding. If mortality of the test fish exceeds 5% in the 7 days prior to the test, or the fish show signs of disease or stress during a two-day period immediately preceding the test, they will be euthanized or held for an additional 14-day period to ensure that the fish are healthy. At test initiation, the minnows will be collected from holding or acclimation tanks and transferred to the test chambers.

During the holding period, the test fish will be fed at least once daily. The diet will consist of live or frozen brine shrimp nauplii (*Artemia sp.*), frozen brine shrimp, and/or commercial food. Fish will not be fed for at least two days prior to the test or during the test. Specifications for acceptable levels of contaminants in fish diets have not been established. However, there are no known levels of contaminants reasonably expected to be present in the diet that are considered to interfere with the purpose or conduct of the test.

Dilution Water

Water used for the culturing and testing of fathead minnows will be obtained from a well approximately 40 meters deep located on the Wildlife International, Ltd. site. The water will be passed through a sand filter and pumped into a 37,800-L storage tank where the water will be aerated with spray nozzles. Prior to use the water will be filtered to 0.45 µm in order to remove fine particles. Water used for culturing and testing is characterized as moderately hard. Typical values for hardness, alkalinity, pH and specific conductance are approximately:

Hardness, mg/L as $CaCO_3$	145
Alkalinity, mg/L as $CaCO_3$	190
pH	8.1
Specific Conductance, µmhos/cm (µS/cm)	330

Hardness, alkalinity, pH and specific conductance will be measured weekly to monitor the consistency of the well water. Means and ranges of the measured parameters for the four-week period preceding the test will be provided in the final report. Analyses will be performed at least once annually to determine the concentrations of selected organic and inorganic constituents of the well water and results of the most recent GLP-compliant analyses will be summarized in the final report.

Test Apparatus

Test chambers will be one-gallon glass jars with Teflon®-lined lids. The jars will be completely filled with test solution to minimize headspace. Test chambers will be positioned in an environmental chamber or temperature-controlled water bath to maintain a temperature of 22 ± 2°C. Test chambers will be covered and labelled with the project number and test concentration.

Environmental Conditions

Ambient room light will be used to illuminate the test systems. Fluorescent tubes (e.g., Colortone® 50) that emit wavelengths similar to natural sunlight will be controlled with an automatic timer to provide a photoperiod of 16 hours of light and 8 hours of darkness. A 30-minute transition period of low light intensity will be provided when lights go on and off to avoid sudden changes in light intensity. The light intensity over a control replicate will be measured at the beginning of the test using a SPER Scientific Ltd. light meter or equivalent.

The test will be conducted at a target temperature of 22 ± 2°C. Temperature will be monitored and recorded continuously during the entire test in a beaker of water adjacent to the test using a Fulscope ER/C Recorder (1900 J Series Model A) or equivalent. Recorder measurements will be verified with a liquid-in-glass thermometer prior to test initiation.

Dissolved oxygen, pH and temperature will be measured in each replicate of the treatment and control groups at test initiation, before and after each renewal, and at test termination. Dissolved oxygen will be measured using a Thermo Orion Model 850Aplus dissolved oxygen meter, or equivalent. Measurements of pH will be made using a Thermo Orion Model 720Aplus pH meter, or equivalent. Temperature measurements will be made using a liquid-in-glass thermometer.

Hardness, alkalinity, total organic carbon (TOC) and specific conductance will be measured in a sample of the dilution water collected at test initiation. Hardness and alkalinity measurements will be made based on procedures in *Standard Methods for the Examination of Water and Wastewater* (6). Specific conductance will be measured using a Yellow Springs Instruments Model 33 Salinity-Conductivity-Temperature meter, or equivalent. Total organic carbon will be measured using a Shimadzu Model 5000 TOC analyzer, or equivalent.

Observations pertaining to test substance solubility (e.g., surface slicks, precipitates, etc.) also will be recorded.

Biological Measurements

Observations of mortality and clinical signs of toxicity will be made between 0-24, and at 24, 48, 72 and 96 hours ± 1 hour. Fish will be considered dead if there is no visible movement (e.g., gill movements) and if touching of the caudal peduncle produces no reaction. Dead fish will be removed at each observation period. All clinical observations will be noted.

Sampling for Analytical Measurements

Water samples will be decanted from each WAF preparation vessel at the beginning of the test, and collected from each replicate test chamber after 24 hours and at the end of the test to determine concentrations of compounds and elements of interest in petroleum coke (see Table 1). In the event that 100% mortality occurs in any treatment, then sampling of that treatment will terminate following the next sampling interval. Samples will be collected at mid-depth from each test chamber, analyzed immediately or placed in a glass container with zero headspace and stored under refrigeration until analyzed. Samples will be analyzed for the components of petroleum coke listed in Table 1. The sample scheme is summarized below:

PROPOSED NUMBERS OF VERIFICATION SAMPLES

Experimental Group	0 Hours	24 Hours (Old)	96 Hours (Old)
Control	1	2	2
Level 1-Low Concentration	1	2	2
Level 2	1	2	2
Level 3	1	2	2
Level 4	1	2	2
Level 5	1	2	2
	6	12	12

Total Number of Verification Samples = 30

The above numbers of samples represent those collected from the test and do not include quality control (QC) samples such as matrix blanks and fortifications prepared and analyzed during the analytical chemistry phase of the study. At the discretion of the Study Director, water samples from one or more appropriate test chambers will be collected and analyzed if an analytical error in sampling or analysis is suspected. The reason for the additional samples will be documented in the raw data and summarized in the final report.

Analytical Chemistry

Chemical analysis of the samples will be performed by Wildlife International, Ltd. The analytical method used will be based upon chromatographic methodology and/or ICP analysis for metals. The methodology used to analyze the test samples will be documented in the raw data and summarized in the final report.

Conditions for the Validity of the Test

The following criteria will be used to judge the validity of the test:

1) mortality of the fish in the negative control group will not exceed 10% by the end of the test, and
2) the dissolved oxygen concentration will be at least 60% the air-saturation value throughout the test.

Data Analysis

The mortality pattern in the definitive multiple concentration test will be used to calculate, if possible, the 24, 48, 72 and 96-hour LL50 concentrations. The LL50 values will be based on nominal loading rates and calculated using the computer software of C.E. Stephan (7). The program was designed to calculate the LL50 value and the 95% confidence interval by probit analysis, the moving average method, and the binomial probability method (8,9,10). The method used will depend upon the mortality pattern and its suitability for each method. Additional analysis of data may be conducted if deemed appropriate by the Study Director. The results of the analysis will be documented in the raw data and summarized in the final report. A no-observed-effect level (NOEL) will be reported if it is defined by the dose-response pattern. The maximum WAF level causing 0% mortality and the minimum WAF level causing 100% mortality will be presented, if possible.

RECORDS TO BE MAINTAINED

Records to be maintained for data generated by Wildlife International, Ltd. will include, but not be limited to:

1. A copy of the signed protocol.
2. Identification and characterization of the test substance, if provided by the Sponsor.
3. Dates of initiation and termination of the test.
4. Fathead minnow history, holding and acclimation records.
5. Stock solution calculation and preparation, if applicable.
6. Observations.
7. Water chemistry results (e.g., alkalinity and hardness).
8. If analytical verification of the test substance concentrations is done, the methods used to analyze test substance concentrations and the results of analytical measurements.
9. Statistical calculations, if applicable.
10. Test conditions (light intensity, photoperiod, etc.).
11. Calculation and preparation of test concentrations.
12. Results of range-finding tests, if performed.
13. Copy of final report.

FINAL REPORT

A final report of the results of the study will be prepared by Wildlife International, Ltd. The report will include, but will not be limited to the following, when applicable:

1. Name and address of the facility performing the study.
2. Dates upon which the study was initiated and completed, and the definitive experimental start and termination dates.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Objective and procedure, as stated in the approved protocol, including a copy of the final protocol, and all amendments and deviations to the protocol.
5. The test chemical identification including name, chemical abstract number or code number, strength, purity, composition, and other information provided by the Sponsor.
6. Stability and solubility of the test chemical under the conditions of administration, if provided by the Sponsor.
7. A description of the methods used to conduct the test.
8. A description of the test organisms, including the source, scientific name, age or life stage, lengths and weights of a representative group of test organisms and feed types.

9. A description of the preparation of the test solutions.
10. The methods used to allocate organisms to test chambers and begin the test, the number of organisms and chambers per treatment, the duration of the test and environmental conditions during the test.
11. A description of circumstances that may have affected the quality or integrity of the data.
12. The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study.
13. A description of the transformations, calculations, and operations performed on the data, a summary and analysis of the biological data, analytical chemistry data, and a statement of the conclusions drawn from the analyses.
14. Statistical methods used to evaluate the data, and copies of the output from the statistics program.
15. A graph of the concentration-mortality curve at the end of the test, if possible. If the data is conducive to evaluation by probit analysis, the slope of the concentration-response curve will be reported.
16. The signed and dated reports of each of the individual scientists or other professionals involved in the study, if applicable.
17. The location where raw data and final report are to be stored.
18. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made and the dates of any findings reported to the Study Director and Management.
19. If it is necessary to make corrections or additions to a final report after it has been accepted, such changes will be made in the form of an amendment issued by the Study Director. The amendment will clearly identify the part of the final report that is being amended and the reasons for the amendment, and will be signed by the Study Director.

CHANGING OF PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and approved by the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations filed with the raw data. All changes to and deviation from the protocol will be indicated in the final report.

GOOD LABORATORY PRACTICES

This study will be conducted in accordance with OECD Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17 (3) and TSCA Good Laboratory Practice Standards (40 CFR Part 792) (4). Each study conducted by Wildlife International, Ltd. is routinely examined by the Wildlife International, Ltd. Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. A statement of compliance with Good Laboratory Practices will be prepared for all portions of the study conducted by Wildlife International, Ltd. The Sponsor will be responsible for compliance with Good Laboratory Practices for procedures performed by other laboratories (e.g., residue analyses or pathology). Raw data for all work performed at Wildlife International, Ltd. and a copy of the final report will be filed by project number in archives located on the Wildlife International, Ltd. site or at an alternative location to be specified in the final report.

REFERENCES

1 **Organization for Economic Cooperation and Development.** 1992. *Fish, Acute Toxicity Test.* OECD Guideline for Testing of Chemicals. Guideline 203. Paris.

2 **U.S. Environmental Protection Agency.** 1996. Series 850- Ecological Effects Test Guidelines (*draft*), OPPTS Number 850.1075: *Fish Acute Toxicity Test, Freshwater and Marine.*

3 **OECD.** 1998. *OECD Principles of Good Laboratory Practice* ENV/MC/CHEM (98) 17.

4 **Title 40 of the Code of Federal Regulations, Part 792.** 1989. *Toxic Substances Control Act (TSCA) Good Laboratory Practice Standards.*

5 **Eddy, S.** 1974. *The Freshwater Fishes.* Wm. C. Brown Company Publishers, Dubuque, Iowa.

6 **APHA, AWWA, WPCF.** 1998. *Standard Methods for the Examination of Water and Wastewater.* 20th Edition.

7 **Stephan, C.E.** 1978. U.S. EPA, Environmental Research Laboratory, Duluth, Minnesota. Personal communication.

8 **Thompson, W.R.** 1947. *Bacteriological Reviews.* Vol. II, No. 2. Pp. 115-145.

9 **Stephan, C.E.** 1977. "Methods for Calculating an LC50", *Aquatic Toxicology and Hazard Evaluations.* American Society for Testing and Materials. Publication Number STP 634, pp 65-84.

10 **Finney, D.J.** 1971. *Statistical Methods in Biological Assay.* Second edition. Griffin Press, London.

Table 1.

Analytes of Interest in Petroleum Coke

PAH	Metals and Sulfur
Acenaphthene	Nickel
Acenaphthylene	Vanadium
Anthracene	Iron
Benzo(a)anthracene	Copper
Benzo(a)pyrene	Selenium
Benzo(b)fluoranthene	Arsenic
Benzo(g,h,i)perylene	Sulfur
Benzo(k)fluoranthene	
Chrysene	
Dibenzo(a,e)pyrene	
Dibenz(a,h)anthracene	
Fluoranthene	
Fluorene	
Indeno(1,2,3-cd)pyrene	
Naphthalene	
Perylene	
Phenanthrene	
Pyrene	

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: Petroleum Coke: A 96-Hour Static-Renewal Acute Toxicity Test with the Fathead Minnow (*Pimephales promelas*)

PROTOCOL NO.: 472/033004/FAT/SUB472 **AMENDMENT NO.:** 1

SPONSOR: American Petroleum Institute **PROJECT NO.:** 472A-113

EFFECTIVE DATE: March 8, 2005

AMENDMENT: Page 2:

ADD: Proposed Dates: Experimental Start Date (OECD): March 27, 2005
Experimental Start Date (EPA): March 28, 2005
Experimental Termination Date: April 1, 2005
Test Concentrations: 1000 mg/L

REASON: This information was not available at the time the protocol was signed by the Study Director.

AMENDMENT: Experimental Design, Page 3:

CHANGE: Fathead minnows will be exposed to a geometric series of at least five water accommodated fractions (WAF) of the test substance and a negative (dilution water) control for 96 hours. Two replicate test chambers will be maintained for each treatment and control group, with 10 fathead minnows in each test chamber for a total of 20 fathead minnows per treatment level.

TO: Fathead minnows will be exposed to a single water accommodated fraction (WAF) of the test substance and a negative (dilution water) control for 96 hours. The WAF loading rate will be 1000 mg/L. Three replicate test chambers will be maintained for each treatment and control group, with 10 fathead minnows in each test chamber for a total of 30 fathead minnows per treatment level.

REASON: A limit test will be conducted.

AMENDMENT: Experimental Design, Page 4, 1st Paragraph:

DELETE: Generally, each test substance loading rate in the definitive test will be at least 60% of the next higher loading rate unless information concerning the concentration-effect curve indicates that a different dilution factor would be more appropriate.

REASON: Because the test will be run at a single limit concentration, discussion of dilution factors becomes irrelevant.

AMENDMENT: Experimental Design, Page 4, 2nd Paragraph:

CHANGE: Cumulative percent mortality observed in the treatment groups will be used to calculate, when possible, LL50 values at 24, 48, 72 and 96 hours.

TO: Cumulative percent mortality observed in the treatment group will be used to determine whether the 24, 48, 72 and 96-hour LL50 values are greater than or less than the 1000 mg/L WAF loading rate used in the test.

REASON: LL50 values cannot be calculated in a limit test. Therefore, the response observed in the treatment group will provide a relative indication of where the LL50 might lie.

AMENDMENT: Preparation of Water Accommodated Fraction Solutions, Page 4:

CHANGE: The length of the mixing time in the definitive test will be determined based on results of the WAF equilibration trial.

TO: The test solution will be mixed for approximately 24 hours.

REASON: The WAF equilibration trial showed no effect of mixing time on concentrations of the analytes of interest. Therefore, it is not necessary to stir the WAF mixtures beyond 24 hours.

AMENDMENT: Sampling for Analytical Measurements, Page 8:

CHANGE: Replace the entire section with the following.

TO: Water samples will be decanted from each WAF preparation vessel at the beginning of the test, and pooled from each replicate test chamber after 24 hours and at the end of the test to determine concentrations of the compounds and elements of interest in petroleum coke (see Table 1). In the event that 100% mortality occurs in the treatment group, then sampling will cease following the next sampling interval. One set of samples will be collected and analyzed for selected organic compounds, and a second set of samples will be collected and analyzed for selected inorganic elements. Samples will be collected at mid-depth from the test chambers and stored with zero headspace until the end of the test. Samples for organic analyses should be preserved by storage at 4°C. Samples for inorganic analyses should be preserved by treating the sample with nitric acid (HNO_3) to a pH of <2. The sample scheme is summarized below:

PROPOSED NUMBERS OF VERIFICATION SAMPLES

Experimental Group	0-Hours [1]	24-Hours [1] (old)	96-Hours [1] (old)
Control	2	2	2
Treatment (1000 mg/L loading)	2	2	2
	4	4	4

[1] At each sampling interval, one set of samples will be collected and analyzed for selected organic compounds, and a second set of samples will be collected and analyzed for selected inorganic elements.

Total Number of Verification Samples = 12

The above numbers of samples represent those collected from the test and do not include quality control (QC) samples such as matrix blanks and fortifications prepared and analyzed during the analytical chemistry phase of the study. At the discretion of the Study Director, water samples from one or more appropriate test chambers will be collected and analyzed if an analytical error in sampling or analysis is suspected. The reason for the additional samples will be documented in the raw data and summarized in the final report.

REASON: The change to a limit test necessitated a revision in the numbers of samples collected for analytical verification.

AMENDMENT: Data Analyses, Page 9:

CHANGE: The mortality pattern in the definitive multiple concentration test will be used to calculate, if possible, the 24, 48, 72 and 96-hour LL50 concentrations. The LL50 values will be based on nominal loading rates and calculated using the computer software of C.E. Stephan (7). The program was designed to calculate the LL50 value and the 95% confidence interval by probit analysis, the moving average method, and the binomial probability method (8,9,10). The method used will depend upon the mortality pattern and its suitability for each method.

TO: In the definitive limit test, mortality will be used to determine if the 24, 48, 72 and 96-hour LL50 concentrations are greater or less than the nominal limit WAF loading rate.

REASON: Statistical analyses will not be conducted in the limit test.

Wildlife International, Ltd. Project Number 472A-113
Page 4 of 4

AMENDMENT: Table 1, Page 14:

ADD: 1-methylnaphthalene
2- methylnaphthalene

REASON: The Sponsor requested the addition of these two compounds to the list of analytes of interest.

[redacted]

22 March 2005
DATE

22 Mar 05
DATE

3/15/05
DATE

Wildlife International, Ltd.

Project Number 472A-113
Page 1 of 1

AMENDMENT TO STUDY PROTOCOL

STUDY TITLE: Petroleum Coke: A 96-Hour Static-Renewal Acute Toxicity Test with the Fathead Minnow (*Pimephales promelas*)

PROTOCOL NO.: 472/033004/FAT/SUB472 **AMENDMENT NO.:** 2

SPONSOR: American Petroleum Institute **PROJECT NO.:** 472A-113

EFFECTIVE DATE: March 18, 2005

AMENDMENT: Sampling for Analytical Measurements, Page 8 (as amended in Amendment #1):

CHANGE: Samples will be collected at mid-depth from the test chambers and stored with zero headspace until the end of the test. Samples for organic analyses should be preserved by storage at 4°C. Samples for inorganic analyses should be preserved by treating the sample with nitric acid (HNO_3) to a pH of <2.

TO: Samples will be collected at mid-depth from the test chambers and stored until the end of the test. Samples for organic analyses should be preserved by storage at 4°C with zero headspace. Samples for inorganic analyses should be preserved by the addition of sufficient nitric acid (HNO_3) to achieve a final acid concentration of 2%.

REASON: The procedures specified in Amendment #1 did not accurately reflect the procedures to be used for the preservation of samples collected for inorganic analyses.

[redacted]

24 March 2005
DATE

29 Mar 05
DATE

March 24, 2005
DATE

Wildlife International, Ltd.

Project No.: 472A-113
Page 1 of 1

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: Petroleum Coke: A 96-Hour Static-Renewal Acute Toxicity Test with the Fathead Minnow (*Pimephales promelas*)

PROTOCOL NO.: 472/033004/FAT/SUB472

DEVIATION NO.: 1

SPONSOR: American Petroleum Institute

PROJECT NO.: 472A-113

DATE OF DEVIATION: March 28 – 31, 2005

DEVIATION: Dissolved oxygen, pH and temperature were measured in the newly prepared WAF solutions of each treatment and control group, rather than in each replicate, at test initiation and at each 24-hour renewal.

REASON: Error in the protocol. In order for the test chambers to be completely filled with no headspace, no solution could be collected from the replicates at the start of each renewal period. Therefore, the measurements were taken from a sample of the batch WAF solutions. This deviation from the protocol had no adverse impact upon the results or interpretation of the study.

[redacted]

6 April 2005
DATE

7 April 05
DATE

Wildlife International, Ltd.

Project No.: 472A-113
Page 1 of 1

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: Petroleum Coke: A 96-Hour Static-Renewal Acute Toxicity Test with the Fathead Minnow (*Pimephales promelas*)

PROTOCOL NO.: 472/033004/FAT/SUB472

DEVIATION NO.: 2

SPONSOR: American Petroleum Institute

PROJECT NO.: 472A-113

DATE OF DEVIATION: March 31, 2005

DEVIATION: Water samples were not analyzed to determine the concentration of perylene, one analyte of interest in petroleum coke listed in Table 1 of the protocol.

REASON: It was determined in method development work that the analyte co-eluted with another analyte of interest, and the concentration in the water samples would not be evaluated during the definitive test. This deviation from the protocol had no adverse impact upon the results or interpretation of the study.

[redacted]

8 June 2005
DATE

8 June 05
DATE

Wildlife International, Ltd.

Project No.: 472A-113
Page 1 of 1

DEVIATION TO STUDY PROTOCOL

STUDY TITLE: Petroleum Coke: A 96-Hour Static-Renewal Acute Toxicity Test with the Fathead Minnow (*Pimephales promelas*)

PROTOCOL NO.: 472/033004/FAT/SUB472 **DEVIATION NO.:** 3

SPONSOR: American Petroleum Institute **PROJECT NO.:** 472A-113

DATE OF DEVIATION: September 12, 2005

DEVIATION: The summary of the water analysis for selected organic and inorganic constituents that was included in the study report was from the most recent water analysis, but was not from the most recent GLP-compliant analysis, as indicated in the protocol.

REASON: The periodic water analyses are no longer conducted under GLP Standards. The most recent GLP-compliant analysis was conducted in 2002. The most recent non-GLP analysis conducted in 2004 was included in the report since it was considered to be more representative of the dilution water used in the test. This deviation from the protocol had no adverse impact upon the results or interpretation of the study.

[redacted]

09 June 2006
DATE

09 June 06
DATE

Appendix 3

Test Article Selection

THE PACE CONSULTANTS INC.
Post Office Box 53473 Houston, Texas 77052 853/351-7800 Fax 853/351-7887
A Member of Jacobs Engineering Group

February 22, 2001

[redacted]
American Petroleum Institute
1220 L Street, NW
Washington, D.C. 20005-4070

Attached is Pace's report covering Task 1 and 2 entitled "U.S. Delayed Coker Petroleum Coke Quality Survey 1998-1999."

We would be pleased to answer any questions concerning this work for API. Please contact me at 832/351-7811 or email [redacted]

For PACE

[redacted]

[redacted]
Attachment

U.S. DELAYED COKER PETROLEUM COKE QUALITY SURVEY 1998-1999

INTRODUCTION

In 1998 the United States Environmental Protection Agency (EPA) challenged chemical producers and importers to provide voluntarily basic toxicity information on their high production volume (HPV) chemicals, defined as those chemicals which are produced in or imported to the U.S. in amounts greater than 1 million pounds per year. The goal of the HPV Challenge Program is to ensure that the American public has access to basic information about the hazards associated with chemicals manufactured and used in the greatest quantities in the United States. It is designed to generate the complete hazard screening data for HPV commercial chemicals.

The American Petroleum Institute (API) serves as administrator of the Petroleum HPV Testing Group, a consortium made up of 72 member companies from API, the National Petrochemical & Refiners Association (NPRA), the Gas Producers Association (GPA) and the Asphalt Institute. These companies represent 92% of the nation's refinery capacity. The Petroleum HPV Testing Group has sponsored 396 substances produced and used by the nation's petroleum industry to meet the EPA's HPV challenge.

Pace was retained by the API HPV Testing Group to assist in identifying potential sources of U.S. petroleum coke samples that could be used in the HPV testing program. As the first step in this process, Pace undertook a review of its quarterly petroleum coke production data to help characterize current U.S. petroleum coke production qualities. Pace has now completed the review of its 1998 and 1999 quarterly petroleum coke production data for all U.S.-based delayed cokers. The results of this review are discussed below.

METHODOLOGY

Pace's petroleum coke production database was used to determine quality characteristics of petroleum coke produced by U.S. refineries. Pace has conducted a survey of U.S. petroleum coker production on a quarterly basis since the second quarter of 1983. Refineries provide the bulk of the data, but some data are also gathered from other market participants. These data are maintained in a database from which the 1998 and 1999 quarterly data were extracted for this study. It was decided that data analysis would concentrate on delayed cokers (excluding needle cokers) since for 1999 our delayed coker data set includes 92+% of all the petroleum coke produced in the United States. Accordingly, fluid and Flexicokers[1] were removed from the data set.

Needle cokers were removed from the delayed coker database because needle cokers represent a special subset of delayed coking production. Needle coke differences include:

---

[1] Flexicoke is a proprietary coking process developed by Exxon. It involves partially gasifying fluid coke.

1. Needle coke quality is much higher than other delayed coke
2. Needle coke is produced using different feedstock & coking operational procedures because it is a product, not a by-product like other delayed cokes
3. The quantity of needle coke produced is very small
4. Needle coke is handled very carefully due to its high price (typically > $350/metric ton)

SUMMARY AND DATA ANALYSIS

These data were analyzed to determine the ton-weighted average petroleum coke qualities of sulfur (wt%), nickel (ppm), vanadium (ppm), and volatile material (wt%). All data are presented on a dry basis. The results are presented in Table 1 below.

TABLE 1

U.S. DELAYED PETROLEUM COKE QUALITY SUMMARY
TON-WEIGHTED QUARTERLY AVERAGES

Quarter	Sulfur, Wt% 1998	Sulfur, Wt% 1999	Nickel, ppm 1998	Nickel, ppm 1999	Vanadium, ppm 1998	Vanadium, ppm 1999	Vol. Mat., Wt% 1998	Vol. Mat., Wt% 1999
1Q	4.15	4.11	286	275	758	801	10.9	10.5
2Q	4.22	4.22	277	283	811	821	10.8	11.0
3Q	4.21	4.21	277	282	811	857	10.9	10.9
4Q	4.21	4.22	282	276	854	852	10.7	10.9
Ton-Wt Avg	4.20	4.19	280	279	809	833	10.8	10.8

Ton-weighted average qualities for each quarter were calculated in the following manner:

$$\frac{\sum (\text{quality value})_{\text{delayed coker}} * (\text{quarterly production})_{\text{delayed coker}}}{\text{Total quarterly production}}$$

Where:

quality value = sulfur, vanadium, nickel or volatile content of petroleum coke produced by each delayed coker

quarterly production = petroleum coke produced by that delayed coker

Pace next reviewed the data to determine a ton-weighted frequency distribution for each of the qualities listed. The results of this analysis are presented in Table 2 and in Figures 1 through 4.

TABLE 2

U.S. DELAYED PETROLEUM COKE QUALITY SUMMARY BY PRODUCTION QUARTILE

Cumulative Production	Sulfur, Wt% 1998	Sulfur, Wt% 1999	Nickel, ppm 1998	Nickel, ppm 1999	Vanadium, ppm 1998	Vanadium, ppm 1999	Vol, Wt% 1998	Vol, Wt% 1999
min.	0.90	0.50	50	5	45	45	7.0	4.0
25%	3.20	3.10	180	185	400	445	10.0	10.0
50%	4.45	4.60	250	250	650	675	10.7	10.7
75%	5.34	5.30	360	400	1205	1200	12.0	12.0
100%	6.90	6.30	568	568	1900	2000	14.0	14.0

Quality quartiles for each year were calculated in the following manner:

> Annual data were sorted according to each specific quality value (e.g., sulfur, vanadium, nickel, and volatile content) and the cumulative production of petroleum coke by delayed coker was calculated. Quartiles were then calculated for the annual production total, and the quality value at the cumulative total that equaled each quartile was used to determine the quality for that quartile.

TRENDS

Comparing the ton-weighted averages to the 50% production quartile (i.e., the median) reveals the following trends:

- The weighted average nickel and vanadium content of U.S. delayed petroleum coke is higher than the median. This is a direct result of the increasing amount of heavy crudes, particularly Mexican and Venezuelan crudes, processed by U.S. refineries. Because these crudes produce petroleum cokes with nickel and vanadium contents that are significantly above the median, they skew the weighted average away from the median.
- Ton-weighted sulfur content is slightly below the median because some cokers produce petroleum cokes that are well below the median sulfur content (i.e., anode-grade coke which is calcined and primarily used to make anodes for the aluminum smelting industry).

FIGURE 1

U.S. DELAYED PETROLEUM COKE SULFUR CONTENT

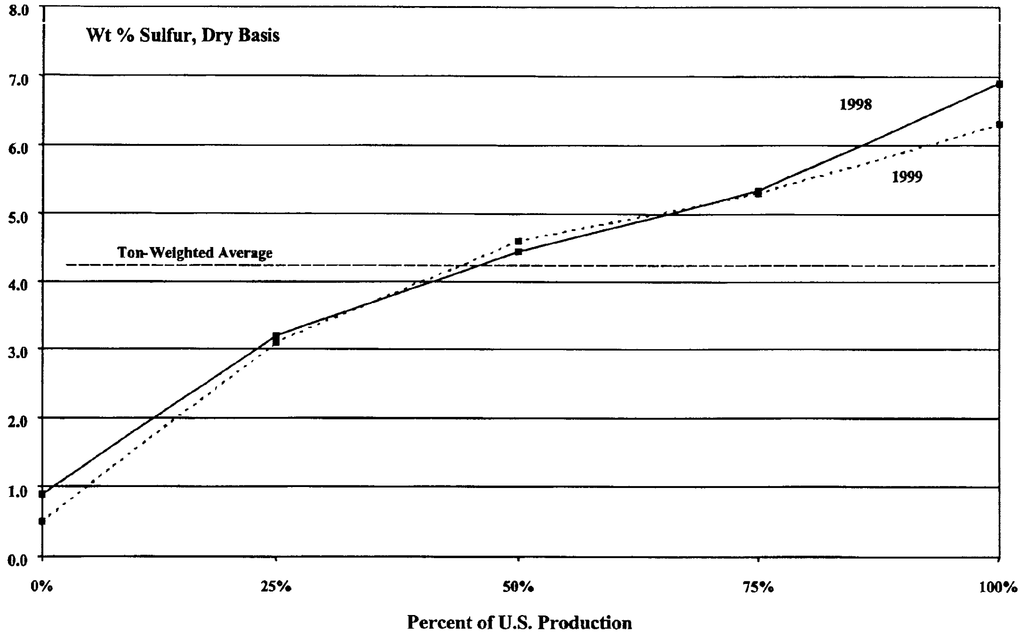

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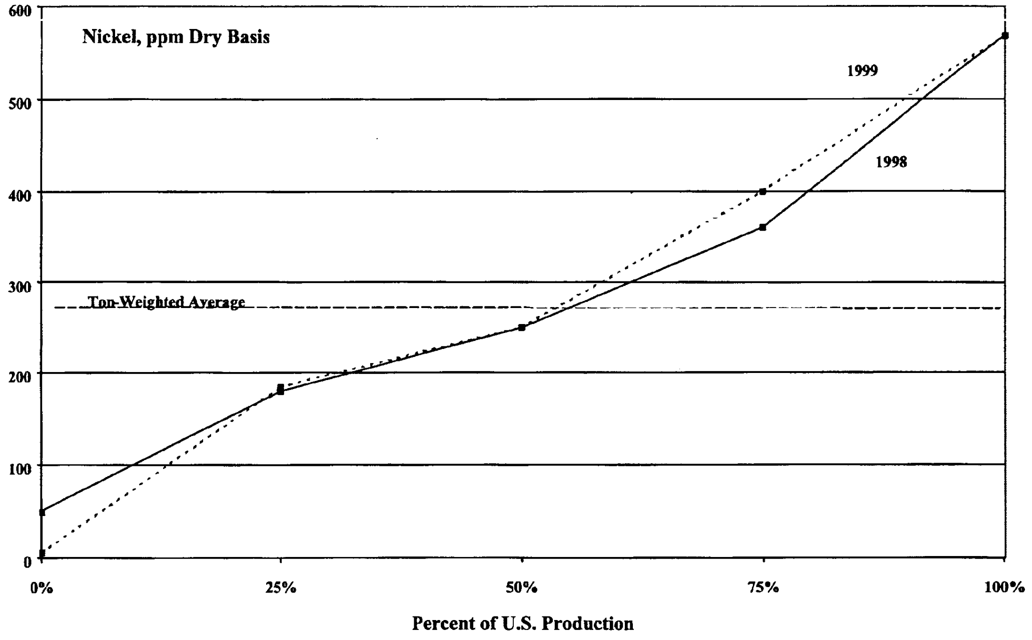
FIGURE 2

U.S. DELAYED PETROLEUM COKE NICKEL CONTENT



THE PACE CONSULTANTS INC.

FIGURE 3

U.S. DELAYED PETROLEUM COKE VANADIUM CONTENT

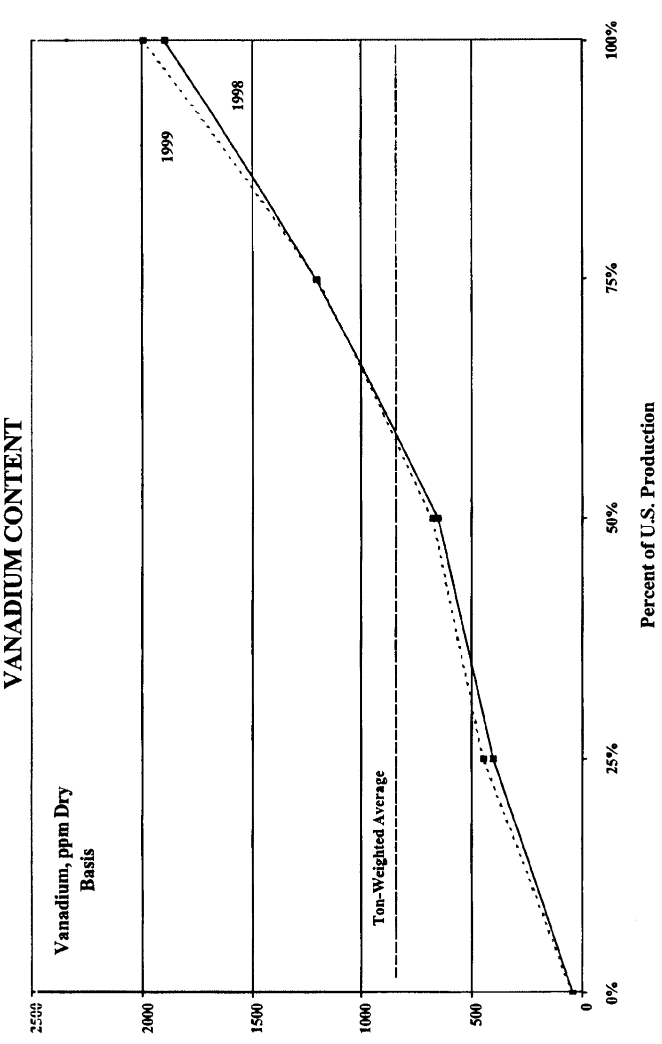


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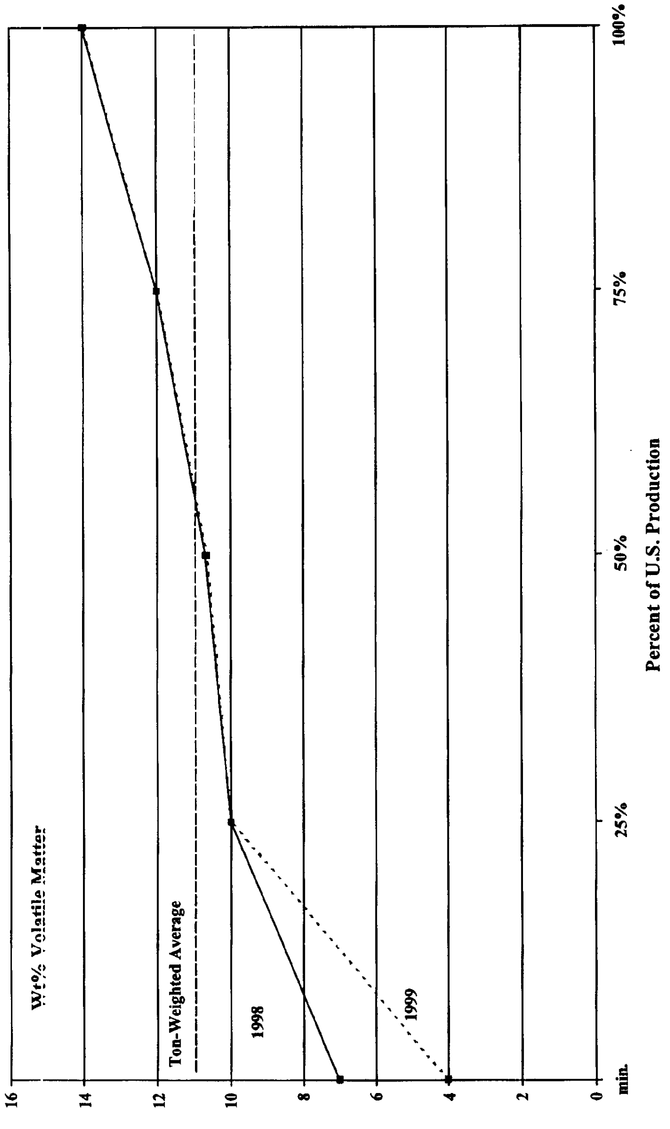
FIGURE 4
U.S. DELAYED PETROLEUM COKE VOLATILE MATTER CONTENT



THE PACE CONSULTANTS INC.

- The sulfur content at the upper and lower ends of the quality spectrum was better in 1999 than in 1998. We believe the lower sulfur content in 1999 was a result of crude production cut-backs by OPEC (Organization of Petroleum Exporting Countries) and other crude oil producers. These producers preferentially reduced the production of their lower quality crude oils in order to minimize the production reductions of their higher quality (i.e. higher priced) crude oils. We see 1999 as an aberration in the general trend of increasing sulfur content in U.S. petroleum cokes.

- We expect the metals content and sulfur content of U.S. petroleum coke will deteriorate beginning in 2001 as new U.S. cokers scheduled to begin operations in the 2000-2002 time frame start up.

- The average volatile matter content is essentially equal to the median.

RECOMMENDATIONS

Pace identified candidate refineries for sampling based on the quality data from the third quarter of 2000, which is the most recent quarter for which data are available. It should be noted that these data may vary slightly from the 1998-1999 averages as increasing amounts of heavy crude are processed. Based on these data, Pace recommends the following candidates for sampling in support of the Petroleum HPV Testing Program:

PETROLEUM HPV TESTING PROGRAM
DELAYED PETROLEUM COKE SAMPLE CANDIDATES

	Candidate A		Candidate B		Candidate C	
	Value	Percentile	Value	Percentile	Value	Percentile
Sulfur, Wt%	6.00	93	5.75	86	5.50	80
Nickel, ppm	500	90	300	58	250	50
Vanadium, ppm	1,500	84	1,200	75	1,000	65
Volatiles, Wt%	10.00	25	12.00	75	13.00	88

PETROLEUM HPV TESTING PROGRAM
DELAYED PETROLEUM COKE SAMPLE CANDIDATES

	Candidate D			Candidate E	
	Value	Percentile		Value	Percentile
Sulfur, Wt%	4.20	43		5.50	80
Nickel, ppm	250	50		350	67
Vanadium, ppm	1,500	84		1,100	70
Volatiles, Wt%	15.00	100		10.00	25

Our analysis indicates that some compromises will have to be made in obtaining a sample for the HPV program since no refinery's petroleum coke is in the upper 75th percentile in all four quality parameters we have evaluated. Additionally, we have spent some time and effort trying to find petroleum cokes which are sampled with automatic sampling equipment that has been bias tested and is operated by an independent laboratory. Unfortunately, we have found that the locations with the best sampling systems have petroleum cokes of generally better quality. Therefore, we do not believe that we will be able to find a "perfect" candidate petroleum coke.

While the sampling at the candidate refineries may not be ideal, the sampling and analysis data have been used for commercial transactions. Substantial quantities of petroleum coke from each of the candidate refineries have been sold in the petroleum coke market. Commercial transactions have relied on the laboratory results for determining quality bonus and penalties and conformance with contract quality specifications. Thus, the samples taken for the HPV study would conform to generally accepted industry sampling practice.

The sampling plan would be to have the sample analyzed for the quality parameters used in this screening analysis (i.e. sulfur, vanadium, nickel, volatile matter) as well as four other commonly tested quality parameters—gross calorific value (Btu/lb), moisture (%), ash (%), and Hardgrove Grindability Index (HGI)–to verify that the sample obtained is similar to the anticipated quality characteristics. This plan would assure that the sample submitted for detailed HPV testing conforms to our quality expectations.

We may not be able to receive authorization from a refinery to use a sample of their petroleum coke for the HPV test. Our present plan would be to approach Refineries B and C regarding obtaining a sample. In the event that these two refineries choose not to participate, then the choice would be either refinery A or E, which have high sulfur and metals but low volatile content or refinery D, which has high vanadium and volatile matter but low sulfur content. (note: each of the five candidate refineries has a different corporate owner).

Pace requests that the HPV Committee confirm Pace's recommended plan to approach refineries B and C regarding obtaining an HPV sample. It is not necessary for the HPV committee to decide now on the preferred refinery to contact in the event that refineries B and C do not wish to participate in the program. However, we would suggest that the committee begin to think about this issue so that decisions can be made expeditiously in the event that refineries B and C choose not participate.

Appendix 4

AVEKA, Inc. Particle Processing Report

AVEKA, Inc.
PARTICLE PROCESSING & CUSTOM RESEARCH

Date: May 29, 2003

Make Order #: 5369

Company Name: API

Contact Person: [redacted]

Material: Green Petroleum Coke

Objective: Task 1: Hammermill, Ball-mill and Classify Petroleum Coke to a mean particle size less than 3.6 microns. Task 2: Crush and Classify petroleum coke to a mean particle size of 2 mm.

Equipment: Homoloid JT Hammermill (SN # JT-694) with 0.0093 screen
5 Gallon Ball-mill with 0.25 inch alumina media
Majac A-12 classifier
Horiba LA-910 Laser Light Scattering Particle Sizer
Marcy 4"x 6" Jaw Crusher
Gilson Sonic Sieve

Receipt: Approximately 80 lbs. of material was received 3-19-03 from Federal Express. Confirmation of receipt (EPL Project Identification 1203-001) was returned upon delivery.

Storage: Petroleum coke was stored at room temperature in sealed polyethylene bags when the material was not being processed.

Processing Procedure:

The green petroleum coke showed high moisture content upon inspection. The high moisture content was indicated by condensation on the inside of the received petroleum coke bags. After consulting with Deborah Herron and Jacobs Consultancy, the material was dried according to ASTM D 3302-00 (Standard Test Method for Total Moisture in Coal).

Task 1

All processes were run at room temperature. The dried petroleum coke was then run through a Homoloid JT Hammermill (SN # JT-694) equipped with a 0.0093 screen.

The resulting hammermilled powder was loaded into 5-gallon ball mills loaded with 0.25 inch ceramic (alumina) media. The loading level in the ball mill was 27 lbs. of media with 5.5 lbs. of petroleum coke.

The mills were rotated at 36 rpm for 17.25 hours. The resulting powder had a mean particle size of 9.56 microns (Attch 1) when tested with the Horiba LA-910 in water.

The oversized petroleum coke material was removed using a Majac A-12 Classifier. The Majac was run at 1800 RPM and 8.5 cfm. The resulting particle size of the petroleum coke was a 3.3 micron mean (Attch. 2) when tested with the Horiba LA-910 in water. The Horiba LA-910 test method for the petroleum coke samples is outlined in Attch. 3.

The final yield of product was 10.5 kg of powder.

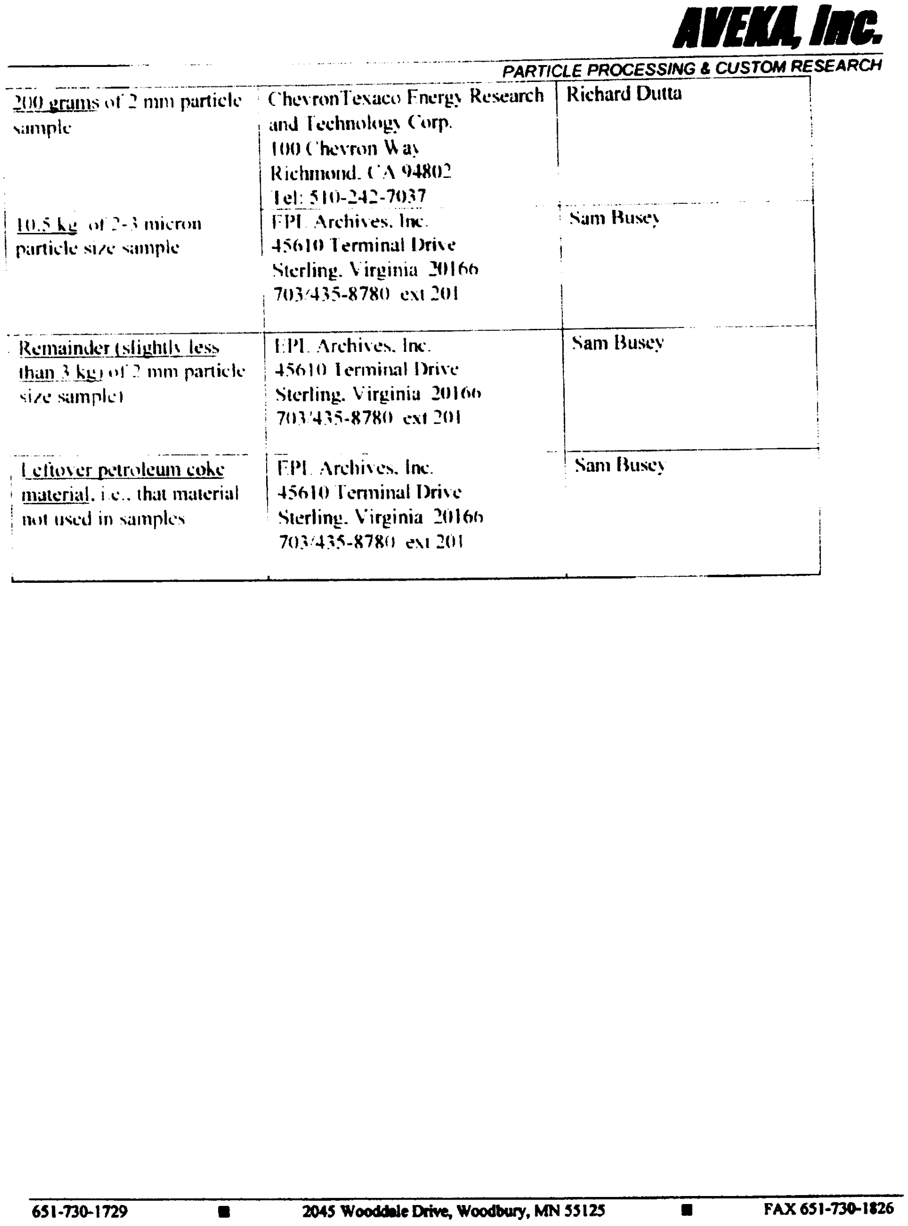
Task 2

All processes were run at room temperature. An 18" Sweco Screener was set-up with a 7 mesh (2.8 mm) top-screen and a 14 mesh (1.4 mm) bottom-screen. Petroleum coke was fed through the screener and 2-mm material was collected from between the top and bottom screen. Oversized petroleum coke was jaw crushed with a Marcy 4"x 6" Jaw Crusher and rescreened. A Gilson Sonic Sieve particle size analysis (Attch. 4) was run on the screened petroleum coke and the results showed 99.4 % of the material between 1.4 mm – 2.8 mm. Final yield was 3.3 kg of 2 mm Petroleum Coke.

Shipping

All samples were shipped UPS Ground. The following is a summary of the sample disposition.

Sample/Amount	Address	Person
200 grams of 2-3 micron particle size sample	ChevronTexaco Energy Research and Technology Corp. 100 Chevron Way Richmond, CA 94802 Tel: 510-242-7037	Richard Dutta



AVEKA, Inc.

PARTICLE PROCESSING & CUSTOM RESEARCH

200 grams of 2 mm particle sample	ChevronTexaco Energy Research and Technology Corp. 100 Chevron Way Richmond, CA 94802 Tel: 510-242-7037	Richard Dutta
10.5 kg of 2-3 micron particle size sample	EPI Archives, Inc. 45610 Terminal Drive Sterling, Virginia 20166 703/435-8780 ext 201	Sam Busey
Remainder (slightly less than 3 kg) of 2 mm particle size sample)	EPI Archives, Inc. 45610 Terminal Drive Sterling, Virginia 20166 703/435-8780 ext 201	Sam Busey
Leftover petroleum coke material, i.e., that material not used in samples	EPI Archives, Inc. 45610 Terminal Drive Sterling, Virginia 20166 703/435-8780 ext 201	Sam Busey

651-730-1729 ■ 2045 Wooddale Drive, Woodbury, MN 55125 ■ FAX 651-730-1826

Attch 1

HORIBA LA-910

PARTICLE SIZE DISTRIBUTION DATA TABLE Standard 04/23/03

File Name: 5369001.DAT Sample Name: Ballmilled 17.25 Hours **ID No: **/04/23-350**

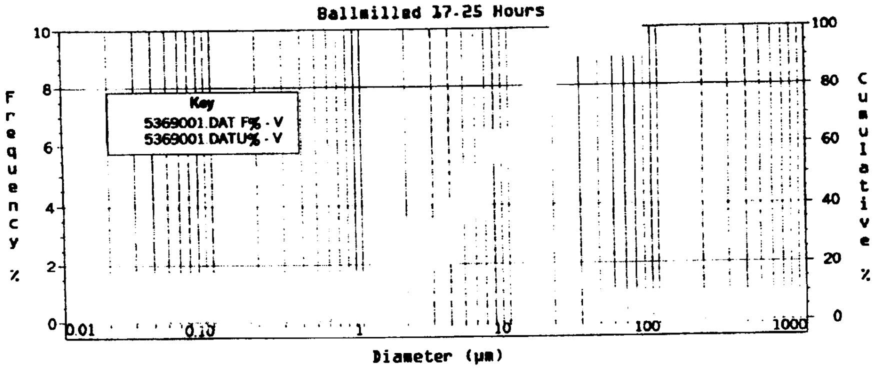
Dist. Form: STANDARD R.R. Index: co.rrjD

Dist. Mode: VOLUME Laser: 65.128 % Lamp: 61.185 %

Circulation: 2 Agitation: 7 U.Sonic ** (min)

Material: Petroleum Coke Source: American Petroleum

Lot No: MO5369 Test No: 5369001



					SIZE(µm)	FREQ%	UNDR%	No.	SIZE(µm)	FREQ%	UNDR%
(1)	0.020	0.0	0.0	(28)	0.766	0.5	0.8	(55)	29.907	1.5	95.4
(2)	0.022	0.0	0.0	(29)	0.877	0.8	1.6	(56)	34.255	1.2	96.6
(3)	0.026	0.0	0.0	(30)	1.005	1.2	2.8	(57)	39.234	0.9	97.5
(4)	0.029	0.0	0.0	(31)	1.151	1.6	4.4	(58)	44.938	0.7	98.2
(5)	0.034	0.0	0.0	(32)	1.318	2.0	6.3	(59)	51.471	0.5	98.8
(6)	0.039	0.0	0.0	(33)	1.510	2.3	8.7	(60)	58.953	0.4	99.2
(7)	0.044	0.0	0.0	(34)	1.729	2.6	11.3	(61)	67.523	0.3	99.5
(8)	0.051	0.0	0.0	(35)	1.981	2.9	14.2	(62)	77.340	0.2	99.7
(9)	0.058	0.0	0.0	(36)	2.269	3.0	17.2	(63)	88.582	0.2	99.9
(10)	0.067	0.0	0.0	(37)	2.599	3.2	20.4	(64)	101.460	0.1	100.0
(11)	0.076	0.0	0.0	(38)	2.976	3.5	23.9	(65)	116.210	0.0	100.0
(12)	0.087	0.0	0.0	(39)	3.409	3.7	27.7	(66)	133.103	0.0	100.0
(13)	0.100	0.0	0.0	(40)	3.905	4.0	31.6	(67)	152.453	0.0	100.0
(14)	0.115	0.0	0.0	(41)	4.472	4.3	35.9	(68)	174.616	0.0	100.0
(15)	0.131	0.0	0.0	(42)	5.122	4.6	40.6	(69)	200.000	0.0	100.0
(16)	0.150	0.0	0.0	(43)	5.867	5.1	45.6	(70)	229.075	0.0	100.0
(17)	0.172	0.0	0.0	(44)	6.720	5.5	51.2	(71)	262.376	0.0	100.0
(18)	0.197	0.0	0.0	(45)	7.697	5.9	57.0	(72)	300.518	0.0	100.0
(19)	0.226	0.0	0.0	(46)	8.816	6.0	63.0	(73)	344.205	0.0	100.0
(20)	0.259	0.0	0.0	(47)	10.097	5.8	68.8	(74)	394.244	0.0	100.0
(21)	0.296	0.0	0.0	(48)	11.565	5.4	74.2	(75)	451.556	0.0	100.0
(22)	0.339	0.0	0.0	(49)	13.246	4.8	79.1	(76)	517.200	0.0	100.0
(23)	0.389	0.0	0.0	(50)	15.172	4.2	83.3	(77)	592.387	0.0	100.0
(24)	0.445	0.0	0.0	(51)	17.377	3.5	86.8	(78)	678.504	0.0	100.0
(25)	0.510	0.0	0.0	(52)	19.904	2.9	89.7	(79)	777.141	0.0	100.0
(26)	0.584	0.1	0.1	(53)	22.797	2.3	92.0	(80)	890.116	0.0	100.0
(27)	0.669	0.2	0.3	(54)	26.111	1.9	93.9	(81)	1019.510	0.0	100.0

```
Median    :   6.533 (µm)  Mean:    9.561 (µm)  Mode:    8.237 (µm)
Std. Dev.:  10.531 (µm)  Span:   10.623
Coef. Var: 110.14%        Spec. Area:  15308 (cm2/cm3)
```

Attch. 2

HORIBA LA-910

PARTICLE SIZE DISTRIBUTION DATA TABLE Standard 05/15/03

File Name: 5369011.DAT Sample Name: Fines ID No: **/04/30-566

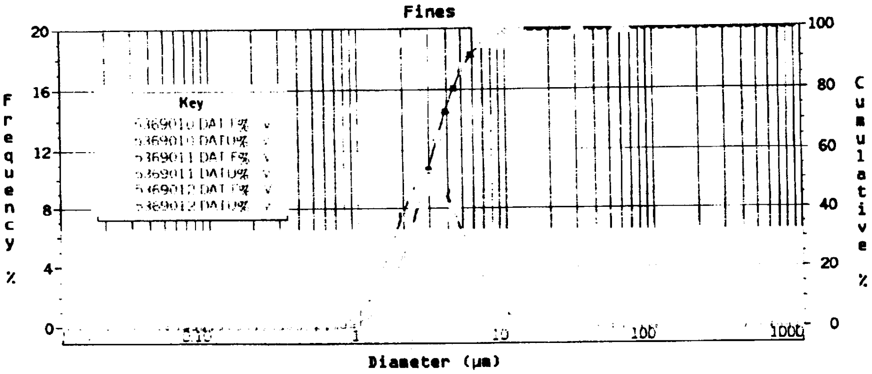
Dist. Form: STANDARD R.R. Index: co.rrj□

Dist. Mode: VOLUME Laser: 85.118 % Lamp: 86.338 %

Circulation: 3 Agitation: 7 U.Sonic OFF (min)

Material: Petroleum Coke Source: American Petroleum

Lot No: MO5369 Test No: 5369004



No.	SIZE (µm)	FREQ%	UNDR%
(1)	0.020	0.0	0.0
(2)	0.022	0.0	0.0
(3)	0.026	0.0	0.0
(4)	0.029	0.0	0.0
(5)	0.034	0.0	0.0
(6)	0.039	0.0	0.0
(7)	0.044	0.0	0.0
(8)	0.051	0.0	0.0
(9)	0.058	0.0	0.0
(10)	0.067	0.0	0.0
(11)	0.076	0.0	0.0
(12)	0.087	0.0	0.0
(13)	0.100	0.0	0.0
(14)	0.115	0.0	0.0
(15)	0.131	0.0	0.0
(16)	0.150	0.0	0.0
(17)	0.172	0.0	0.0
(18)	0.197	0.0	0.0
(19)	0.226	0.0	0.0
(20)	0.259	0.0	0.0
(21)	0.296	0.0	0.0
(22)	0.339	0.0	0.0
(23)	0.389	0.0	0.0
(24)	0.445	0.0	0.0
(25)	0.510	0.0	0.0
(26)	0.584	0.0	0.0
(27)	0.669	0.0	0.0
(28)	0.766	0.2	0.2
(29)	0.877	0.5	0.7
(30)	1.005	1.0	1.7
(31)	1.151	1.7	3.5
(32)	1.318	2.8	6.3
(33)	1.510	4.3	10.6
(34)	1.729	5.9	16.5
(35)	1.981	7.6	24.1
(36)	2.269	9.0	33.0
(37)	2.599	10.1	43.1
(38)	2.976	10.6	53.7
(39)	3.409	10.2	63.8
(40)	3.905	9.0	72.9
(41)	4.472	7.6	80.4
(42)	5.122	6.0	86.5
(43)	5.867	4.6	91.1
(44)	6.720	3.4	94.5
(45)	7.697	2.3	96.8
(46)	8.816	1.5	98.3
(47)	10.097	0.9	99.1
(48)	11.565	0.5	99.6
(49)	13.246	0.2	99.9
(50)	15.172	0.1	100.0
(51)	17.377	0.0	100.0
(52)	19.904	0.0	100.0
(53)	22.797	0.0	100.0
(54)	26.111	0.0	100.0
(55)	29.907	0.0	100.0
(56)	34.255	0.0	100.0
(57)	39.234	0.0	100.0
(58)	44.938	0.0	100.0
(59)	51.471	0.0	100.0
(60)	58.953	0.0	100.0
(61)	67.523	0.0	100.0
(62)	77.340	0.0	100.0
(63)	88.582	0.0	100.0
(64)	101.460	0.0	100.0
(65)	116.210	0.0	100.0
(66)	133.103	0.0	100.0
(67)	152.453	0.0	100.0
(68)	174.616	0.0	100.0
(69)	200.000	0.0	100.0
(70)	229.075	0.0	100.0
(71)	262.376	0.0	100.0
(72)	300.518	0.0	100.0
(73)	344.205	0.0	100.0
(74)	394.244	0.0	100.0
(75)	451.556	0.0	100.0
(76)	517.200	0.0	100.0
(77)	592.387	0.0	100.0
(78)	678.504	0.0	100.0
(79)	777.141	0.0	100.0
(80)	890.116	0.0	100.0
(81)	1019.510	0.0	100.0

Median : 2.840 (µm) Mean: 3.289 (µm) Mode: 2.781 (µm)
Std. Dev.: 1.844 (µm) Span: 2.780
Coef. Var: 56.08% Spec. Area: 23790 (cm2/cm3)

Attch. 3

TEST METHOD FOR API PETROLEUM COKE

Sample Preparation

May 15, 2003

Mix 0.15 – 0.2 grams of petroleum coke with 5-6 grams distilled water. Add TX-100 surfactant to aid dispersion. Mix thoroughly until no large concentrations of sample are evident.

LA-910 Preparation

Fill the test chamber to capacity with 140 ml distilled water. Add 3-4 drops of TX-100 surfactant from a 10% concentrate source, resulting in approximately a .1% diluted total. Select the relative refractive index appropriate for this material (1.61-3.02i). Circulate the solvent using a pump speed of 2-3, subtract the background. Add the sample drop by drop until the laser transmission falls into the acceptable range (70 – 95)% transmittance. Activate the sonicator to aid dispersion, cease sonication when sample is completely dispersed.

Sample Test

Measure the sample three times. Save each measurement. Overlay the three measurements on a graph. If they appear stable, the test is complete. If not, investigate. A steady increase in the laser transmission rate indicates more particles are present from pass to pass. That indicates the sample was not completely dispersed yet. A steady decrease in the laser transmission rate indicates the sample is agglomerating, settling, or dissolving.

Report

Using the Display module, graph the three test runs over one another. A stable test will appear as one line, an unstable condition will clearly show all three runs, indicating instability. If stable, select a run (typically the middle run) and print the complete data table along with the graph.

Author: T.J. Roberts
Lab Manager
Aveka, Inc.
(651) 714-4293 ext 208

Attch. 4

Sample ID: American Petroleum Institute
2mm Pet.Coke

Sieve Analysis

5/29/03

US Standard Mesh Size	Mesh Opening (Microns)	Sieve Weight (Grams)	Sieve Weight + Sample (g)	Weight of Sample (g)	% Sample Above Sieve	[illegible] Under Sieve
7	**2800**	50 951	50 975	0 024	0.31	99.69
8	**2360**	50 741	52 146	1 405	18.18	81.51
10	**2000**	48 772	51 024	2.252	29.14	52.37
12	**1700**	47.324	50.173	2 849	36.86	15.51
14	**1400**	48.450	49 624	1 174	15.19	0.32
catch	**0**	220.018	220.043	0 025	0.32	0.00
			Totals:	7.729	100.00	

Notes/Comments:

MO5369a.xls

Appendix 5

Laboratory Characterization of 2 mm Particle Size Petroleum Coke

Analysis Report

Lancaster Laboratories

ANALYTICAL RESULTS

Prepared for:

Chevron Products Company
940 Hensley St. Bldg. 210

Richmond CA 94801
510-242-8191

Prepared by:

Lancaster Laboratories
2425 New Holland Pike
Lancaster, PA 17605-2425

SAMPLE GROUP

The sample group for this submittal is 857532. Samples arrived at the laboratory on Friday, June 27, 2003. The PO# for this group is 99011184 and the release number is [redacted]

Client Description	**Lancaster Labs Number**
Pet Coke 2mm Solid Sample	**4073301**
Pet Coke Micronized Solid Sample	**4073302**

1 COPY TO Lancaster Laboratories
1 COPY TO Chevron CRTC

[redacted]

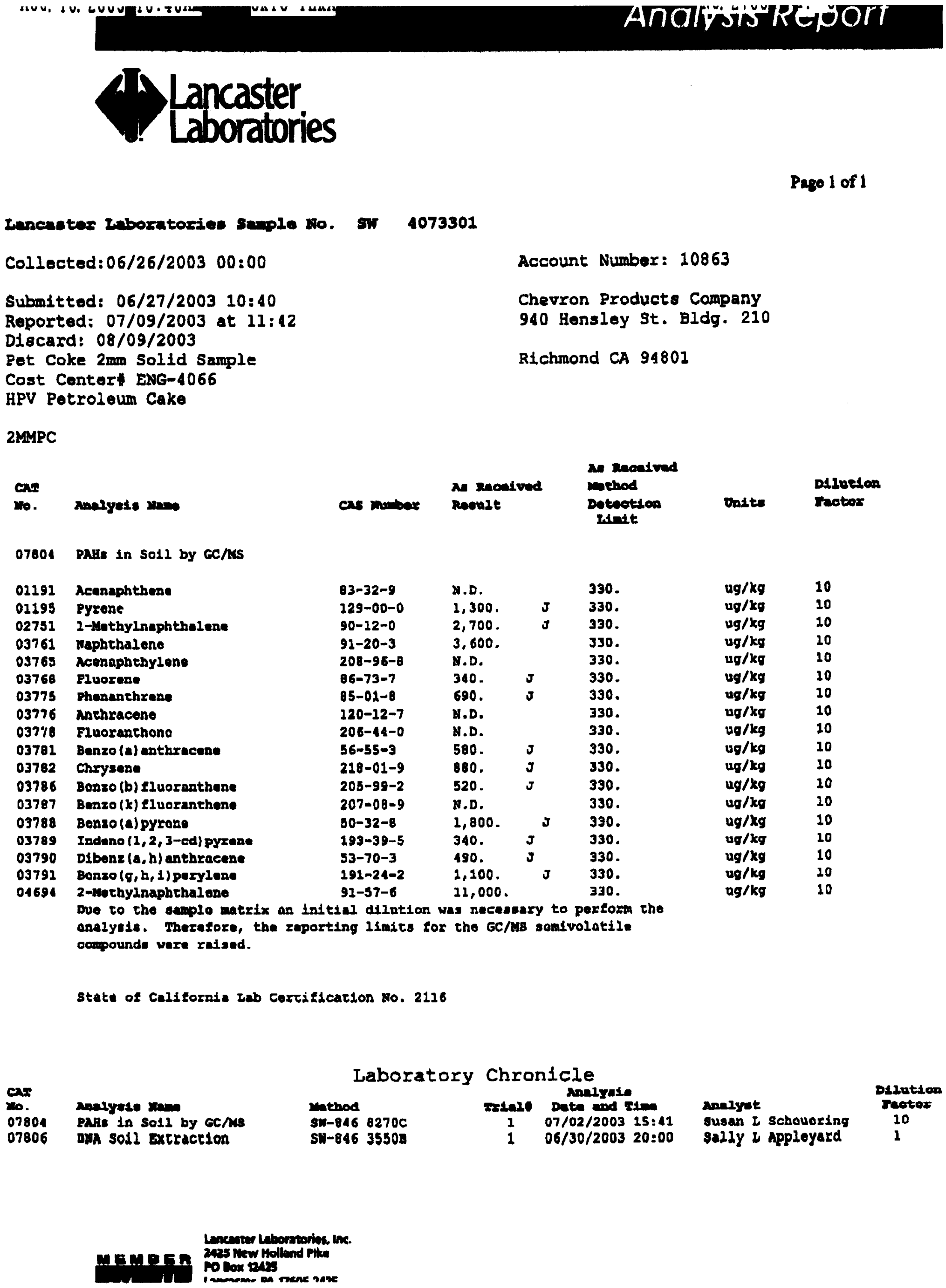
Questions? Contact your Client Services Representative
Alison M O'Connor at (717) 656-2300.

Respectfully Submitted,

[redacted]

A4200

3.66



Analysis Report

Lancaster Laboratories

Page 1 of 1

Lancaster Laboratories Sample No. SW 4073301

Collected:06/26/2003 00:00

Submitted: 06/27/2003 10:40
Reported: 07/09/2003 at 11:42
Discard: 08/09/2003
Pet Coke 2mm Solid Sample
Cost Center# ENG-4066
HPV Petroleum Cake

2MMPC

Account Number: 10863

Chevron Products Company
940 Hensley St. Bldg. 210

Richmond CA 94801

CAT No.	Analysis Name	CAS Number	As Received Result		As Received Method Detection Limit	Units	Dilution Factor
07804	PAHs in Soil by GC/MS						
01191	Acenaphthene	83-32-9	N.D.		330.	ug/kg	10
01195	Pyrene	129-00-0	1,300.	J	330.	ug/kg	10
02751	1-Methylnaphthalene	90-12-0	2,700.	J	330.	ug/kg	10
03761	Naphthalene	91-20-3	3,600.		330.	ug/kg	10
03765	Acenaphthylene	208-96-8	N.D.		330.	ug/kg	10
03768	Fluorene	86-73-7	340.	J	330.	ug/kg	10
03775	Phenanthrene	85-01-8	690.	J	330.	ug/kg	10
03776	Anthracene	120-12-7	N.D.		330.	ug/kg	10
03778	Fluoranthene	206-44-0	N.D.		330.	ug/kg	10
03781	Benzo(a)anthracene	56-55-3	580.	J	330.	ug/kg	10
03782	Chrysene	218-01-9	880.	J	330.	ug/kg	10
03786	Benzo(b)fluoranthene	205-99-2	520.	J	330.	ug/kg	10
03787	Benzo(k)fluoranthene	207-08-9	N.D.		330.	ug/kg	10
03788	Benzo(a)pyrene	50-32-8	1,800.	J	330.	ug/kg	10
03789	Indeno(1,2,3-cd)pyrene	193-39-5	340.	J	330.	ug/kg	10
03790	Dibenz(a,h)anthracene	53-70-3	490.	J	330.	ug/kg	10
03791	Benzo(g,h,i)perylene	191-24-2	1,100.	J	330.	ug/kg	10
04694	2-Methylnaphthalene	91-57-6	11,000.		330.	ug/kg	10

Due to the sample matrix an initial dilution was necessary to perform the analysis. Therefore, the reporting limits for the GC/MS semivolatile compounds were raised.

State of California Lab Certification No. 2116

Laboratory Chronicle

CAT No.	Analysis Name	Method	Trial#	Analysis Date and Time	Analyst	Dilution Factor
07804	PAHs in Soil by GC/MS	SW-846 8270C	1	07/02/2003 15:41	Susan L Schouering	10
07806	BNA Soil Extraction	SW-846 3550B	1	06/30/2003 20:00	Sally L Appleyard	1

MEMBER

Lancaster Laboratories, Inc.
2425 New Holland Pike
PO Box 12425

Analysis Report

Lancaster Laboratories

Page 1 of 2

Quality Control Summary

Client Name: Chevron Products Company Group Number: 857532
Reported: 07/09/03 at 11:42 AM

Laboratory Compliance Quality Control

Analysis Name	Blank Result	Blank MDL	Report Units	LCS %REC	LCSD %REC	LCS/LCSD Limits	RPD	RPD Max
Batch number: 03181SLA026	Sample number(s): 4073301-4073302							
Acenaphthene	N.D.	33.	ug/kg	91		76-109		
Pyrene	N.D.	33.	ug/kg	89		71-110		
1-Methylnaphthalene	N.D.	33.	ug/kg	87		76-101		
Naphthalene	N.D.	33.	ug/kg	87		73-103		
Acenaphthylene	N.D.	33.	ug/kg	94		73-106		
Fluorene	N.D.	33.	ug/kg	93		66-115		
Phenanthrene	N.D.	33.	ug/kg	88		70-107		
Anthracene	N.D.	33.	ug/kg	86		71-107		
Fluoranthene	N.D.	33.	ug/kg	90		69-107		
Benzo(a)anthracene	N.D.	33.	ug/kg	93		74-107		
Chrysene	N.D.	33.	ug/kg	89		72-109		
Benzo(b)fluoranthene	N.D.	33.	ug/kg	95		71-113		
Benzo(k)fluoranthene	N.D.	33.	ug/kg	97		75-112		
Benzo(a)pyrene	N.D.	33.	ug/kg	94		79-111		
Indeno(1,2,3-cd)pyrene	N.D.	33.	ug/kg	88		74-113		
Dibenz(a,h)anthracene	N.D.	33.	ug/kg	95		81-118		
Benzo(g,h,i)perylene	N.D.	33.	ug/kg	92		74-114		
2-Methylnaphthalene	N.D.	33.	ug/kg	90		70-102		

Sample Matrix Quality Control

Analysis Name	MS %REC	MSD %REC	MS/MSD Limits	RPD	RPD MAX	BKG Conc	DUP Conc	DUP RPD	Dup RPD Max
Batch number: 03181SLA026	Sample number(s): 4073301-4073302								
Acenaphthene	107	93	48-132	14	30				
Pyrene	82	68	28-144	12	30				
1-Methylnaphthalene	75	67*	72-100	5	30				
Naphthalene	77	61	38-132	9	30				
Acenaphthylene	108	91	46-128	18	30				
Fluorene	88	75	39-137	14	30				
Phenanthrene	88	74	29-143	13	30				
Anthracene	101	85	35-138	17	30				
Fluoranthene	81	72	19-148	11	30				
Benzo(a)anthracene	89	75	26-144	14	30				
Chrysene	101	90	23-150	9	30				
Benzo(b)fluoranthene	90	74	32-140	16	30				
Benzo(k)fluoranthene	103	88	36-143	16	30				
Benzo(a)pyrene	90	72	23-154	13	30				
Indeno(1,2,3-cd)pyrene	92	78	13-155	15	30				
Dibenz(a,h)anthracene	110	88	19-163	19	30				
Benzo(g,h,i)perylene	99	83	17-152	13	30				
2-Methylnaphthalene	38	19*	32-133	6	30				

*- Outside of specification
(1) The result for one or both determinations was less than five times the LOQ.
(2) The background result was more than four times the spike added.

Analysis Report

Lancaster Laboratories

Page 2 of 2

Quality Control Summary

Client Name: Chevron Products Company Group Number: 857532
Reported: 07/09/03 at 11:42 AM

Sample Matrix Quality Control

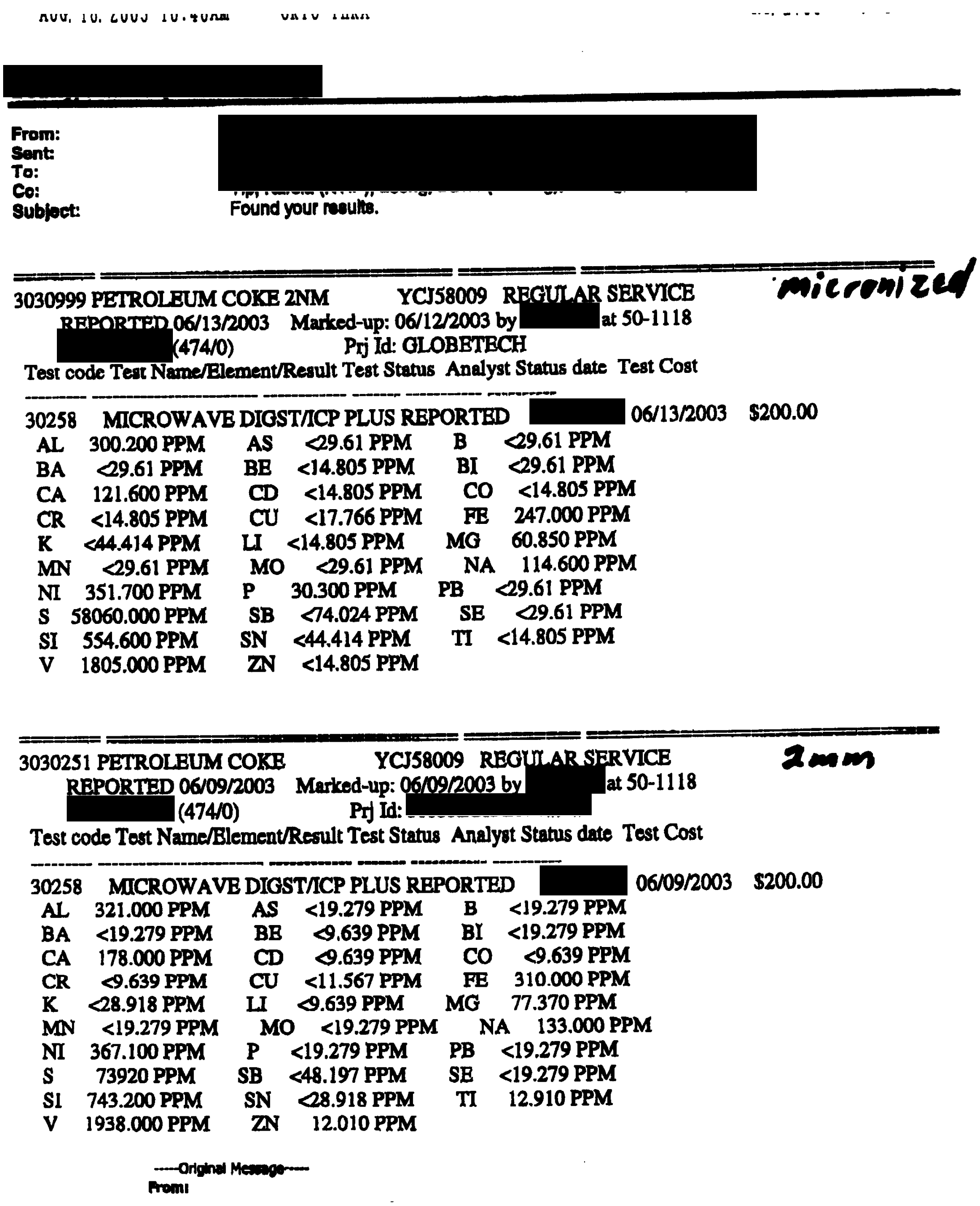
Analysis Name	MS %REC	MSD %REC	MS/MSD Limits	RPD	RPD MAX	BKG Conc	DUP Conc	DUP RPD	Dup RPD Max

Surrogate Quality Control

Analysis Name: PAHs in Soil by GC/MS
Batch number: 031818LA026

	Nitrobenzene-d5	2-Fluorobiphenyl	Terphenyl-d14
4073301	101	108	92
4073302	101	99	84
Blank	87	85	83
LCS	94	92	93
MS	105	107	88
MSD	90	90	78
Limits:	47-128	53-123	39-128

*- Outside of specification
(1) The result for one or both determinations was less than five times the LOQ.
(2) The background result was more than four times the spike added.



AUG. 18. 2003 10:40AM [illegible]

[redacted]

From:
Sent:
To:
Cc:
[redacted]
Subject: Found your results.

3030999 PETROLEUM COKE 2NM YCJ58009 REGULAR SERVICE micronized
REPORTED 06/13/2003 Marked-up: 06/12/2003 by [redacted] at 50-1118
[redacted] (474/0) Prj Id: GLOBETECH
Test code Test Name/Element/Result Test Status Analyst Status date Test Cost

30258 MICROWAVE DIGST/ICP PLUS REPORTED [redacted] 06/13/2003 $200.00

AL	300.200 PPM	AS	<29.61 PPM	B	<29.61 PPM
BA	<29.61 PPM	BE	<14.805 PPM	BI	<29.61 PPM
CA	121.600 PPM	CD	<14.805 PPM	CO	<14.805 PPM
CR	<14.805 PPM	CU	<17.766 PPM	FE	247.000 PPM
K	<44.414 PPM	LI	<14.805 PPM	MG	60.850 PPM
MN	<29.61 PPM	MO	<29.61 PPM	NA	114.600 PPM
NI	351.700 PPM	P	30.300 PPM	PB	<29.61 PPM
S	58060.000 PPM	SB	<74.024 PPM	SE	<29.61 PPM
SI	554.600 PPM	SN	<44.414 PPM	TI	<14.805 PPM
V	1805.000 PPM	ZN	<14.805 PPM		

3030251 PETROLEUM COKE YCJ58009 REGULAR SERVICE 2mm
REPORTED 06/09/2003 Marked-up: 06/09/2003 by [redacted] at 50-1118
[redacted] (474/0) Prj Id: [redacted]
Test code Test Name/Element/Result Test Status Analyst Status date Test Cost

30258 MICROWAVE DIGST/ICP PLUS REPORTED [redacted] 06/09/2003 $200.00

AL	321.000 PPM	AS	<19.279 PPM	B	<19.279 PPM
BA	<19.279 PPM	BE	<9.639 PPM	BI	<19.279 PPM
CA	178.000 PPM	CD	<9.639 PPM	CO	<9.639 PPM
CR	<9.639 PPM	CU	<11.567 PPM	FE	310.000 PPM
K	<28.918 PPM	LI	<9.639 PPM	MG	77.370 PPM
MN	<19.279 PPM	MO	<19.279 PPM	NA	133.000 PPM
NI	367.100 PPM	P	<19.279 PPM	PB	<19.279 PPM
S	73920 PPM	SB	<48.197 PPM	SE	<19.279 PPM
SI	743.200 PPM	SN	<28.918 PPM	TI	12.910 PPM
V	1938.000 PPM	ZN	12.010 PPM		

-----Original Message-----
From:

1

Appendix 6

Specific Conductance, Hardness, Alkalinity and pH of Well Water Measured
During the 4-Week Period Immediately Preceding the Test

Parameter	Mean	Range
Specific Conductance (µmhos/cm)	294 (N = 4)	275 – 305
Hardness (mg/L as $CaCO_3$)	131 (N = 4)	128 – 136
Alkalinity (mg/L as $CaCO_3$)	178 (N = 4)	174 – 180
pH	8.4 (N = 4)	8.3 – 8.4

Appendix 7

Analyses of Pesticides, Organics and Metals in Wildlife International, Ltd. Well Water[1]

Pesticides and Organics			
Component	Measured Concentration (µg/L)	Component	Measured Concentration (µg/L)
Aldrin	< 0.0099	Heptachlor Epoxide	< 0.0099
Alpha BHC	< 0.0099	Malathion	< 2.0
Beta BHC	< 0.040	Merphos	< 2.0
Bolstar	< 2.0	Methoxychlor	< 0.099
Chlordane	< 0.50	Methyl Parathion	< 2.0
Coumaphos	< 3.0	Mevinphos	< 2.0
Delta BHC	< 0.0099	Mirex	< 0.050
Demeton-O	< 2.0	Naled	< 3.0
Demeton-S	< 2.0	o,p-DDD	< 0.020
Diazinon	< 2.0	o,p-DDE	< 0.020
Dichlorvos	< 2.0	o,p-DDT	< 0.020
Dieldrin	< 0.020	p,p-DDD	< 0.020
Disulfoton	< 2.0	p,p-DDE	< 0.020
Dursban (Chlorpyrifos)	< 2.0	p,p-DDT	< 0.025
Endosulfan I	< 0.0099	PCB-1016	< 0.50
Endosulfan II	< 0.042	PCB-1221	< 1.2
Endosulfan Sulfate	< 0.020	PCB-1232	< 0.89
Endrin	< 0.020	PCB-1242	< 0.50
EPN	< 4.0	PCB-1248	< 0.50
Ethion	< 2.0	PCB-1254	< 0.50
Ethoprop	< 2.0	PCB-1260	< 0.50
Ethyl Parathion	< 2.0	Phorate	< 2.0
Famphur	< 2.0	Ronnel	< 2.0
Fensulfothion	< 4.0	Stirophos	< 2.0
Fenthion	< 2.0	Telodrin	< 0.0099
Gamma BHC – Lindane	< 0.0099	Tokuthion	< 2.0
Guthion (Azinphos-methyl)	< 4.0	Toxaphene	< 0.99
HCB	< 0.099	Trichloronate	< 2.0
Heptachlor	< 0.0099	Trithion	< 2.0

[1] Analyses performed by Lancaster Laboratories on samples collected on December 22, 2004.

Appendix 7 (Continued)

Analyses of Pesticides, Organics and Metals in Wildlife International, Ltd. Well Water[1]

Metals			
Component	Measured Concentration (mg/L)	Component	Measured Concentration (mg/L)
Aluminum	< 0.200	Magnesium	12.7
Antimony	< 0.0200	Manganese	< 0.0050
Arsenic	< 0.0100	Mercury	< 0.00020
Barium	< 0.0050	Nickel	< 0.0100
Beryllium	< 0.0050	Nitrate Nitrogen	< 0.50
Bromide	< 2.5	Nitrite Nitrogen	< 0.50
Cadmium	< 0.0050	Potassium	6.64
Calcium	31.1	Selenium	< 0.0100
Chloride	6.9	Silver	< 0.0050
Chromium	< 0.0050	Sodium	19.7
Cobalt	< 0.0050	Sulfate	5.5
Copper	< 0.0100	Thallium	< 0.0200
Fluoride	< 0.50	Vanadium	< 0.0050
Iron	< 0.200	Zinc	< 0.0200
Lead	< 0.0200		

[1] Analyses performed by Lancaster Laboratories on samples collected on December 22, 2004.

Appendix 8

Results of WAF Equilibration Trial

Polyaromatic Hydrocarbon Analysis

Water accommodated fractions (WAFs) were analyzed for the presence of the 19 polyaromatic hydrocarbons (PAHs) after mixing for 24, 48, 72 and 96 hours. No PAHs were detected in any WAF samples. Results of PAH analyses in WAFs are presented in Wildlife International, Ltd. Project Number 472C-104.

Metals Analysis

Water accommodated fractions (WAFs) were analyzed for the presence of the six metals and sulfur after mixing for 24, 48, 72 and 96 hours. Except for a trace of iron contamination in one test vessel, no metals were detected in any WAF samples. Sulfur was not detected above the background level in the freshwater used. Results of the metals analyses in WAFs are presented in Wildlife International, Ltd. Project Number 472C-105.

Appendix 9

The Analysis of Organic Constituents in Petroleum Coke in Freshwater

Appendix 9.1

Analytical Method Flowchart for the Analysis of PAH Components of Petroleum Coke in Freshwater by HPLC

FLOWCHART FOR THE ANALYSIS OF WATER SOLUBLE COMPONENTS OF PETROLEUM COKE IN FRESHWATER

Prepare samples in freshwater using volumetric flasks, volumetric pipettes, gas tight syringes and culture tubes. Freshwater served as the matrix blanks.

↓

Dilute samples, as necessary, with freshwater such that the final sample concentrations fall within the calibration standard range.

↓

Transfer samples and standards to autosampler vials for analysis by either HPLC/UV or fluorescence detection.

Appendix 9.2

Typical HPLC Operational Parameters

INSTRUMENT:	Agilent Model 1100 High Performance Liquid Chromatograph (HPLC) with a either an Agilent Series 1100 Variable Wavelength Detector or a Jasco Model FP-1520 Fluorescence Detector
ANALYTICAL COLUMN:	YMC-Pack ODS AM (150 mm x 4.6 mm, 3 µm particle size)
STOP TIME:	35 minutes
FLOW RATE:	1.00 mL/minute
OVEN TEMPERATURE:	40°C
MOBILE PHASE:	SOLVENT A: 0.1% H_3PO_4 SOLVENT B: CH_3CN

GRADIENT:

Time (min)	%A	%B	Flow (mL/min)
0.01	40.0	60.0	1.000
1.00	40.0	60.0	1.000
30.00	0.0	100.0	1.000
30.10	40.0	60.0	1.000
35.00	40.0	60.0	1.000

INJECTION VOLUME: 100 µL

APPROXIMATE RETENTION TIMES:

Naphthalene = 6.8 min.
Acenaphthylene = 7.8 min.
1-Methylnaphthalene = 8.9 min.
2-Methylnaphthalene = 9.2 min.
Fluorene = 9.8 min.
Acenaphthene = 9.9 min.
Phenanthrene = 10.7 min.
Anthracene = 11.4 min.
Fluoranthene = 13.0 min.
Pyrene = 13.8 min.
Chrysene = 16.2 min.
Benz(a)anthracene = 16.4 min.
Benzo(b)fluoranthene = 19.2 min.
Benzo(k)fluoranthene = 19.5 min.
Benzo(a)pyrene = 20.2 min.
Dibenz(a,h,)anthracene = 21.8 min.
Indeno(1,2,3-cd)pyrene = 23.1 min.
Benzo(g,h,i)perylene = 23.4 min.
Dibenzo(a,e)pyrene = 25.2 min.

PRIMARY ANALYTICAL WAVELENGTHS: UV = 220 nm; Fluorescence = 340 nm to 425 nm

Appendix 9.3

Analytical Stocks and Standards Preparation

For all compounds received from AccuStandard, with the exception of Benzo(g,h,i)perylene, Benzo(k)fluoranthene and Fluoranthene, the mass received was quantitatively transferred to a 100-mL class A volumetric flask using methanol. These primary stock solution concentrations were 0.100 mg/mL. Benzo(g,h,i)perylene, Benzo(k)fluoranthene and Fluoranthene were quantitatively transferred to a 200-mL class A volumetric flask using methanol. These primary stock solution concentrations were 0.05 mg/mL.

A stock of Dibenzo(a.e)pyrene (received from Cambridge Isotope Labs) was prepared by weighing 0.01000 g on an analytical balance, transferred to a 100-mL class A volumetric flask and brought to volume using tetrahydrofuran. This primary stock solution concentration was 0.100 mg/mL.

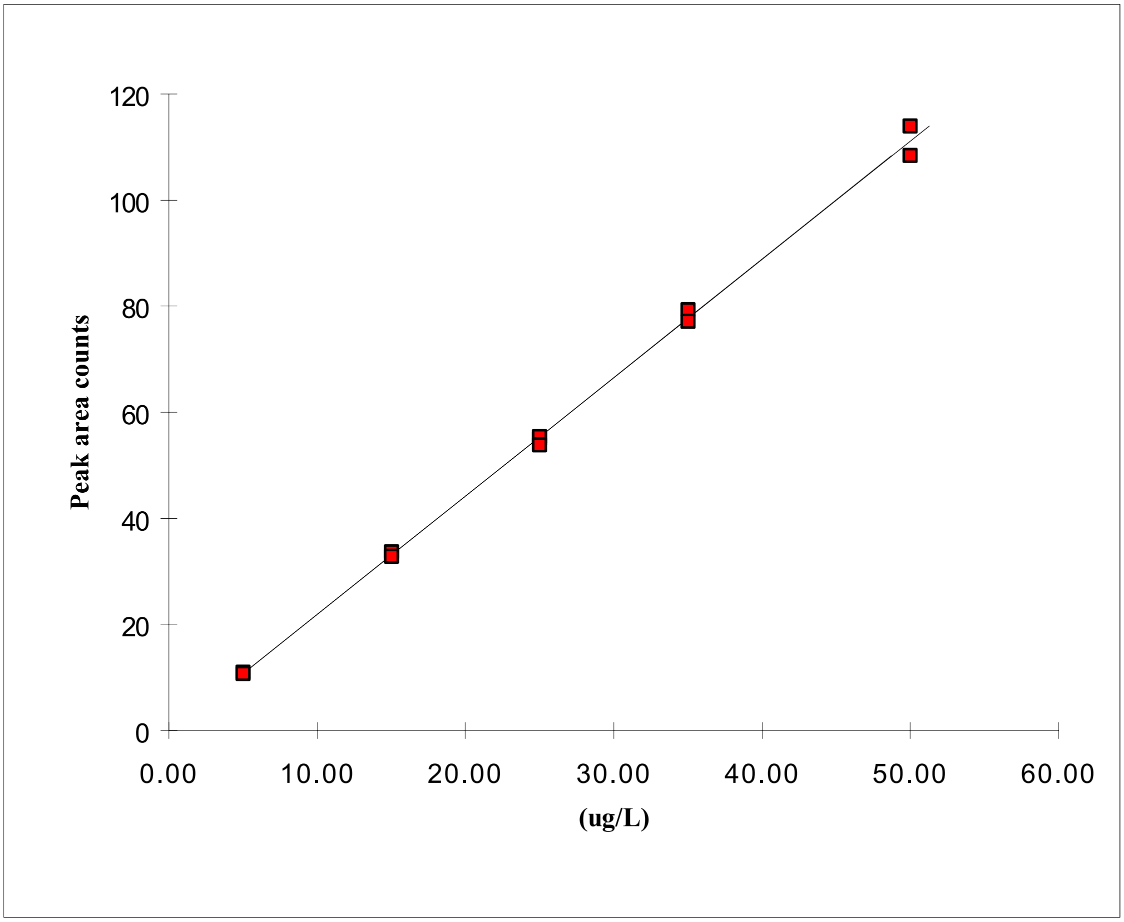
Stocks of 2-methylnaphthylene and 1-methynaphthylene (received from ChemService) were prepared by weighing 0.1004 g and 0.1003 g, respectively, on an analytical balance. The test materials were transferred to 100-mL class A volumetric flasks and brought to volume using methanol. These primary stock solutions contained 1.00 mg/mL of the test material and were diluted in methanol to prepare 0.100 mg/mL stock solutions.

Aliquots (1.00 mL) of the 0.100 mg/mL primary stocks and 2.00 mL of the 0.05 mg/L, were added to a 100-mL class A volumetric flask and brought to volume with methanol. The following shows the dilution scheme for the set of calibration standards:

Stock Concentration (mg/L)	Aliquot (µL)	Final Volume (mL)	Standard Concentration (µg/L)
1.00	50.0	10.0	5.00
1.00	150	10.0	15.0
1.00	250	10.0	25.0
1.00	350	10.0	35.0
1.00	500	10.0	50.0

Appendix 9.4

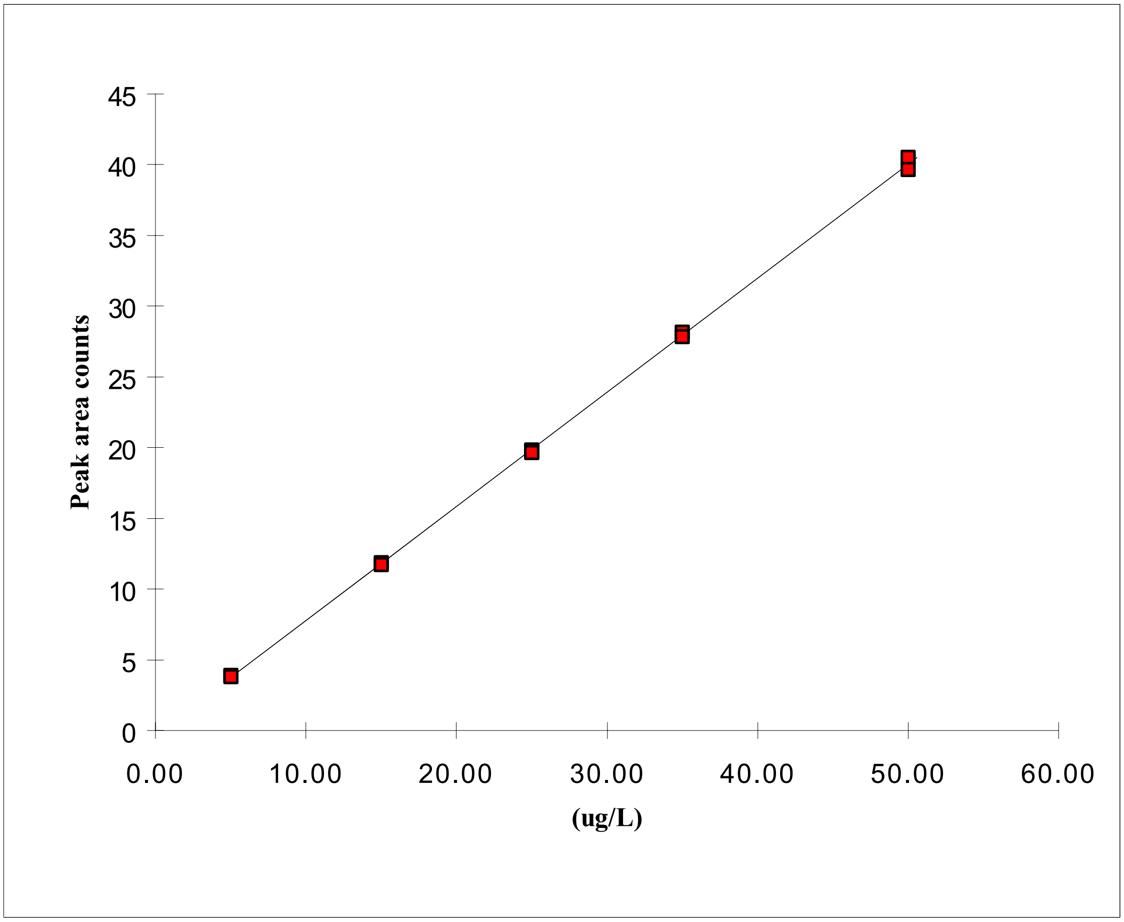
Calibration Curve for Naphthalene Analyzed by HPLC/UV



Slope = 2.2322; Y-intercept = -0.4404; R^2 = 0.9982

Appendix 9.5

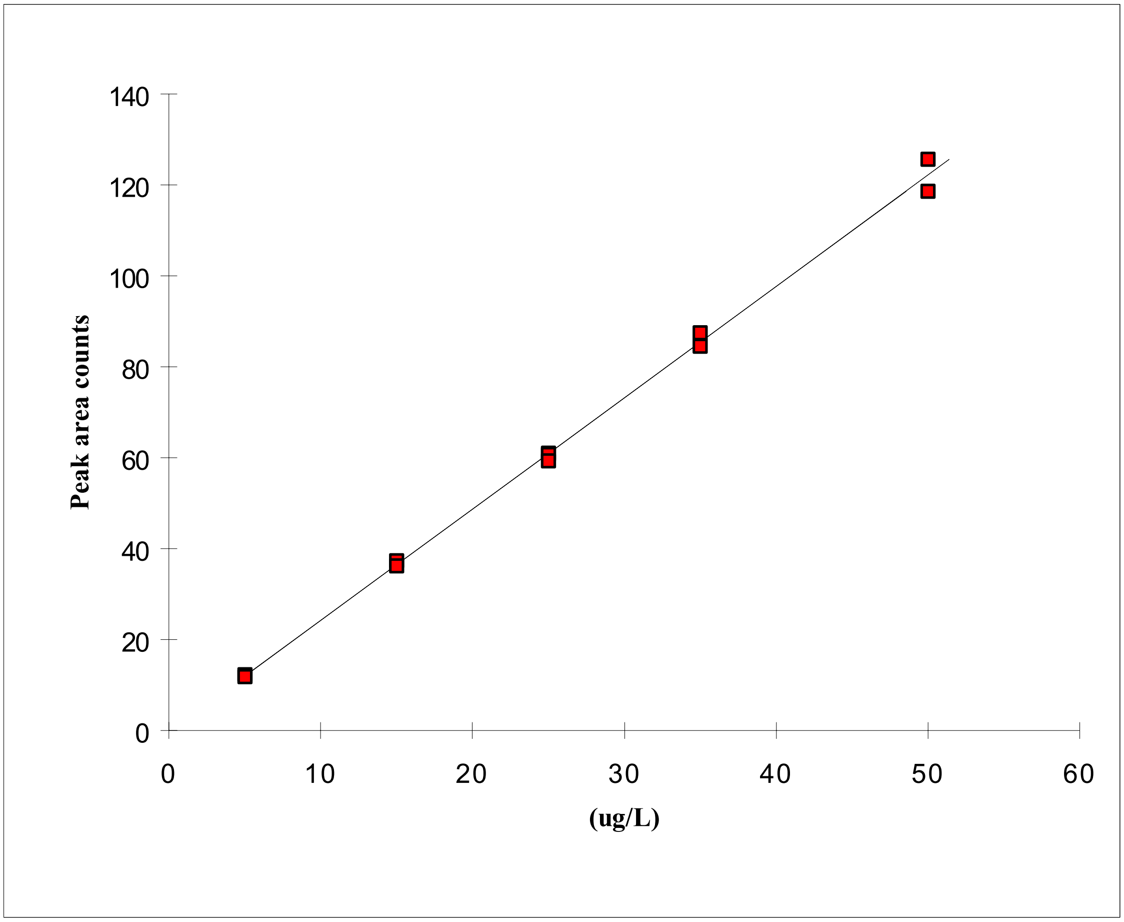
Calibration Curve for Acenaphthylene Analyzed by HPLC/UV



Slope = 0.8067; Y-intercept = -0.2906; R^2 = 0.9997

Appendix 9.6

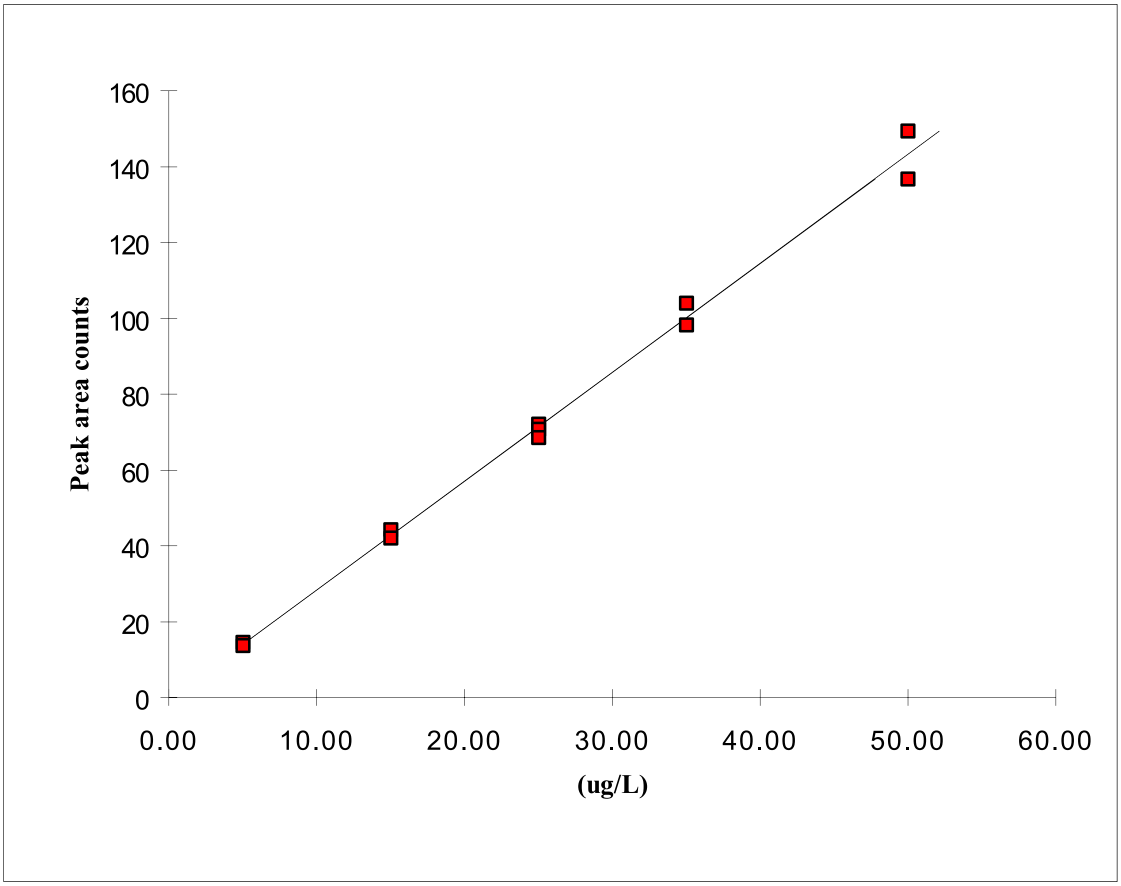
Calibration Curve for 1-Methylnaphthalene Analyzed by HPLC/UV



Slope = 2.4497; Y-intercept = -0.3127; R^2 = 0.9978

Appendix 9.7

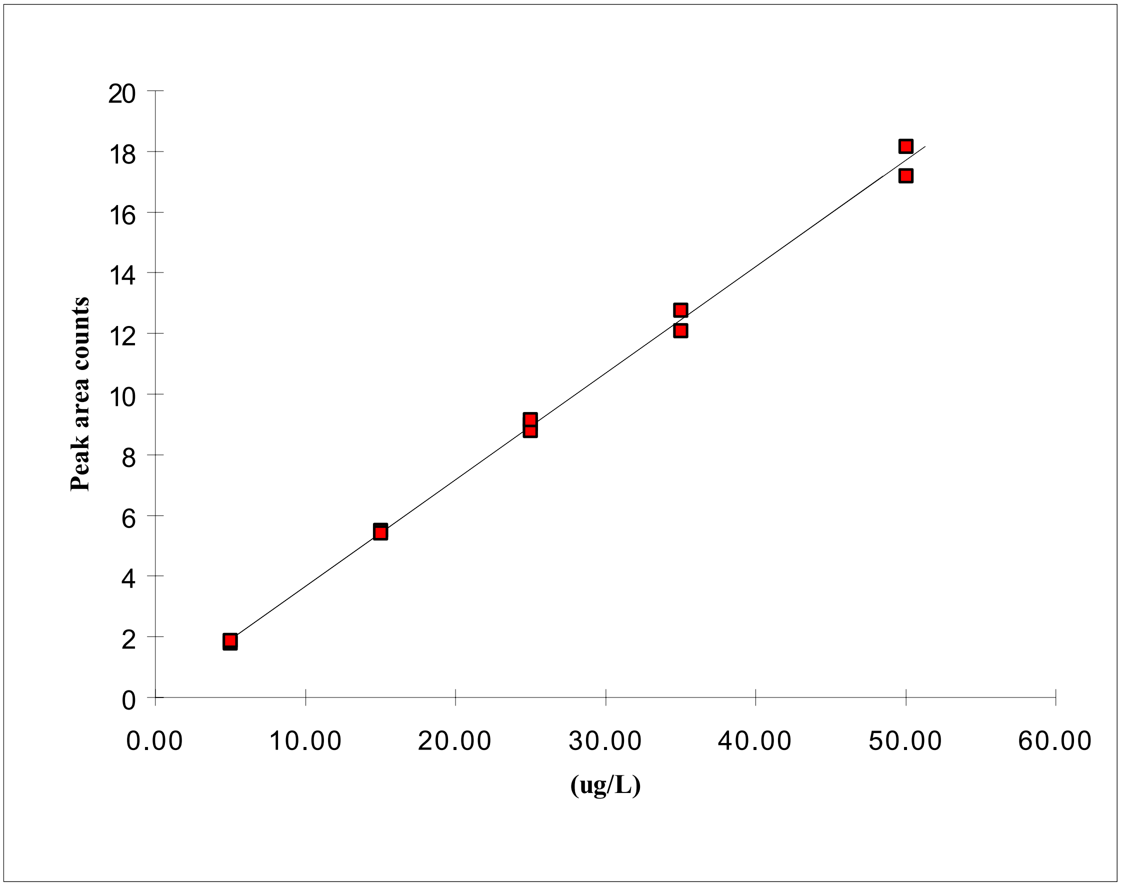
Calibration Curve for 2-Methylnaphthalene Analyzed by HPLC/UV



Slope = 2.8707; Y-intercept = -0.3799; R^2 = 0.9946

Appendix 9.8

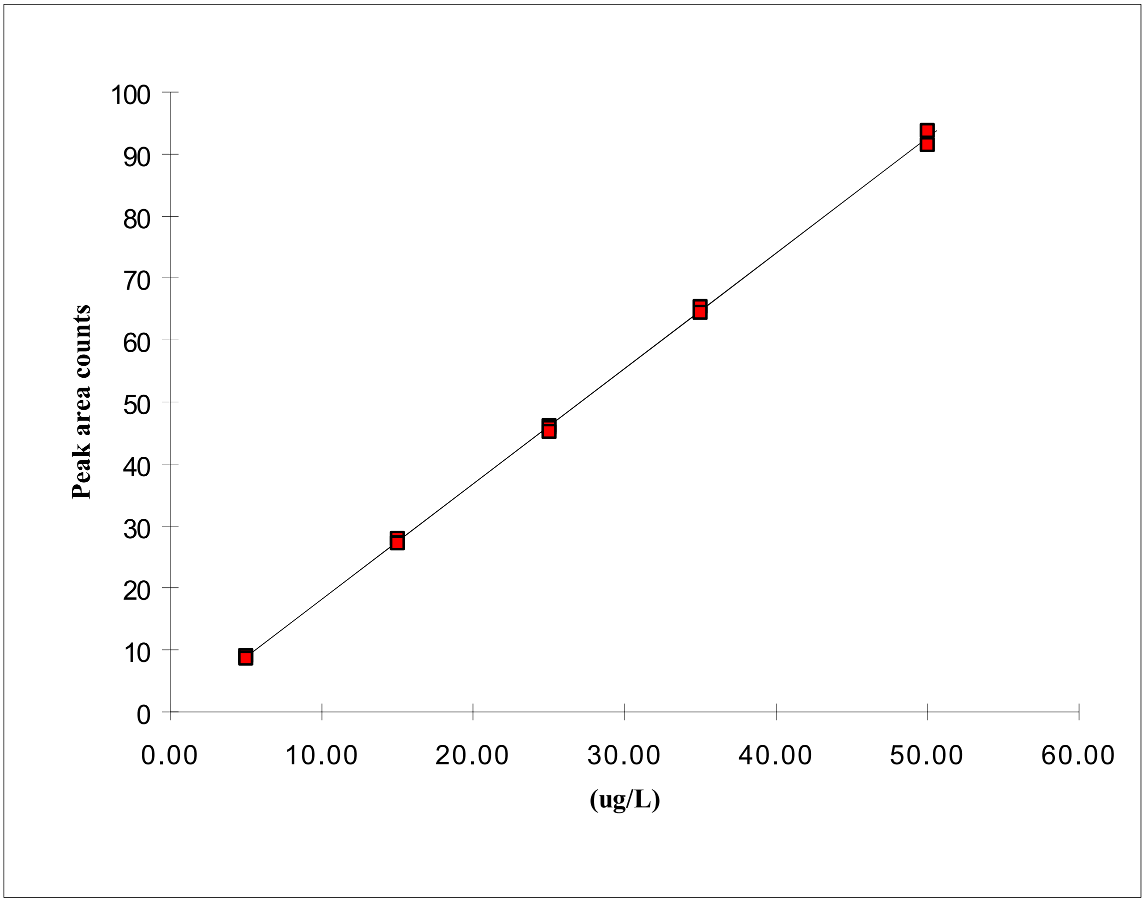
Calibration Curve for Fluorene Analyzed by HPLC/UV



Slope = 0.3512; Y-intercept = 0.1531; R^2 = 0.9973

Appendix 9.9

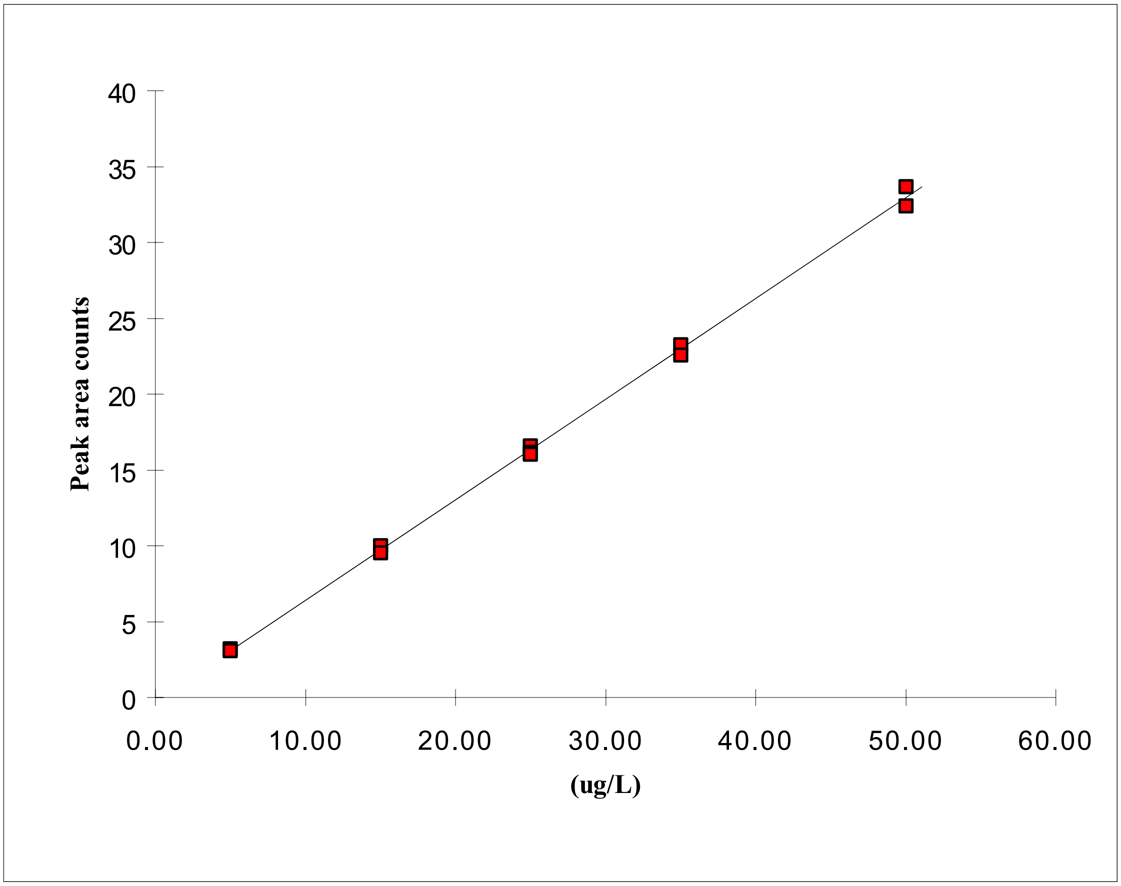
Calibration Curve for Acenaphthene Analyzed by HPLC/UV



Slope = 1.8616; Y-intercept = -0.4561; $R^2 = 0.9995$

Appendix 9.10

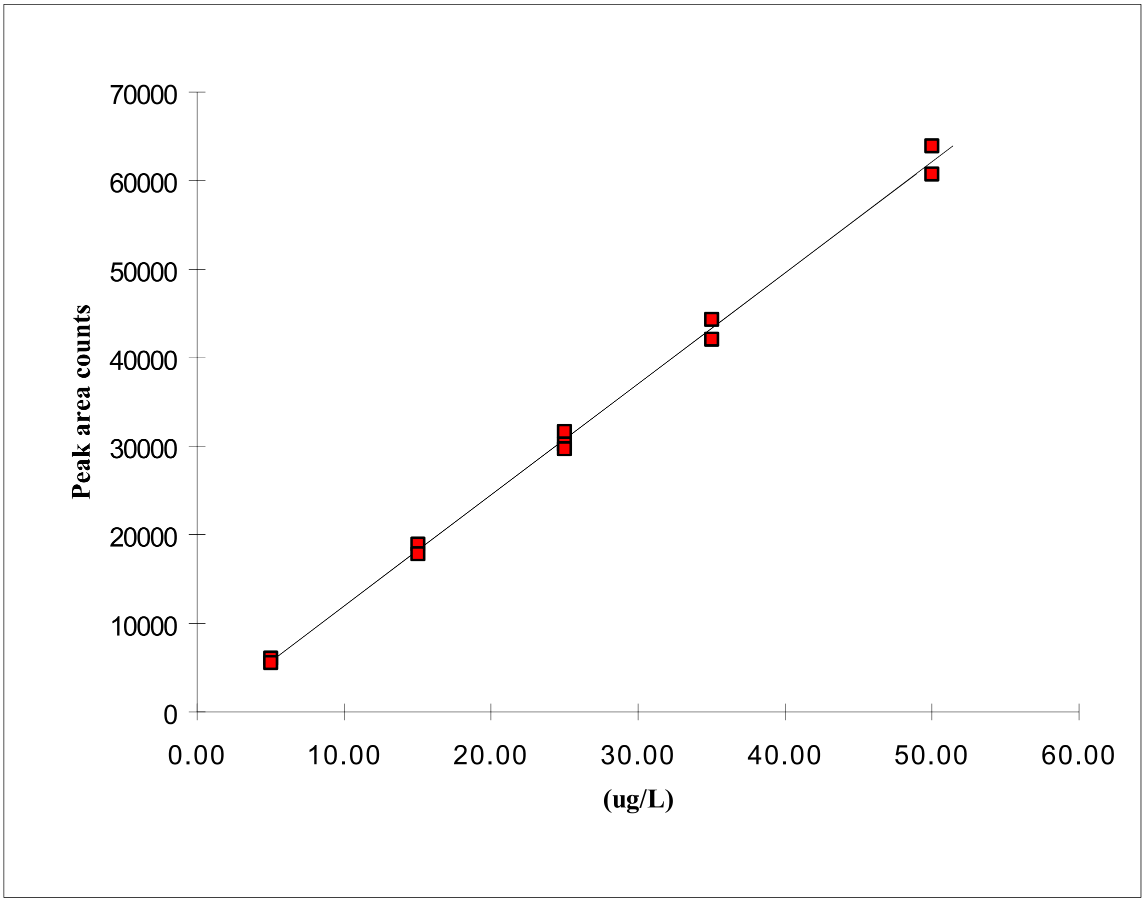
Calibration Curve for Phenanthrene Analyzed by HPLC/UV



Slope = 0.6635; Y-intercept = -0.2373; R^2 = 0.9987

Appendix 9.11

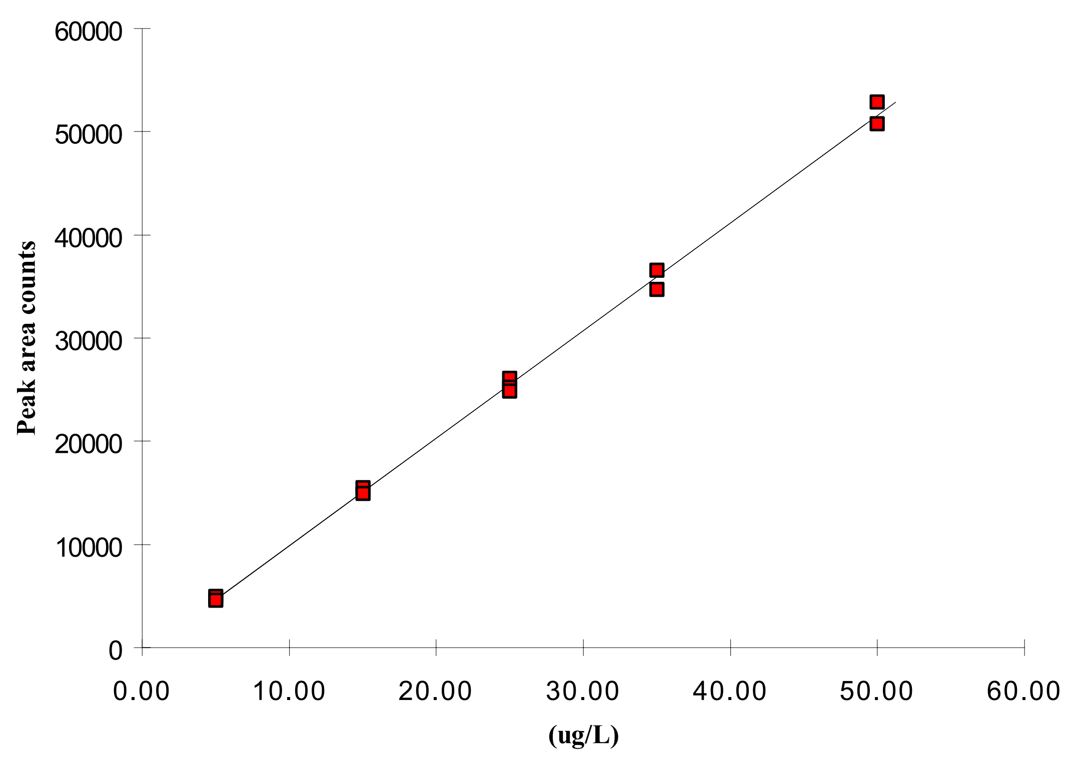
Calibration Curve for Anthracene Analyzed by HPLC with Fluorescence Detection



Slope = 1253.8411; Y-intercept = -565.6560; R^2 = 0.9972

Appendix 9.12

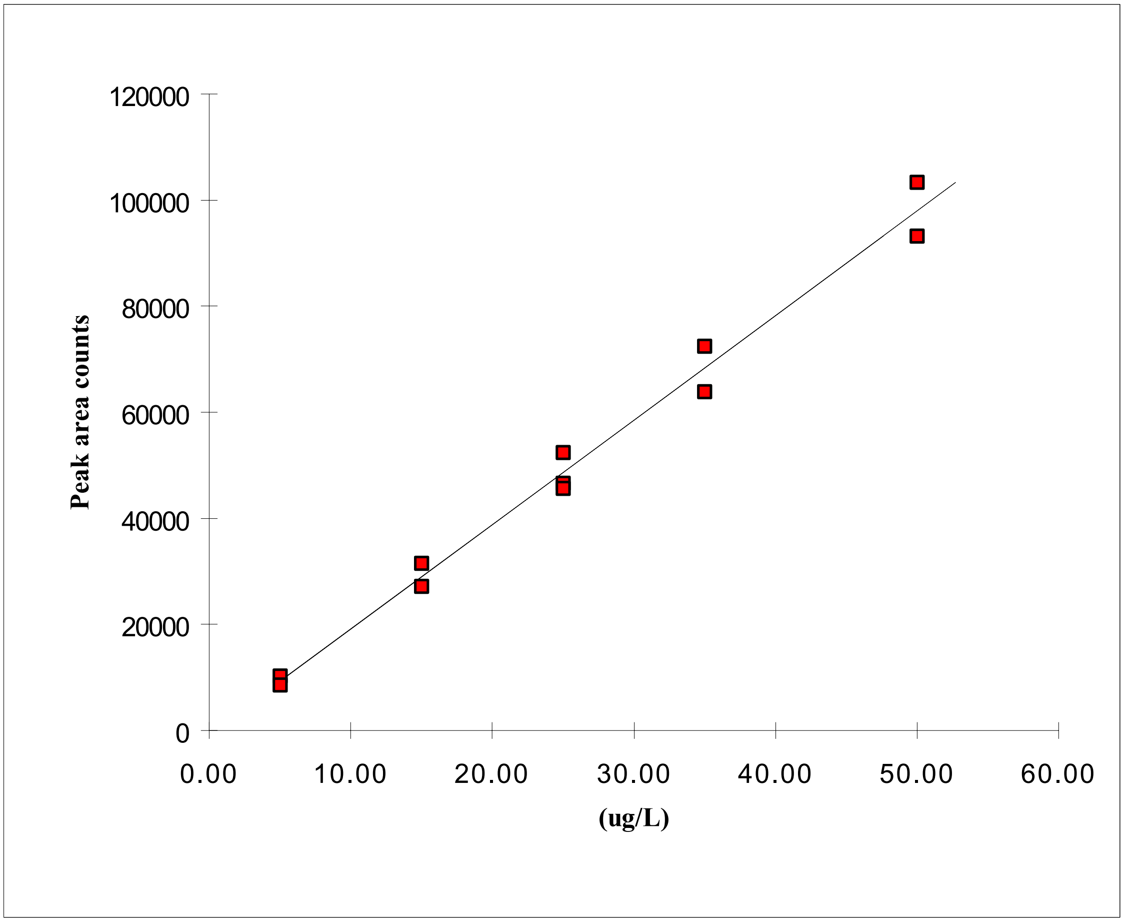
Calibration Curve for Fluoranthene Analyzed by HPLC with Fluorescence Detection



Slope = 1042.4177; Y-intercept = -540.0548; R^2 = 0.9980

Appendix 9.13

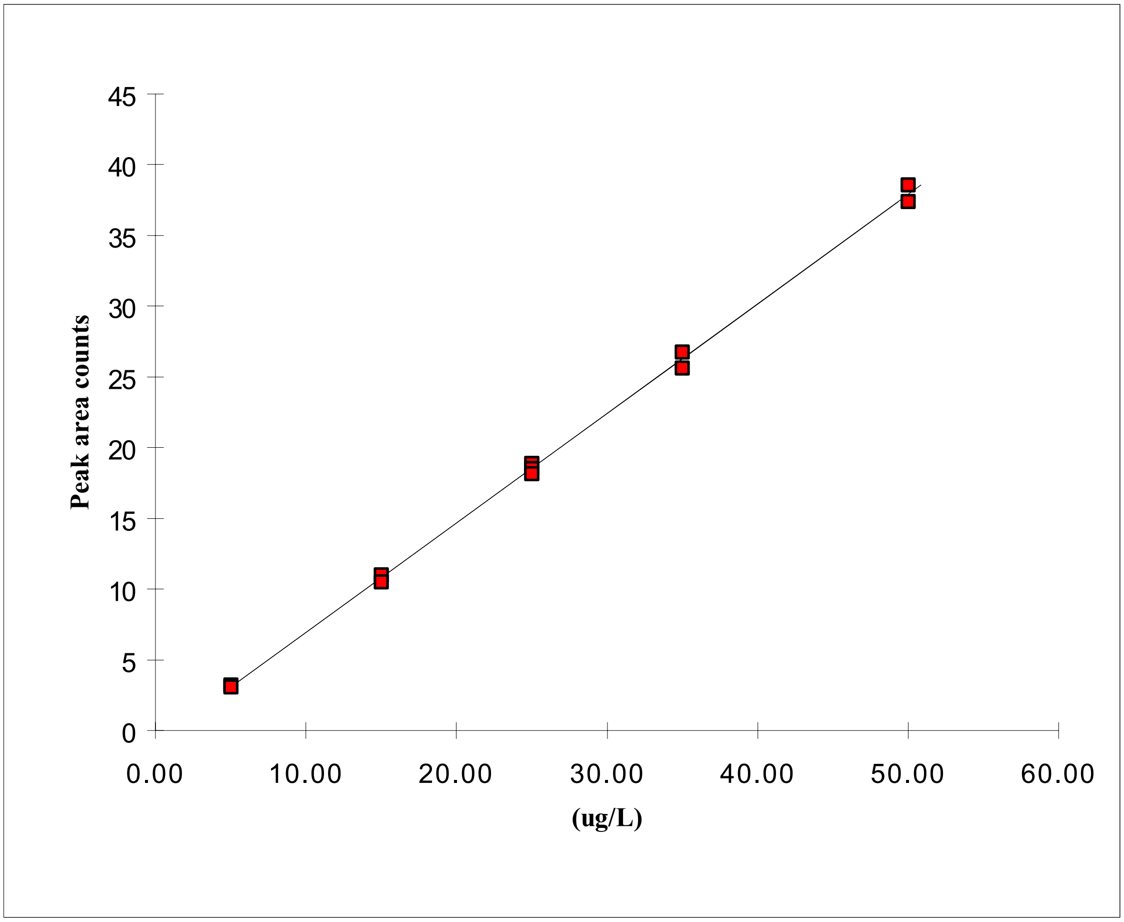
Calibration Curve for Pyrene Analyzed by HPLC with Fluorescence Detection



Slope = 1970.5471; Y-intercept = -612.0144; R^2 = 0.9868

Appendix 9.14

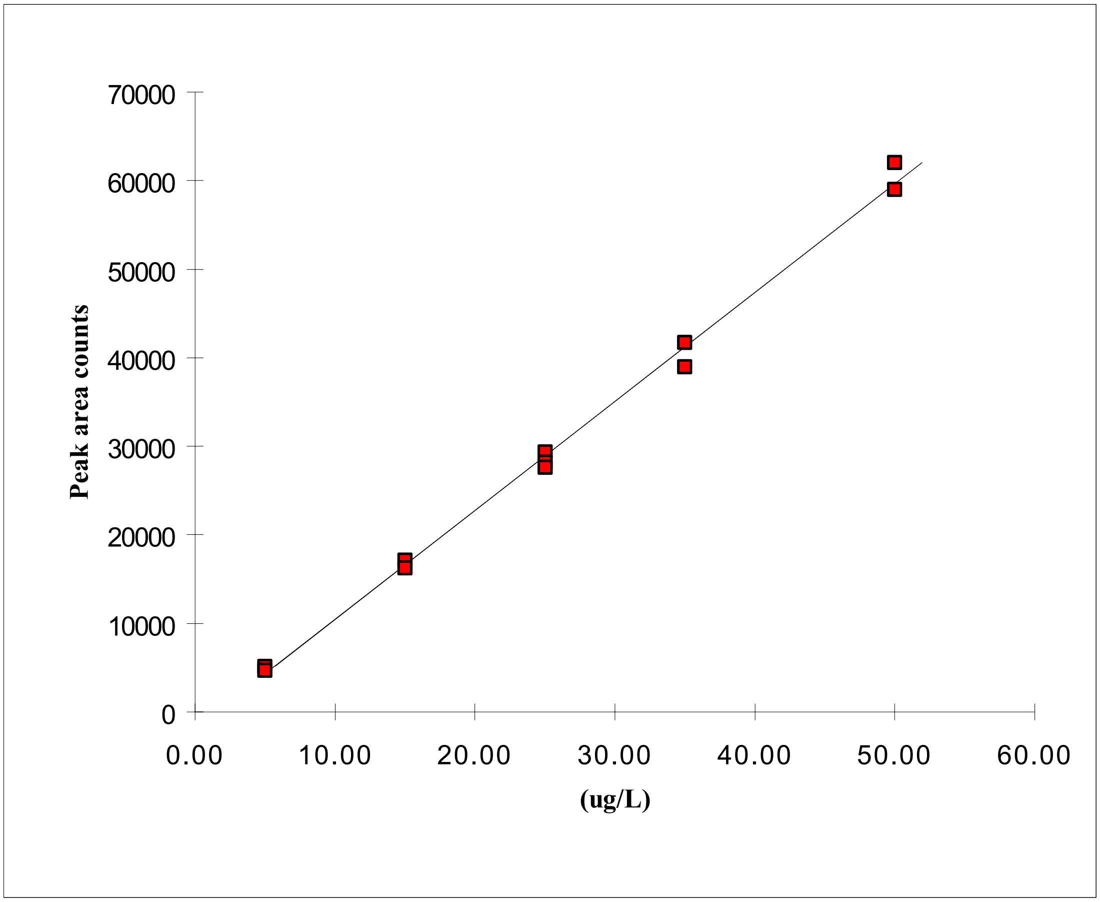
Calibration Curve for Chrysene Analyzed by HPLC/UV



Slope = 0.7739; Y-intercept = -0.8058; R^2 = 0.9988

Appendix 9.15

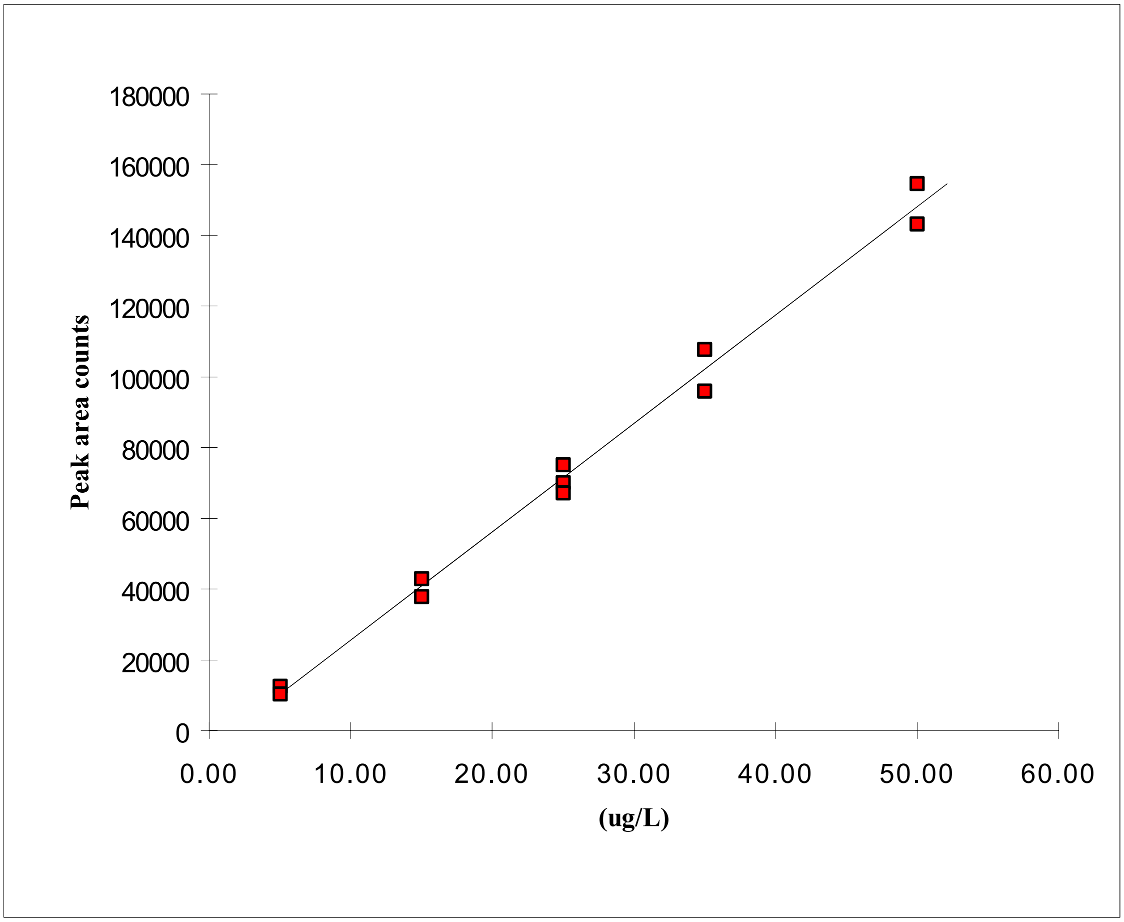
Calibration Curve for Benz(a)anthracene Analyzed by HPLC with Fluorescence Detection



Slope = 1230.1097; Y-intercept = -1859.3667; R^2 = 0.9959

Appendix 9.16

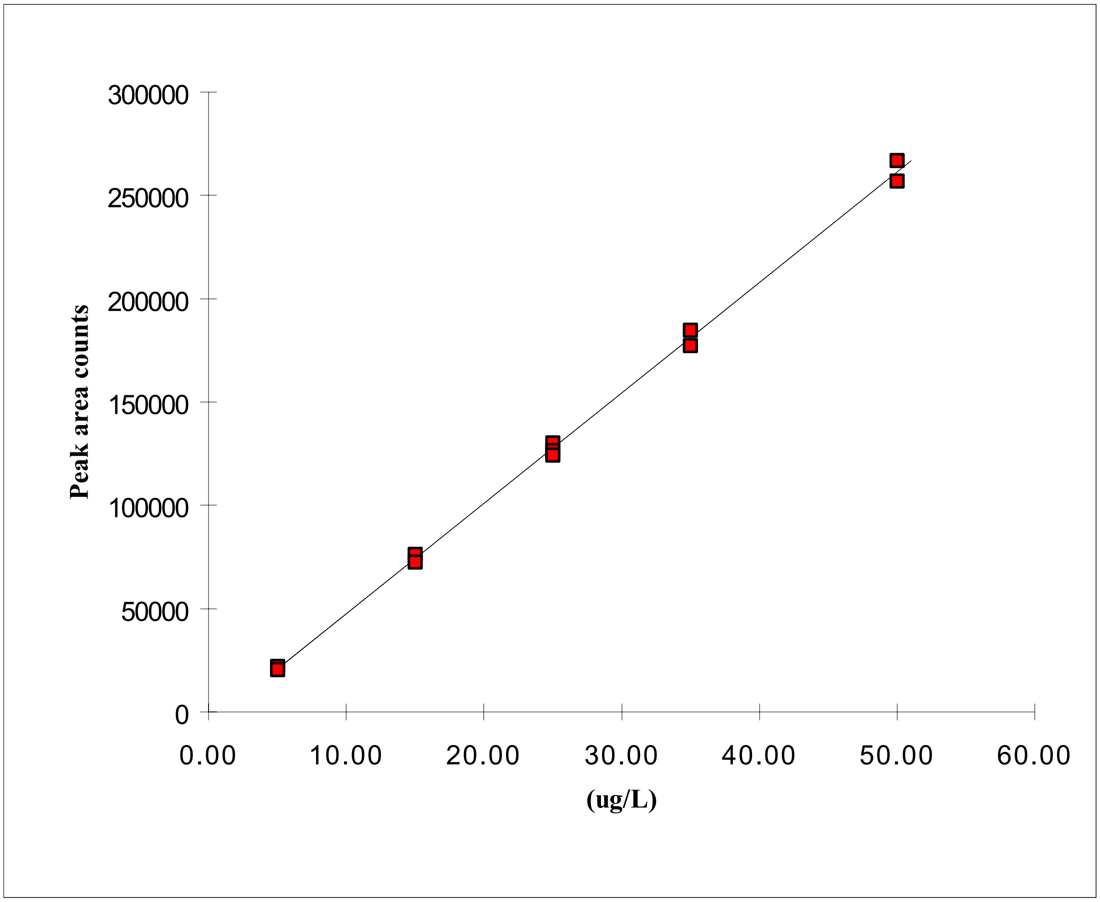
Calibration Curve for Benzo(b)fluoranthene Analyzed by HPLC with Fluorescence Detection



Slope = 3064.6087; Y-intercept = -5042.8430; R^2 = 0.9919

Appendix 9.17

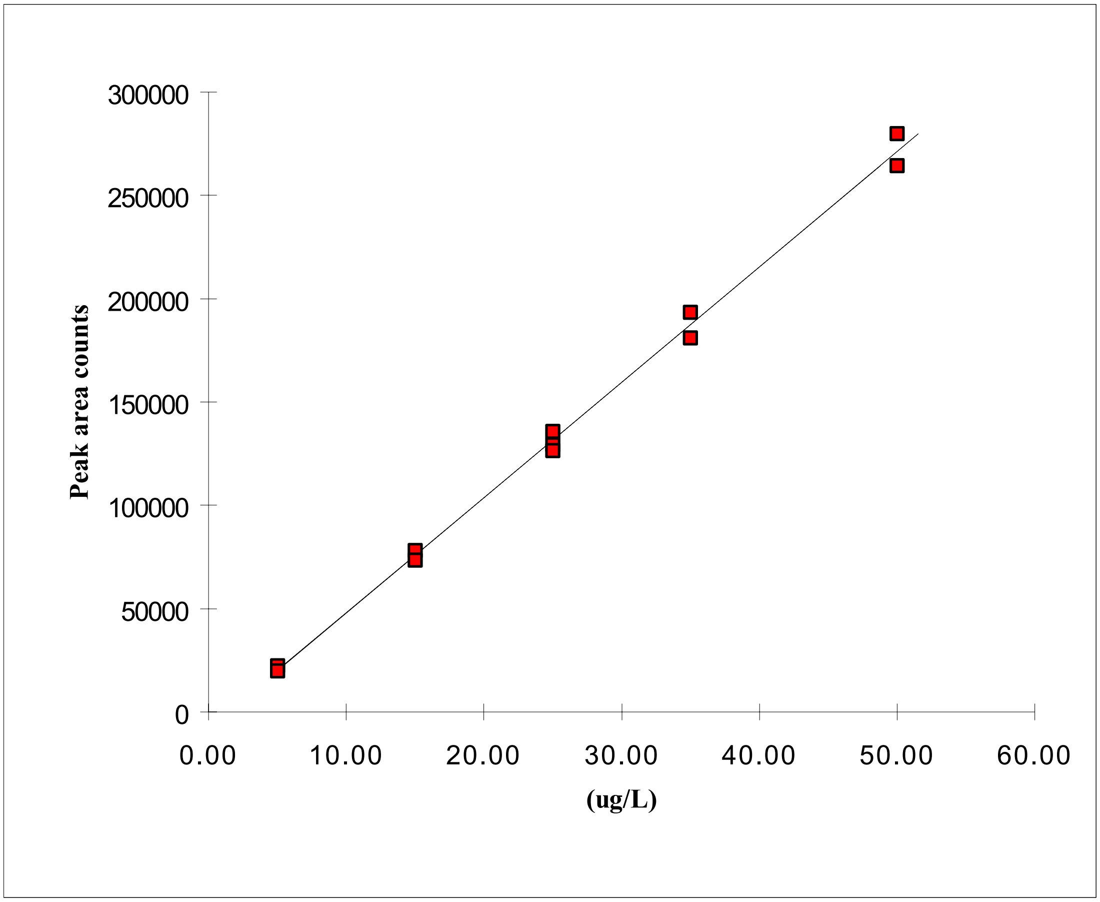
Calibration Curve for Benzo(k)fluoranthene Analyzed by HPLC with Fluorescence Detection



Slope = 5349.2089; Y-intercept = -6041.2933; $R^2 = 0.9985$

Appendix 9.18

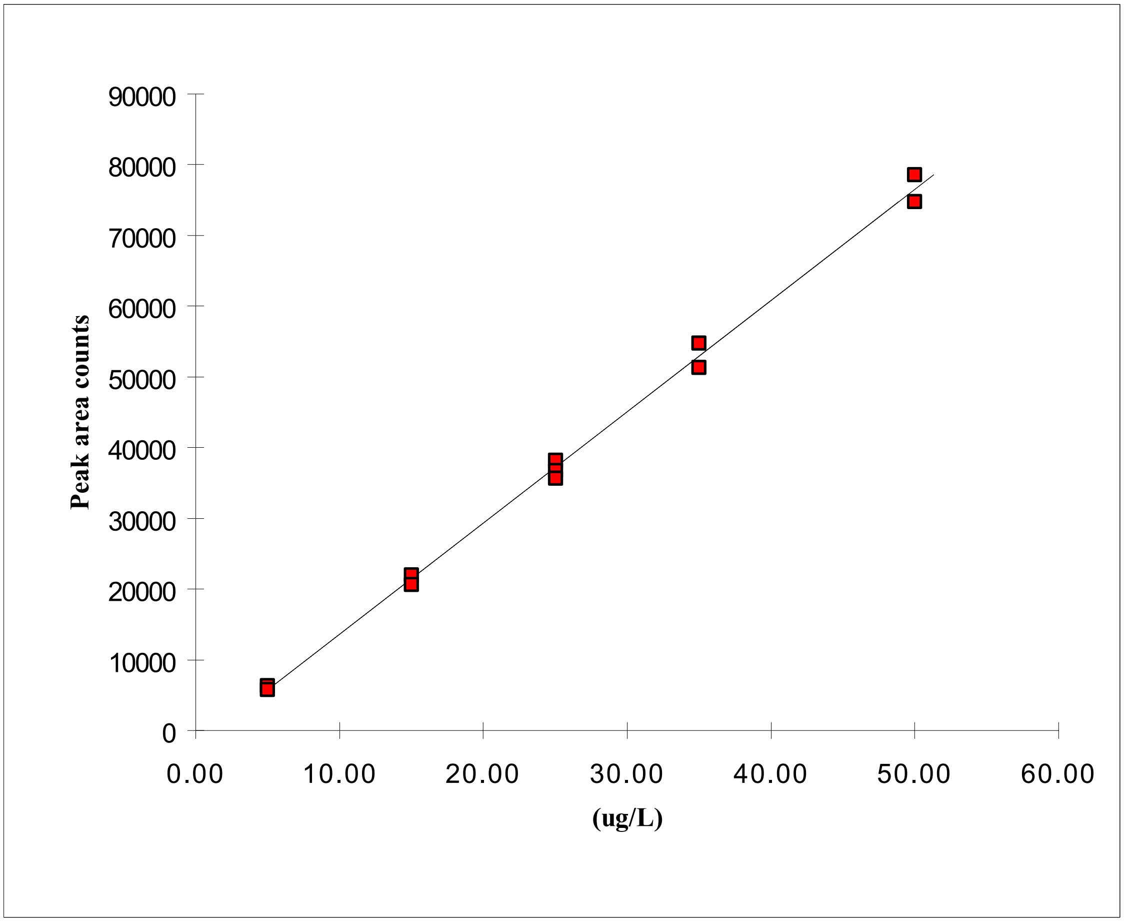
Calibration Curve for Benzo(a)pyrene Analyzed by HPLC with Fluorescence Detection



Slope = 5580.2133; Y-intercept = -7844.6799; R^2 = 0.9966

Appendix 9.19

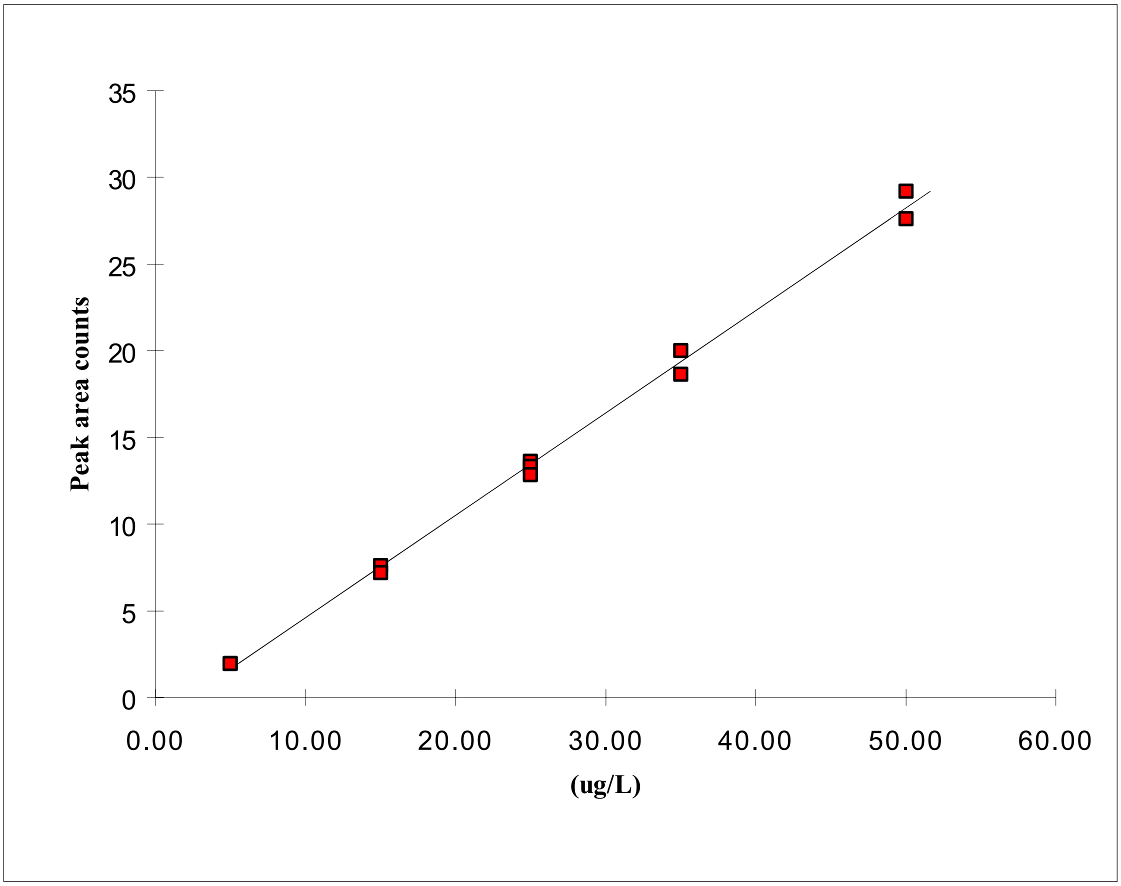
Calibration Curve for Dibenz(a,h)anthracene Analyzed by HPLC with Fluorescence Detection



Slope = 1572.9685; Y-intercept = -2116.8339; $R^2 = 0.9970$

Appendix 9.20

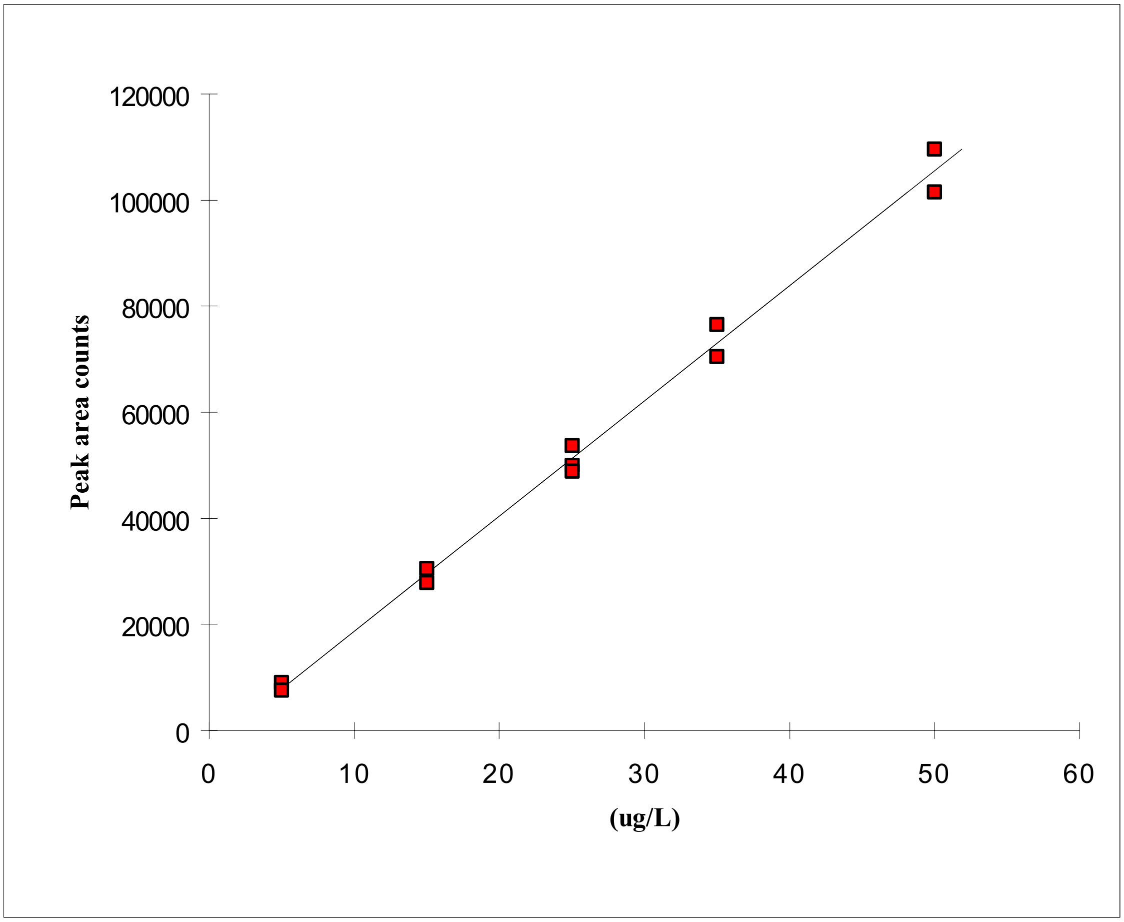
Calibration Curve for Indeno(1,2,3-cd)pyrene Analyzed by HPLC/UV



Slope = 0.5902; Y-intercept = -1.2878; R^2 = 0.9965

Appendix 9.21

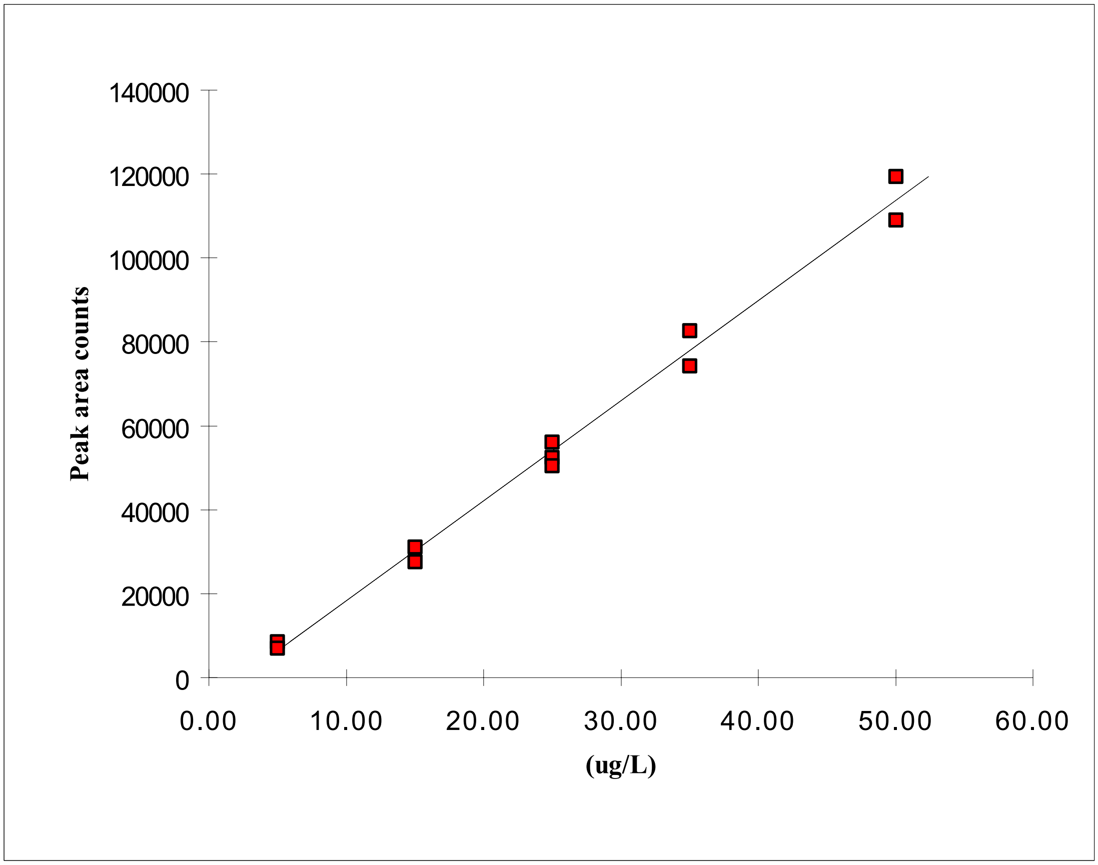
Calibration Curve for Benzo(g,h,i)perylene Analyzed by HPLC with Fluorescence Detection



Slope = 2170.5372; Y-intercept = -2986.2517; R^2 = 0.9939

Appendix 9.22

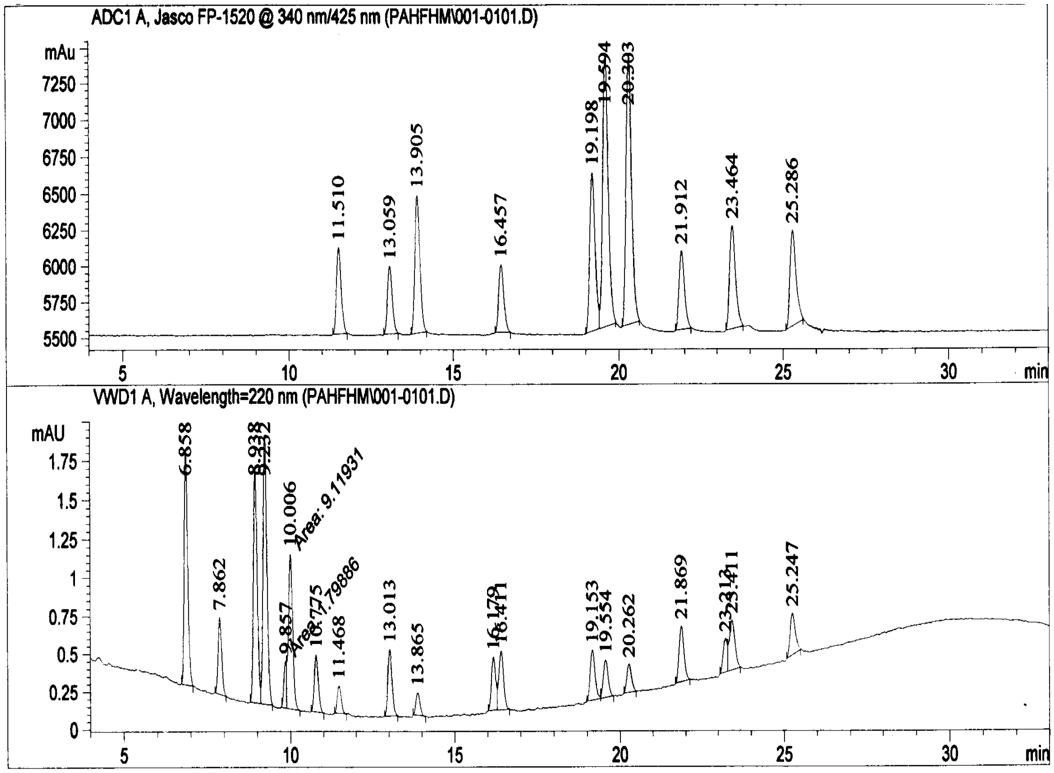
Calibration Curve for Dibenzo(a,e)pyrene Analyzed by HPLC with Fluorescence Detection



Slope = 2382.6701; Y-intercept = -5470.0587; R^2 = 0.9913

Appendix 9.23

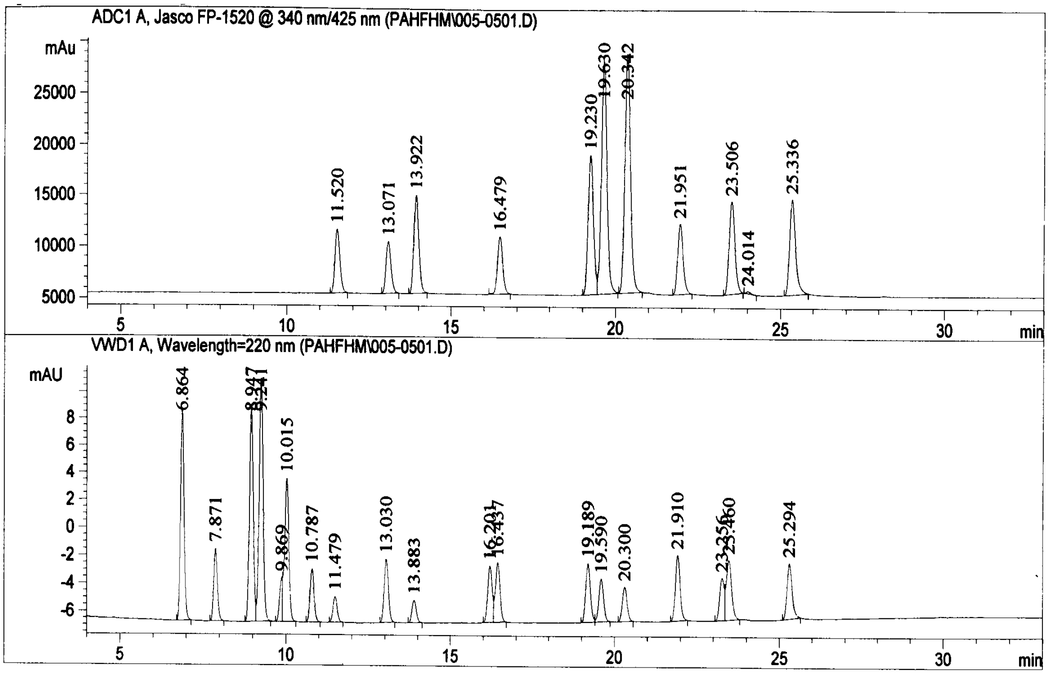
Representative Chromatograms of Low-level Calibration Standards
Analyzed by HPLC/UV and Fluorescence Detection



Nominal Concentration: 5.00 µg/L

Appendix 9.24

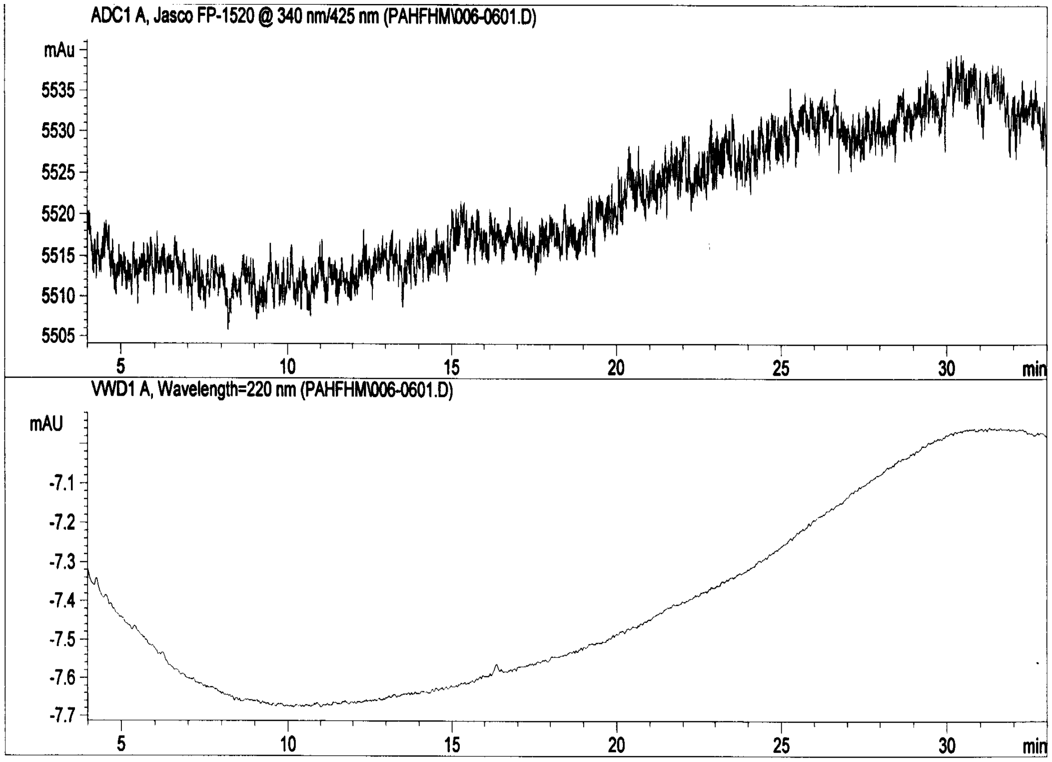
Representative Chromatograms of High-level Calibration Standards
Analyzed by HPLC/UV and Fluorescence Detection



Nominal Concentration: 50.0 µg/L

Appendix 9.25

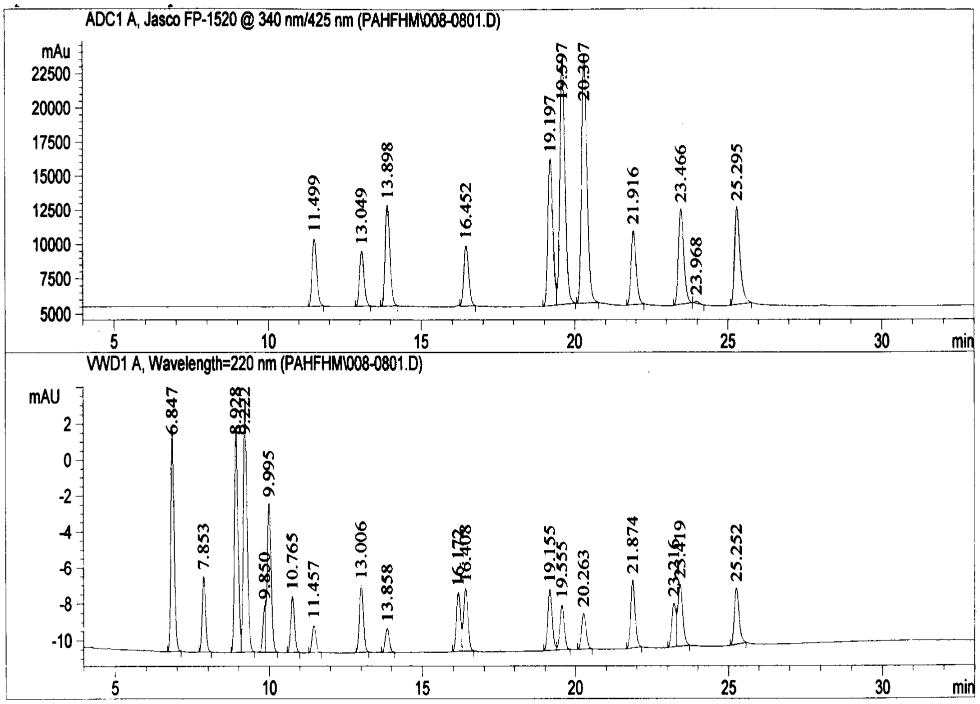
Representative Chromatograms of a Matrix Blank Sample
Analyzed by HPLC/UV and Fluorescence Detection



Sample Number 472A-113-MAB-1.

Appendix 9.26

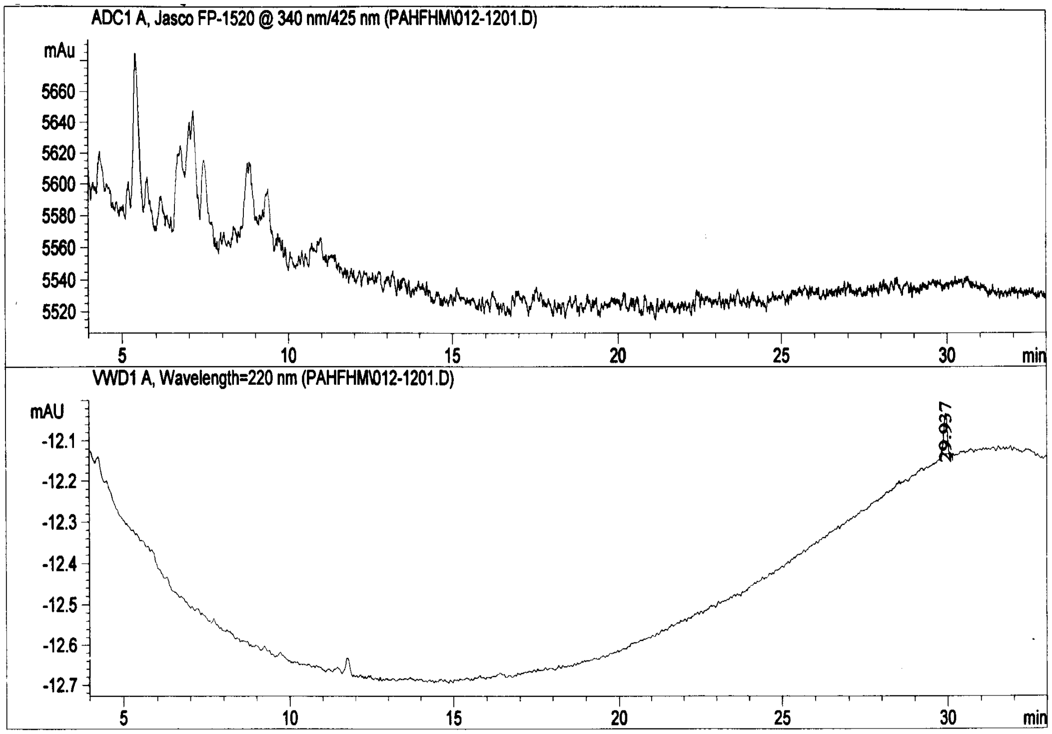
Representative Chromatograms of a Matrix Fortification Sample
Analyzed by HPLC/UV and Fluorescence Detection



Sample Number 472A-113-MAS-2. Nominal Concentration: 40.0 µg/L

Appendix 9.27

Representative Chromatograms of a Test Sample
Analyzed by HPLC/UV and Fluorescence Detection



Sample Number 472A-113-2. Nominal Concentration: 1000 mg/L WAF.

Appendix 10

The Analysis of Inorganic Constituents in Petroleum Coke in Freshwater

Appendix 10.1

Analytical Method Flowchart for the Analysis of As, Cu, Fe, Ni, Se, V and S in Freshwater Analyzed by ICP-AES

Prepare quality control (QC) samples concurrent with the analyses of test samples as follows: Prepare matrix fortification samples by spiking the requisite volume(s) of the appropriate combined and/or individual element stock solution(s) directly into freshwater. Perform fortifications with calibrated micropipettors and graduated centrifuge tubes. Bring to final volume with freshwater. The matrix blank consists of unfortified freshwater.

↓

Partially fill a graduated centrifuge tube with each sample. Using a calibrated micropipettor, fortify each QC and test sample with 200 µL of concentrated nitric acid. Bring to final volume with the sample. For samples not requiring further dilution into the calibration range of the ICP-AES methodology, submit for ICP-AES analysis.

↓

For those samples requiring dilution into the calibration range of the ICP-AES methodology, perform dilutions using graduated centrifuge tubes, calibrated micropipettor(s), and 2% (v/v) nitric acid in freshwater solution. Mix dilutions well and transfer into separate, labeled, 15-mL centrifuge tubes. Submit for ICP-AES analysis.

Appendix 10.2

Typical ICP-AES Operational Parameters for the Analysis of
As, Cu, Fe, Ni, Se, V and S in Freshwater

Instrument:	Perkin-Elmer Optima 3000 DV Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES)	
Sample Introduction System:	Cetac U-5000AT^{+} Ultrasonic Nebulizer	
Analytical Wavelengths:	As	188.979 nm
	Cu	224.700 nm
	Fe	239.562 nm
	Ni	231.604 nm
	Se	196.026 nm
	S	180.669 nm
	V	292.402 nm
Plasma:	Plasma Gas Flow:	15 L/min Ar
	Auxiliary Gas Flow:	0.5 L/min Ar
	Nebulizer Gas Flow:	0.7 L/min Ar
	RF Power:	1300 W
Pump:	Sample Flow Rate:	2.00 mL/min
	Sample Flush Time:	15 sec
	Wash Rate:	2.00 mL/min
	Wash Time:	60 sec
	Wash Frequency:	Between Samples
Spectrometer:	View Mode:	Axial
	Read Delay:	60 sec
	Read Time:	Min: 10.000 sec Max: 20.000 sec
	Read Replicates:	3
	Peak Algorithm:	Peak Area
	Points/Peak:	3
	Background Correction:	2-Point

Appendix 10.3

Analytical Stocks and Standards Preparation

A combined stock solution containing the seven elements of interest was prepared either directly from the procured Spex primary standards, or from single element secondary stock preparations. The combined stock solutions were used to prepare external calibration standards and matrix fortification samples. Volumetric flasks and calibrated micropipettors were used for all preparations. Preparation details for the stocks were as follows:

A single component secondary stock for vanadium was first prepared from a 10x dilution of the 1.00 mg/mL V primary standard using 2% (v/v) nitric acid in reagent grade water (2:98 HNO_3: H_2O) dilution solvent. The nominal concentration of the resultant vanadium stock was 0.100 mg V/mL. A combined secondary stock in 2:98 HNO_3: H_2O dilution solvent was then prepared as follows:

Element	Primary Stock Concentration (mg/mL)	Aliquot (mL)	Final Volume (mL)	Secondary Stock Concentration (mg/L)
As	1.00	1.00		10.0
Cu	1.00	1.00		10.0
Fe	1.00	0.500	100	5.00
Ni	1.00	0.500		5.00
Se	1.00	10.0		100
V	0.100	0.200		0.200

This combined secondary stock solution was used to prepare a set of calibration standards, each in 2% (v/v) nitric acid in Wildlife International, Ltd. freshwater (2:98 HNO_3: FW) dilution solvent and diluted to a 100-mL final volume, using the following dilution scheme:

Stock Aliquot:	0.100 mL	0.250 mL	0.500 mL	0.750 mL	1.00 mL
Element	Standard Concentration (µg/L)	Standard Concentration (µg/L)	Standard Concentration (µg/L)	Standard Concentration (µg/L)	Standard Concentration (µg/L)
As	10.0	25.0	50.0	75.0	100
Cu	10.0	25.0	50.0	75.0	100
Fe	5.00	12.5	25.0	37.5	50.0
Ni	5.00	12.5	25.0	37.5	50.0
Se	100	250	500	750	1000
V	0.200	0.500	1.00	1.50	2.00

Appendix 10.3 (Continued)

Analytical Stocks and Standards Preparation

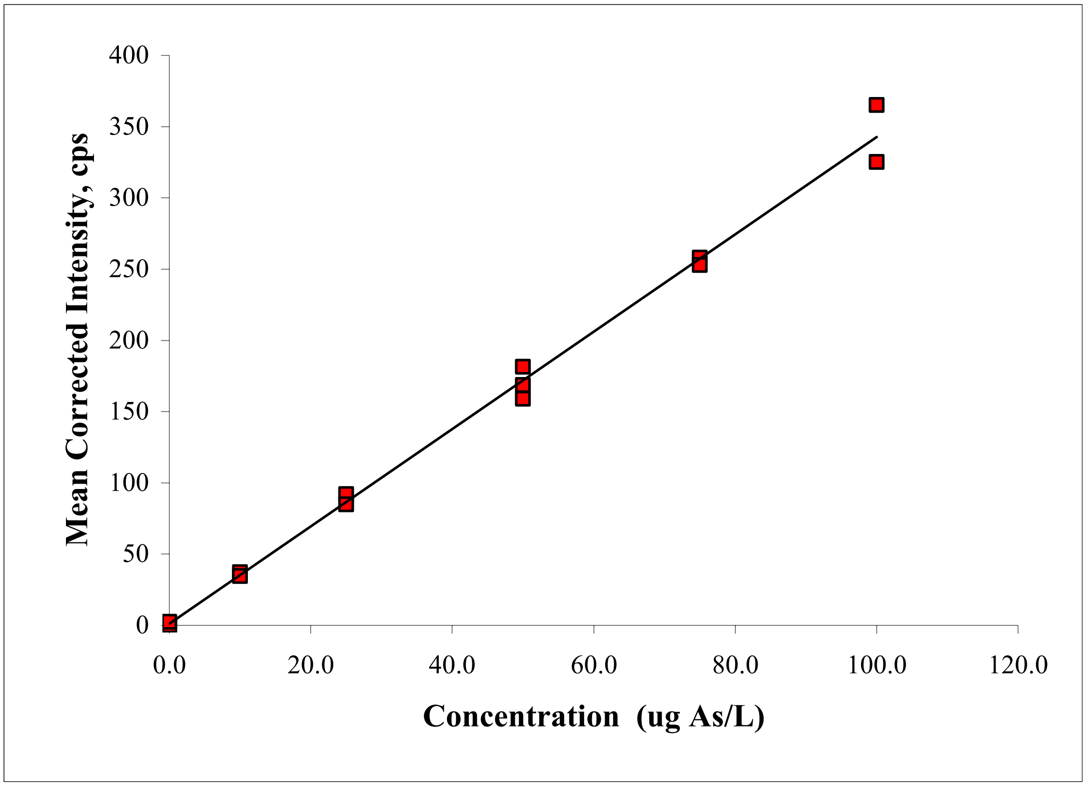
A separate set of sulfur calibration standards, each in 2:98 HNO_3: FW dilution solvent, were prepared from the 10.0-mg S/mL primary standard using the following dilution scheme:

Stock Concentration (mg S/mL)	Aliquot (µL)	Final Volume (mL)	Standard Concentration (mg S/L)
10.0	50.0	100	5.00
10.0	100	100	10.0
10.0	250	100	25.0
10.0	350	100	35.0
10.0	500	100	50.0

The 2:98 HNO_3: FW dilution solvent, prepared concurrently with these calibration standards was also utilized as a calibration/reagent blank.

Appendix 10.4

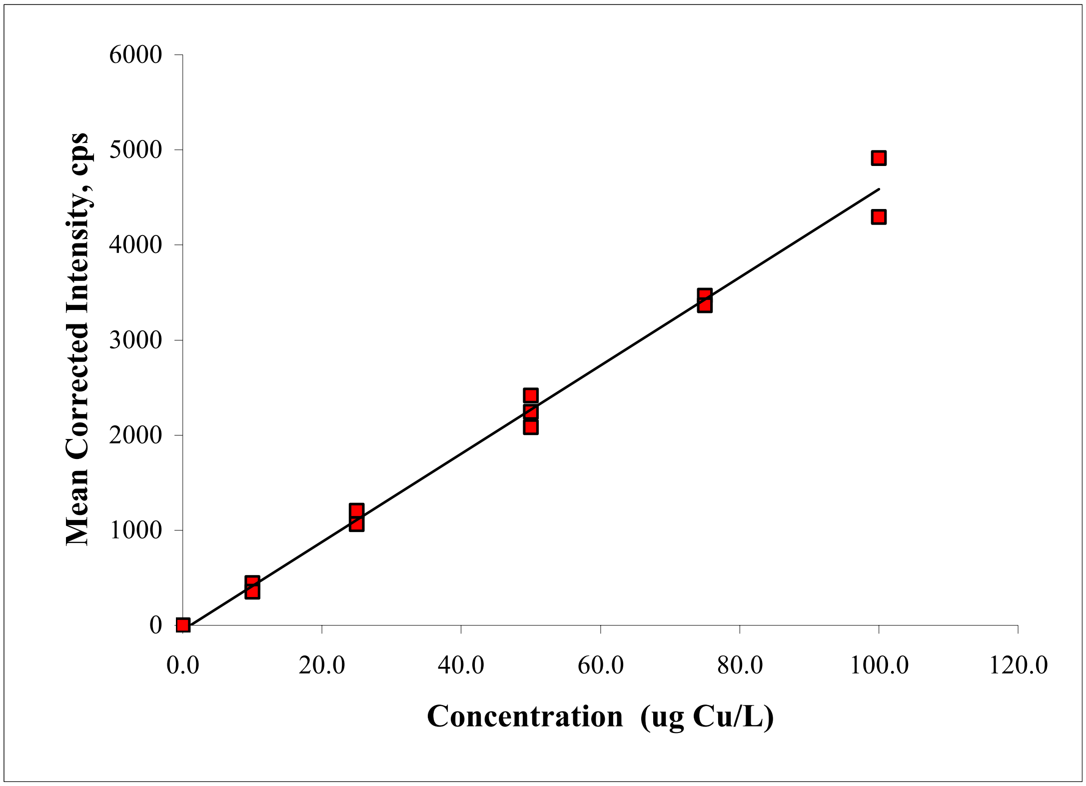
Calibration Curve for Arsenic Analyzed by ICP-AES



Slope = 3.4155; Y-intercept = 1.13574; $R^2 = 0.9936$

Appendix 10.5

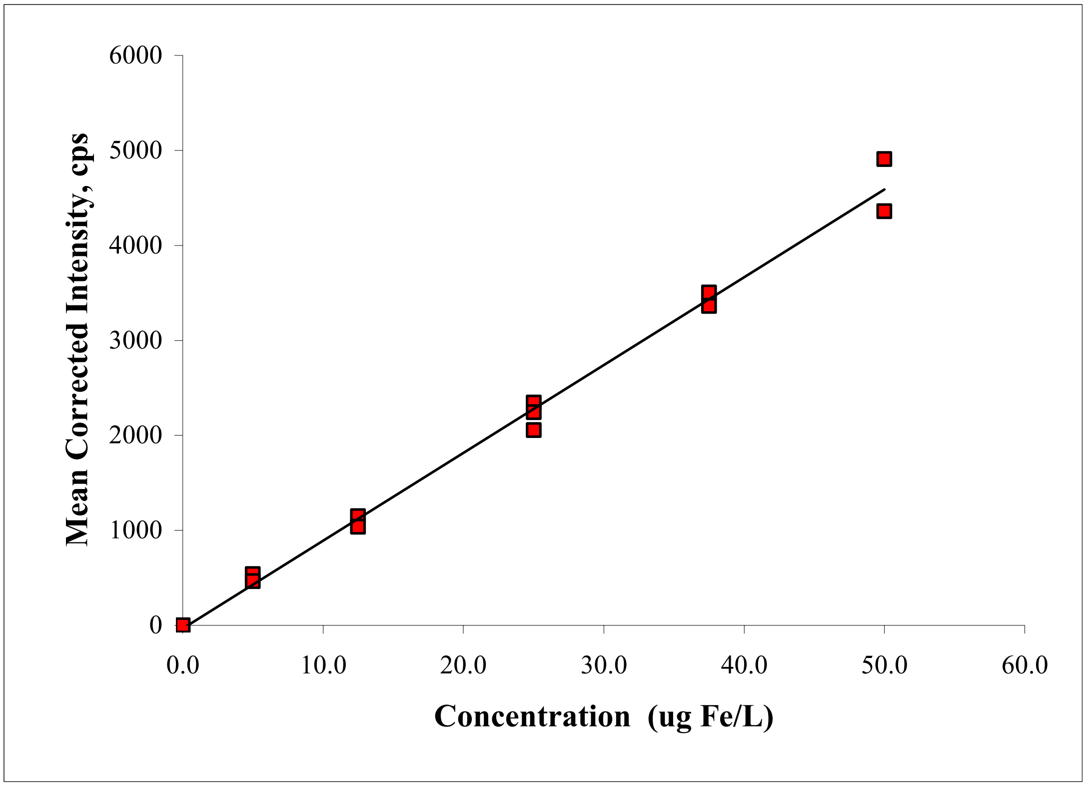
Calibration Curve for Copper Analyzed by ICP-AES



Slope = 46.380; Y-intercept = -51.450; R^2 = 0.9917

Appendix 10.6

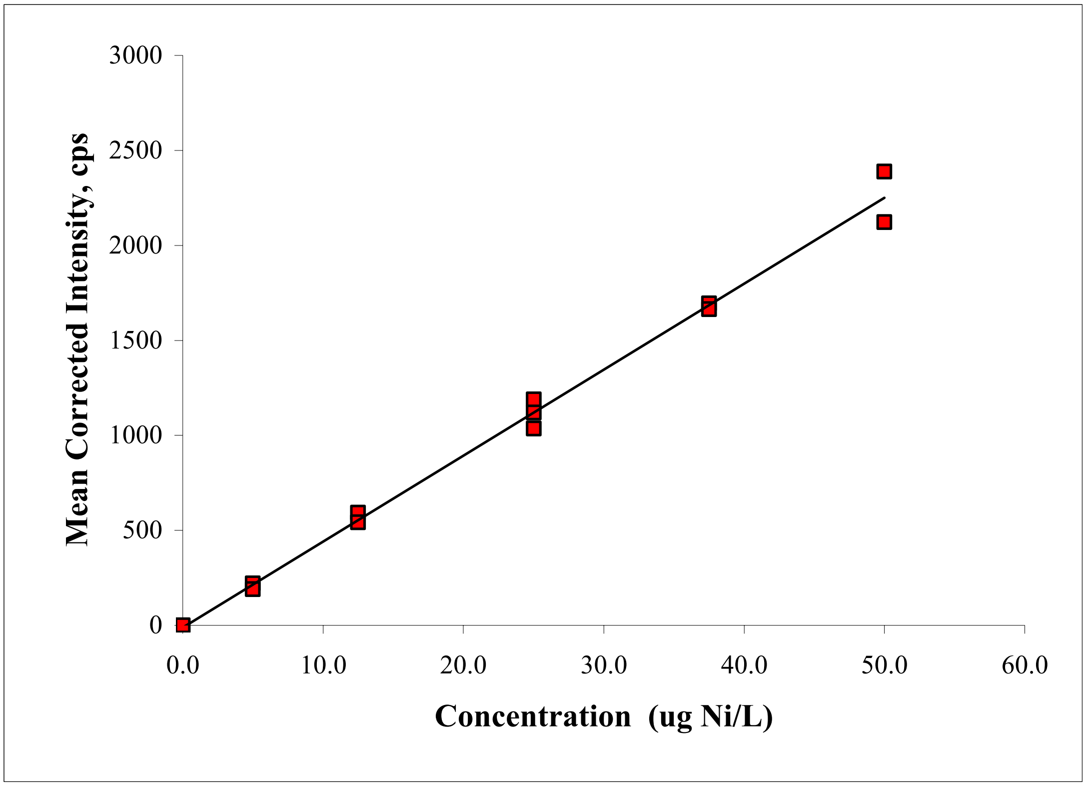
Calibration Curve for Iron Analyzed by ICP-AES



Slope = 92.447; Y-intercept = -34.038; R^2 = 0.9925

Appendix 10.7

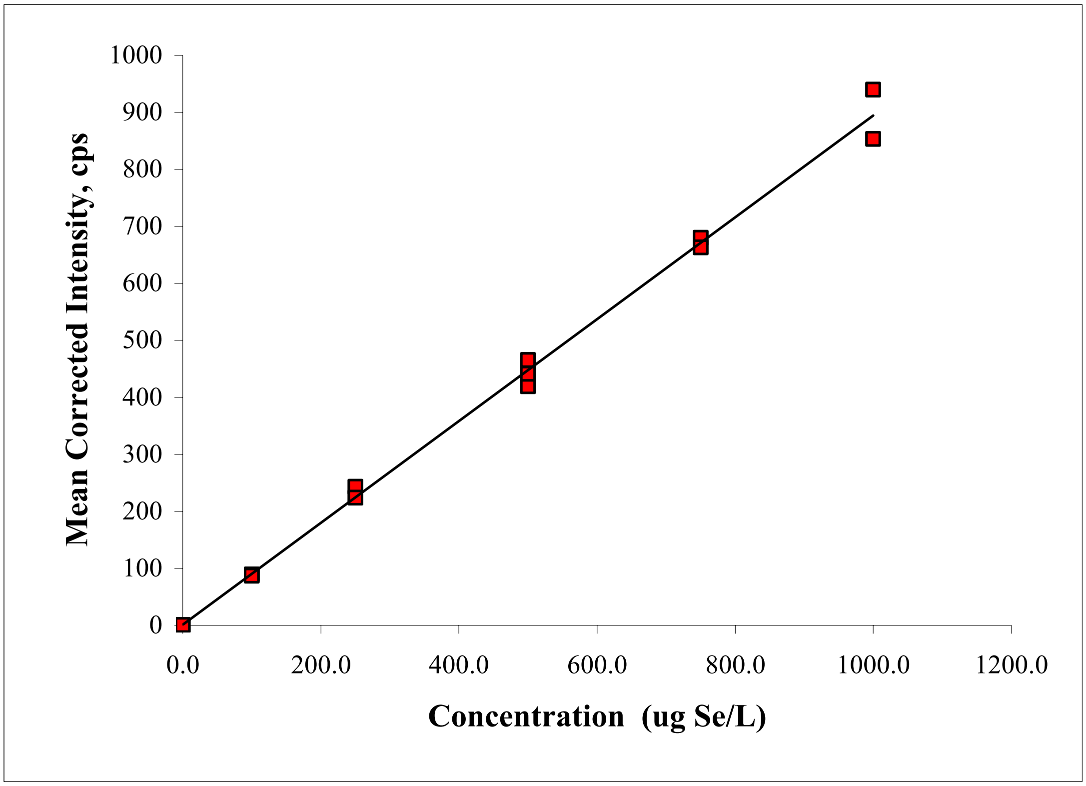
Calibration Curve for Nickel Analyzed by ICP-AES



Slope = 45.242; Y-intercept = -11.5802; $R^2 = 0.9936$

Appendix 10.8

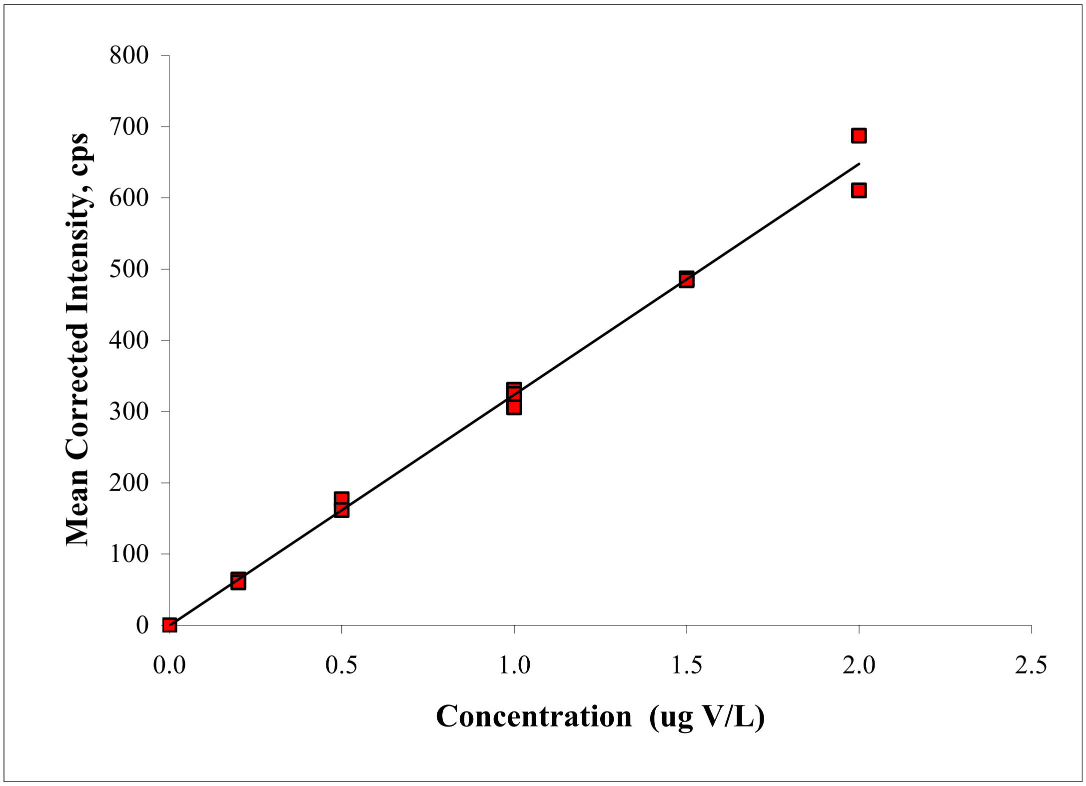
Calibration Curve for Selenium Analyzed by ICP-AES



Slope = 0.8938; Y-intercept = 0.9289; R^2 = 0.9955

Appendix 10.9

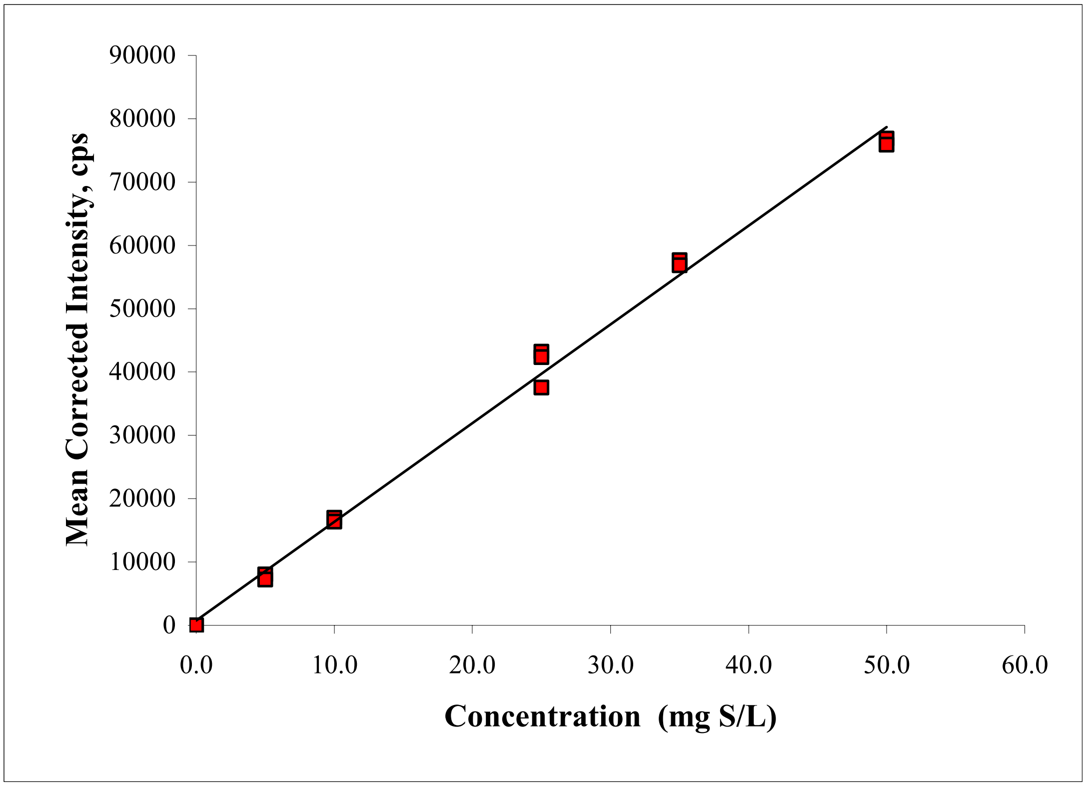
Calibration Curve for Vanadium Analyzed by ICP-AES



Slope = 324.15; Y-intercept = -0.4253; R^2 = 0.9944

Appendix 10.10

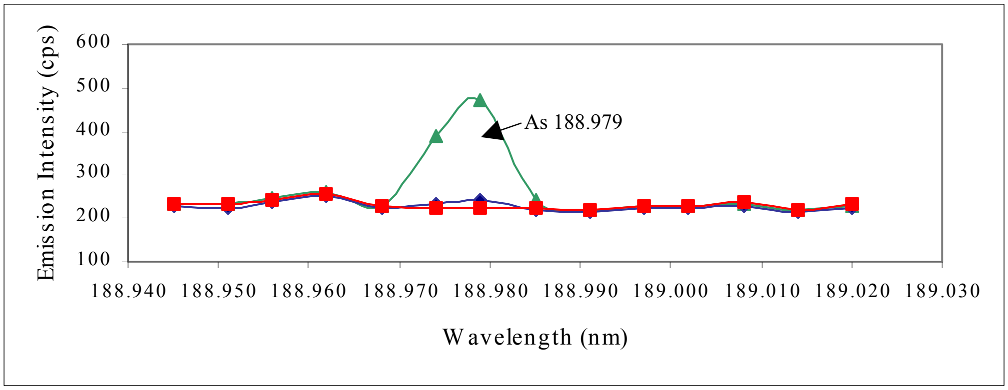
Calibration Curve for Sulfur Analyzed by ICP-AES

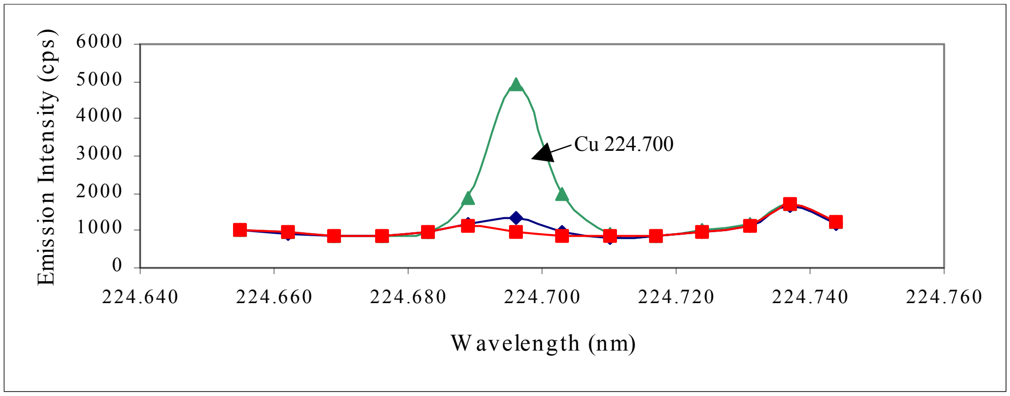


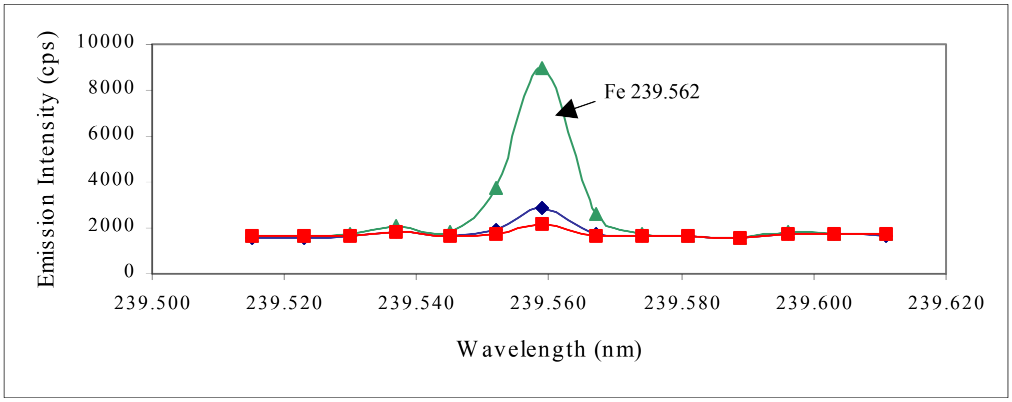
Slope = 1557.9; Y-intercept = 781.1; R^2 = 0.9949

Appendix 10.11

Representative Emission Spectra for Arsenic (top), Copper (middle) and Iron (bottom) in Low- and High-Level Calibration Standards Prepared in Freshwater and Analyzed by ICP-AES



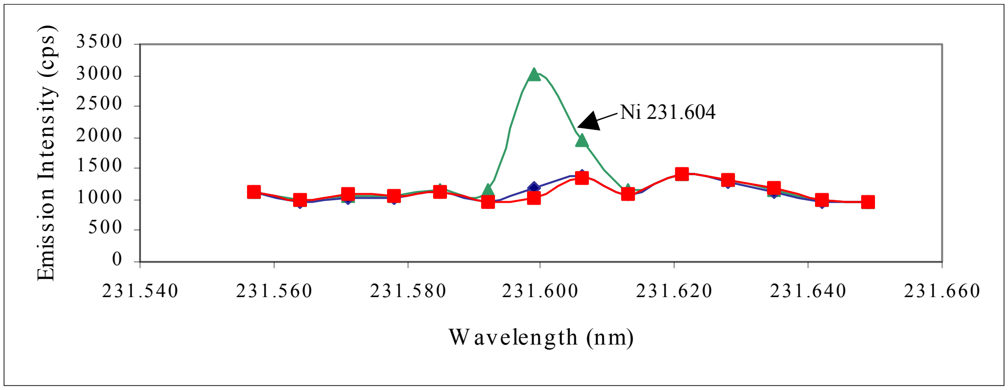


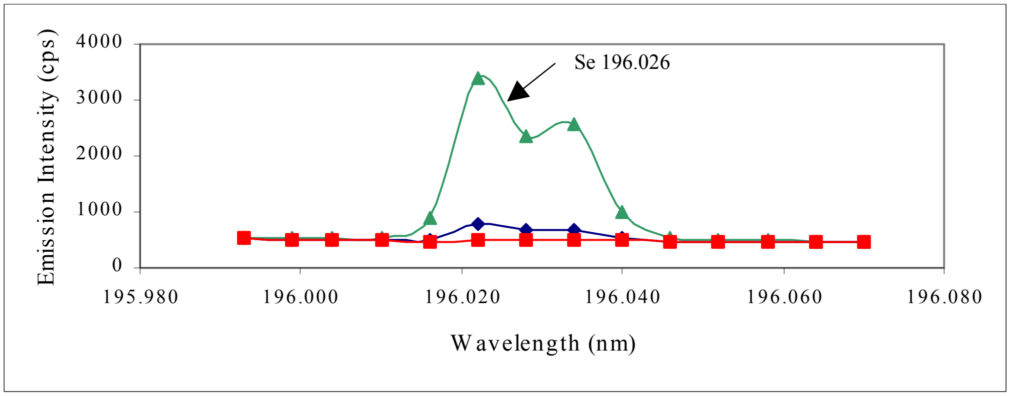


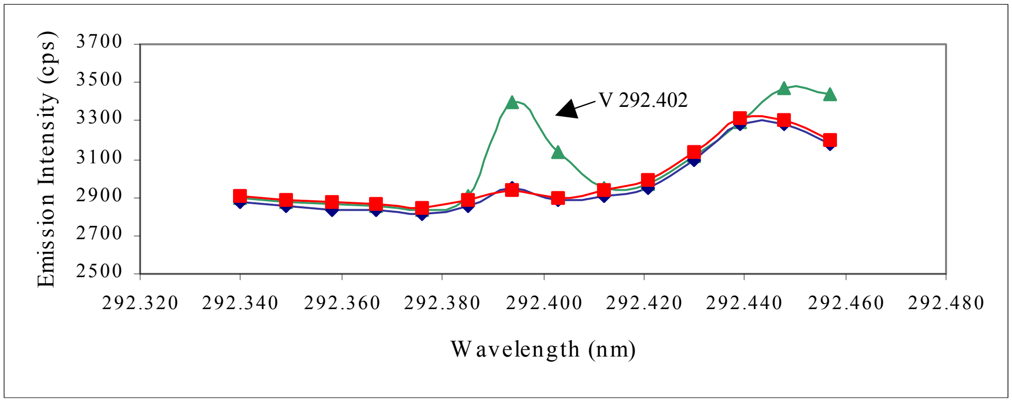
Squares = freshwater calibration blank (I.D.: 472A-112,113-M-0); Diamonds = low-level standard (I.D.: 472A-112,113-M-1); Triangles = high-level standard (I.D.: 472A-112,113-M-5). Nominal concentrations for As, Cu and Fe = 10.0, 10.0 and 5.00 µg/L and 100, 100 and 50.0 µg/L, in the low- and high-level standards, respectively.

Appendix 10.12

Representative Emission Spectra for Nickel (top), Selenium (middle) and Vanadium (bottom) in Low- and High-Level Calibration Standards Prepared in Freshwater and Analyzed by ICP-AES



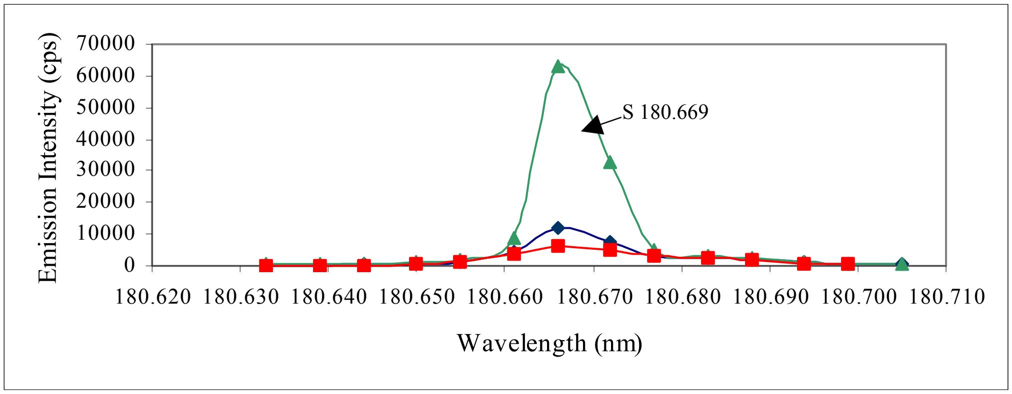




Squares = freshwater calibration blank (I.D.: 472A-112,113-M-0); Diamonds = low-level standard (I.D.: 472A-112,113-M-1); Triangles = high-level standard (I.D.: 472A-112,113-M-5). Nominal concentrations for Ni, Se and V = 5.00, 100 and 0.200 µg/L and 50.0, 1000 and 2.00 µg/L, in the low- and high-level standards, respectively.

Appendix 10.13

Representative Emission Spectra for Sulfur in Low- and High-Level Calibration Standards Prepared in Freshwater and Analyzed by ICP-AES.



Squares = freshwater calibration blank (I.D.: 472A-112,113-S-0); Diamonds = low-level standard (I.D.: 472A-112,113-S-1); Triangles = high-level standard (I.D.: 472A-112,113-S-5). Nominal concentrations for S = 5.00 and 50.0 mg/L in the low- and high-level standards, respectively.

Appendix 10.14

Example Calculations

The analytical result and percent recovery for sample number 472A-113-MAS-1 for vanadium, nominal concentration of 1.00 μg/L in freshwater, were calculated using the following equations:

$$\text{Vanadium (}\mu\text{g/L) in sample} = \frac{\text{Mean Corrected Intensity - (Y-intercept)}}{\text{Slope}} \text{ X Dilution factor}$$

Mean Corrected Intensity = 324.3
Y-intercept = -0.4253
Slope = 324.15
Dilution Factor = 1.02

$$\text{Concentration of Vanadium (}\mu\text{g/L) in sample} = \frac{324.3 - (-0.4253)}{324.15} \text{ X } 1.02$$

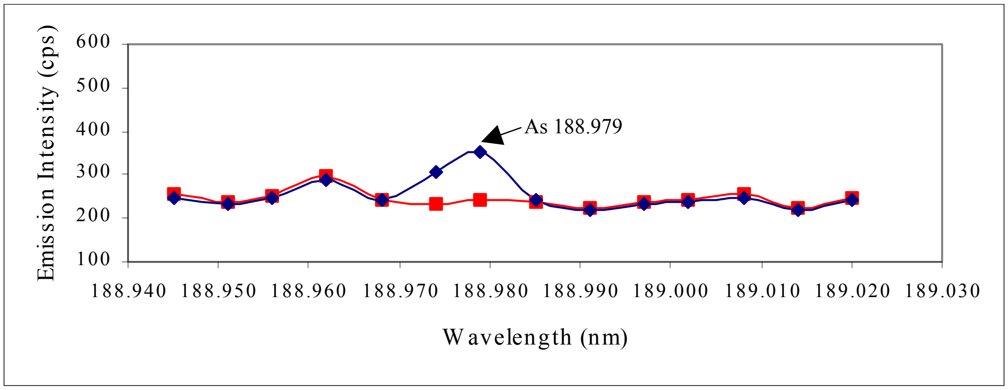
Concentration of Vanadium in sample (μg/L) = 1.02

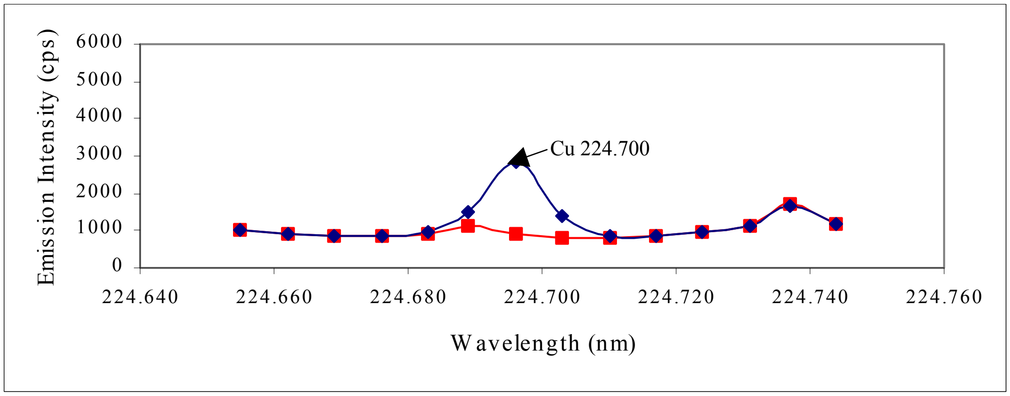
$$\text{Percent of nominal concentration} = \frac{1.02\ (\mu\text{g/L})}{1.00\ (\mu\text{g/L})} \text{ X } 100$$

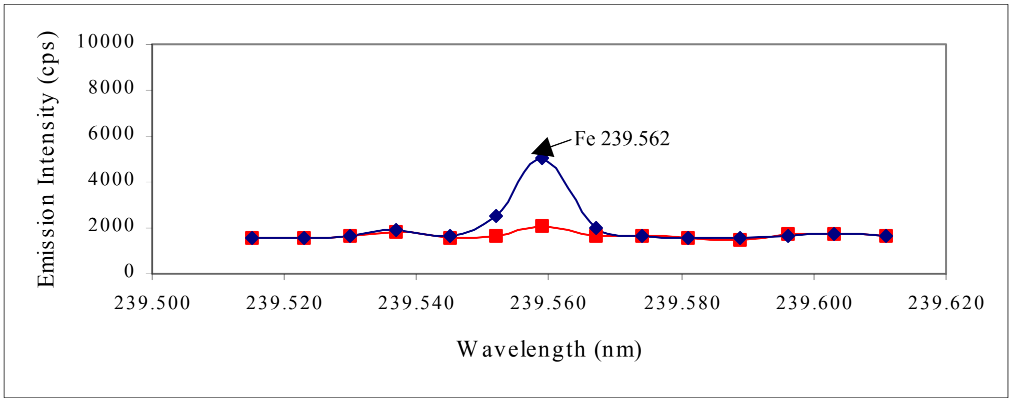
Percent of nominal concentration = 102%

Appendix 10.15

Emission Spectra for Arsenic (top), Copper (middle) and Iron (bottom) in Matrix Blank and Matrix Fortification Samples Prepared in Freshwater and Analyzed by ICP-AES



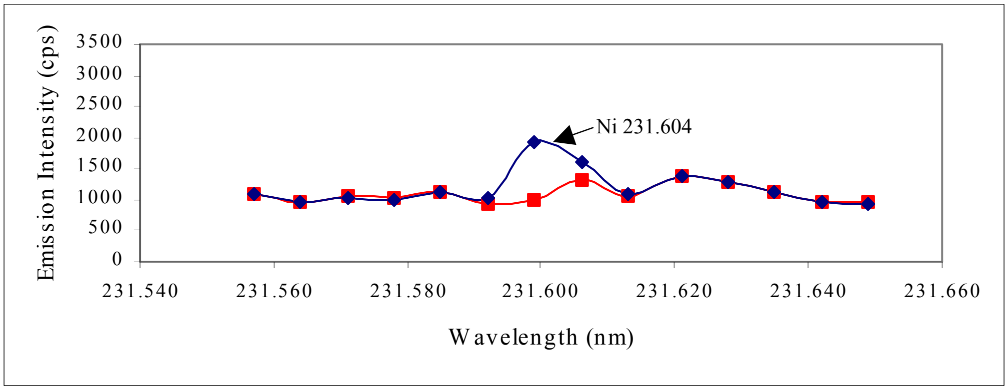


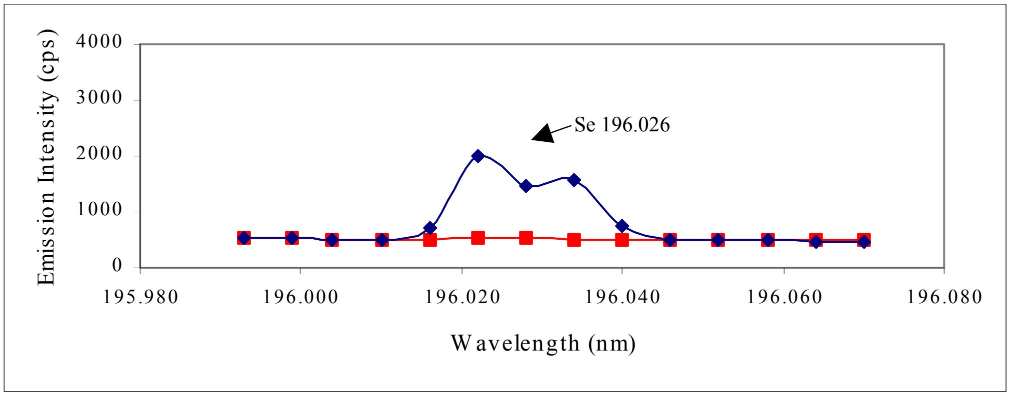


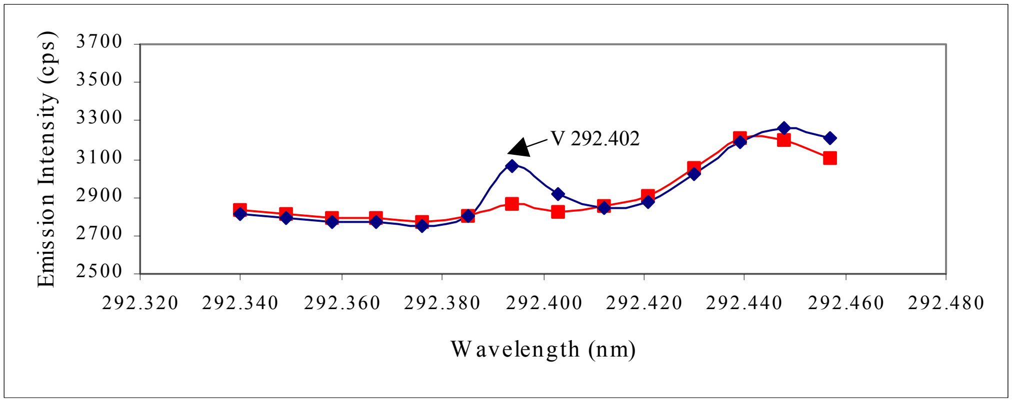
Squares = freshwater matrix blank (I.D.: 472A-113-MAB-1, $D_f = 1.02x$); Diamonds = matrix fortification (I.D.: 472A-113-MAS-1, $D_f = 1.02x$). Nominal concentrations for As, Cu and Fe in matrix fortification sample = 50.0, 50.0 and 25.0 µg/L, respectively.

Appendix 10.16

Emission Spectra for Nickel (top), Selenium (middle) and Vanadium (bottom) in Matrix Blank and Matrix Fortification Samples Prepared in Freshwater and Analyzed by ICP-AES



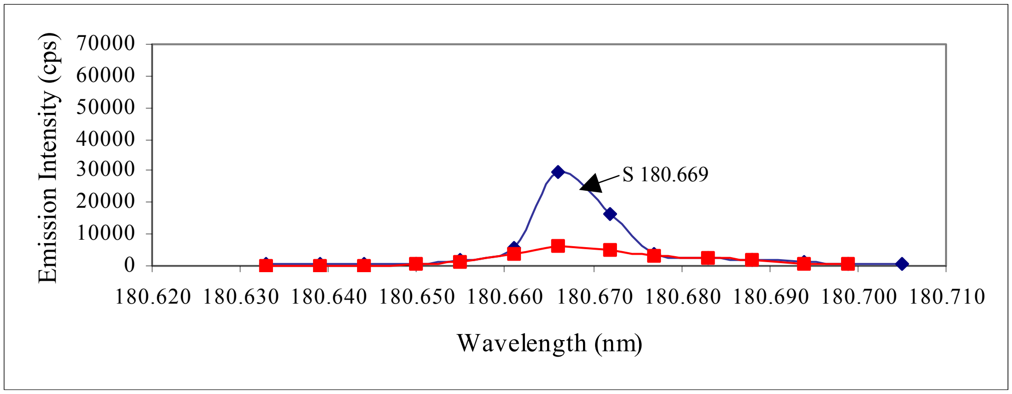




Squares = freshwater matrix blank (I.D.: 472A-113-MAB-1, $D_f = 1.02x$); Diamonds = matrix fortification (I.D.: 472A-113-MAS-1, $D_f = 1.02x$). Nominal concentrations for Ni, Se and V in matrix fortification sample = 25.0, 500 and 1.00 µg/L, respectively.

Appendix 10.17

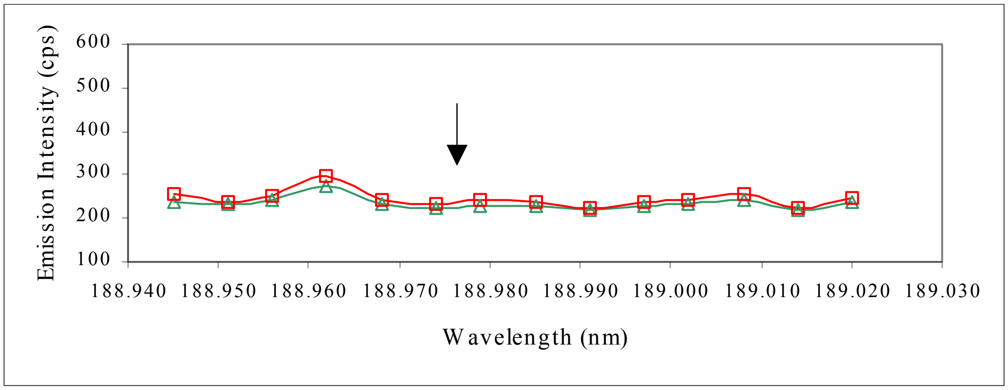
Emission Spectra for Sulfur in Matrix Blank and Matrix Fortification Samples
Prepared in Freshwater and Analyzed by ICP-AES

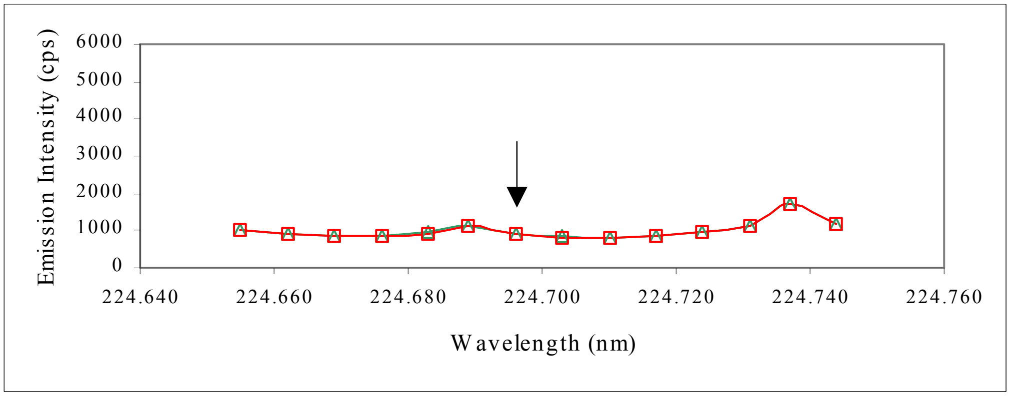


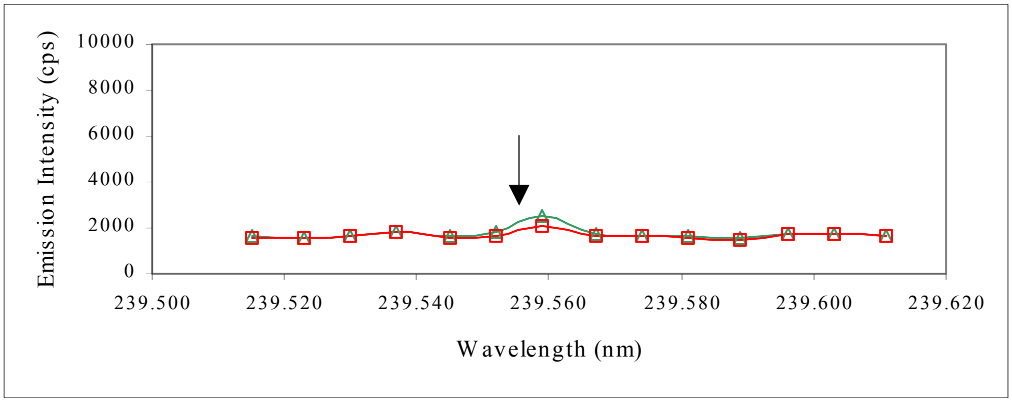
Squares = freshwater matrix blank (I.D.: 472A-113-MAB-1, D_f = 1.02x); Diamonds = matrix fortification (I.D.: 472A-113-MAS-1, D_f = 1.02x). Nominal concentrations for S in matrix fortification sample = 20.0 mg/L.

Appendix 10.18

Representative Emission Spectra of Arsenic (top), Copper (middle) and Iron (bottom) in a Test Sample Analyzed by ICP-AES



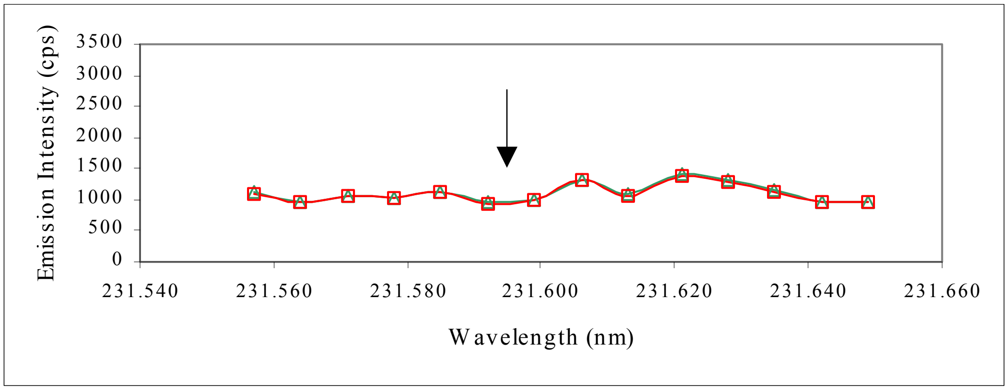


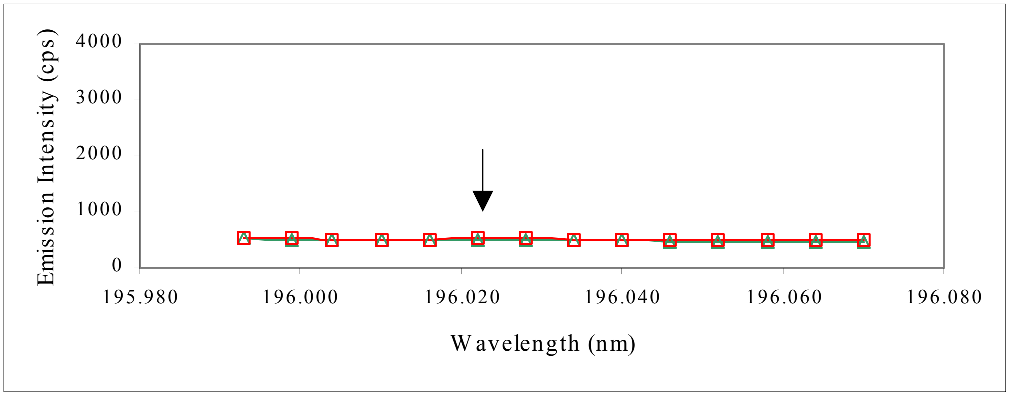


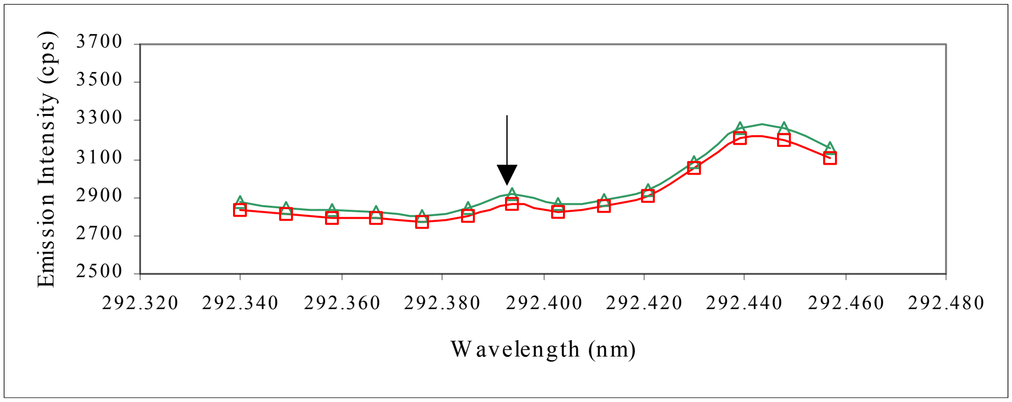
Squares = freshwater calibration blank (I.D.: 472A-112,113-M-0); Triangles = 0 hour WAF test sample (472A-113-4, 1000 mg/L petroleum coke nominal concentration). The arrows indicate expected wavelength for each element response.

Appendix 10.19

Representative Emission Spectra of Nickel (top), Selenium (middle) and Vanadium (bottom) in a Test Sample Analyzed by ICP-AES



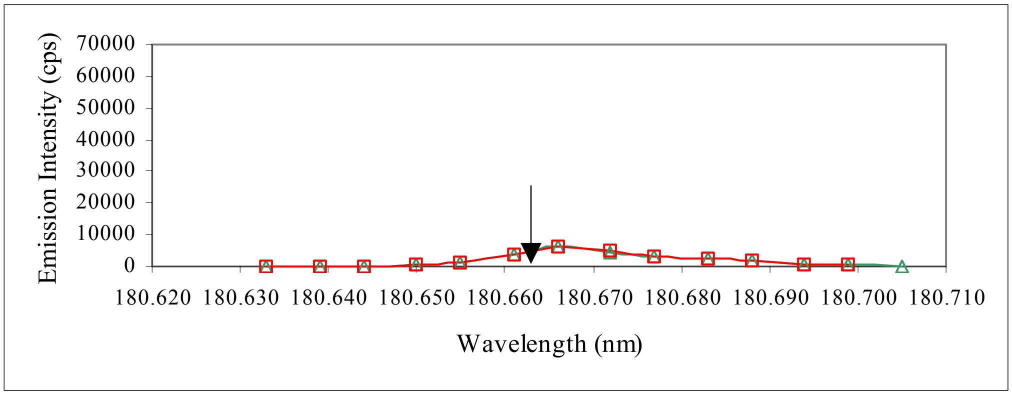




Squares = freshwater calibration blank (I.D.: 472A-112,113-M-0); Triangles = 0 hour WAF test sample (472A-113-4, 1000 mg/L petroleum coke nominal concentration). The arrows indicate expected wavelength for each element response.

Appendix 10.20

Representative Emission Spectra of Sulfur in a Test Sample Analyzed by ICP-AES



Squares = freshwater matrix blank (I.D.: 472A-113-MAB-1, D_f = 1.02x); Triangles = 0 hour WAF test sample (472A-113-4, 1000 mg/L petroleum coke nominal concentration). The arrow indicates the expected wavelength for sulfur response above background levels.

Appendix 11

Personnel Involved in the Study

The following key Wildlife International, Ltd. personnel were involved in the conduct or management of this study:

1. [redacted]
2. [redacted]
3. [redacted]
4. [redacted]
5. [redacted]
6. [redacted]

Appendix 12

Report Amendment

1.	Original Report:	Title Page
	Amended Report:	The amended report date was added. The total number of pages was changed from 148 to 162
	Reason:	To indicate that the report was amended and note change in pagination.
2.	Original Report:	Page 2
	Amended Report:	The amended report date was added and new signatures and dates were added.
	Reason:	To show the amended report date and to provide new signatures and dates for the amended report.
3.	Original Report:	Page 3
	Amended Report:	The audit dates for the amended report were added and a new signature and date were added.
	Reason:	To show the amended report audit dates and to provide a new signature and date for the amended report.
4.	Original Report:	Page 4
	Amended Report:	New signatures and dates were added.
	Reason:	To provide new signatures and dates for the amended report.
5.	Original Report:	Page 8
	Amended Report:	The Table of Contents was updated to show the addition of Appendix 3, renumber all appendices from Appendix 3 through the end of the report and added the Report Amendment appendix (Appendix 12).
	Reason:	The Sponsor requested that Appendix 3 be added to the final report.
6.	Original Report:	Pages 14-18
	Amended Report:	The appendix reference numbers were modified.
	Reason:	The Sponsor requested that Appendix 3 be added to the final report, therefore, all appendices thereafter had to be renumbered.
7.	Original Report:	Page 80
	Amended Report:	Appendix 3 was added to the report.
	Reason:	The Sponsor requested that Appendix 3 be added to the final report.

Appendix 12
(continued)

Report Amendment

8.	Original Report:	Pages 80-148
	Amended Report:	Appendix 3 was added to the report, therefore all appendices thereafter were renumbered.
	Reason:	The Sponsor requested that Appendix 3 be added to the final report.

AMENDMENT SIGNATURES:

[redacted]

10 April 2007
Date

10 April 07
Date

4/26/2007
Date

4/10/2007
Date