



Laboratory Procedure Manual

Analyte: **Monohydroxy-Polycyclic Aromatic Hydrocarbons (OH-PAHs)**

Matrix: **Urine**

Method: **Isotope Dilution Gas Chromatography/High Resolution Mass Spectrometry (GC/HRMS)**

Method No.: **09-OD**

Revised: **10/03/2006**

as performed by:

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Important Information for Users

The Centers for Disease Control and Prevention (CDC) periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

This document details the Lab Protocol for NHANES 2003–2004 data.

A tabular list of the released analytes follows:

Data File Name	Variable Name	SAS Label
I31pah_c	URXPO1	1-naphthol (ng/L)
	URXPO2	2-naphthol
	URXPO3	3-fluorene
	URXPO4	2-fluorene
	URXPO5	3-phenanthrene
	URXPO6	1-phenanthrene
	URXPO7	2-phenanthrene
	URXPO8	1-benzo[c] phenanthrene
	URXP10	1-pyrene
	URXP11	2-benzo[c] phenanthrene
	URXP12	1-benzo[a] anthracene
	URXP13	6-chrysene
	URXP14	3-benzo[c] phenanthrene
	URXP15	3-chrysene
	URXP16	3-benz[a] anthracene
	URXP17	9-fluorene
	URXP19	4-phenanthrene
	URXP20	1-chrysene
	URXP21	2-chrysene
	URXP22	4-chrysene

	URXP24	3-benzo(a)pyrene
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1. Clinical Relevance and Summary of Test Principle

a. Clinical Relevance

Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous environmental contaminants formed during incomplete combustion processes. Many of them have been identified as suspected human carcinogens (1). Common routes of occupational exposure may include work involving diesel fuels and coal tars such as paving and roofing. Possible environmental exposures include smoking, diet, smog and forest fires (2, 3). Application of this method to analyze samples obtained from participants in the National Health and Nutrition Examination Survey (NHANES) will help determining the reference range of these chemicals in general U.S. population, aged 6 years and higher.

b. Test Principle

The specific analytes measured in this method are monohydroxy-PAH (OH-PAH). The procedure involves enzymatic hydrolysis of urine, extraction, derivatization and analysis using capillary gas chromatography combined with high resolution mass spectrometry (GC-HRMS) (4, 5). This method uses isotope dilution with carbon-13 labeled internal standards. Ions from each analyte and each carbon-13 labeled internal standard are monitored, and the abundances of each ion are measured. The ratios of these ions are used as criteria for evaluating the data. The analytes measured in this procedure are shown in Table 1. By evaluating these analytes in urine, a measurement of the body burden from PAH exposure is obtained.

Table 1. Analytes measured, their parent compounds, and their abbreviations.

No.	Parent PAH	Metabolite/Analyte	Abbreviation
1	Naphthalene	1-hydroxynaphthalene	1-NAP
2	Naphthalene	2-hydroxynaphthalene	2-NAP
3	Fluorene	9-hydroxyfluorene	9-FLUO
4	Fluorene	2-hydroxyfluorene	2-FLUO
5	Fluorene	3-hydroxyfluorene	3-FLUO
6	Phenanthrene	1-hydroxyphenanthrene	1-PHEN
7	Phenanthrene	2-hydroxyphenanthrene	2-PHEN
8	Phenanthrene	3-hydroxyphenanthrene	3-PHEN
9	Phenanthrene	4-hydroxyphenanthrene	4-PHEN
11	Fluoranthene	3-hydroxyfluoranthene	3-FLRAN
12	Pyrene	1-hydroxypyrene	1-PYR

No.	Parent PAH	Metabolite/Analyte	Abbreviation
13	Benzo[c]phenanthrene	1-hydroxybenzo[c]phenanthrene	1-BCP
14	Benzo[c]phenanthrene	2-hydroxybenzo[c]phenanthrene	2-BCP
15	Benzo[c]phenanthrene	3-hydroxybenzo[c]phenanthrene	3-BCP
16	Benz[a]anthracene	1-hydroxybenz[a]anthracene	1-BAA
17	Benz[a]anthracene	3-hydroxybenz[a]anthracene	3-BAA
18	Benz[a]anthracene	9-hydroxybenz[a]anthracene	9-BAA
19	Chrysene	1-hydroxychrysene	1-CHRY
20	Chrysene	2-hydroxychrysene	2-CHRY
21	Chrysene	3-hydroxychrysene	3-CHRY
22	Chrysene	4-hydroxychrysene	4-CHRY
23	Chrysene	6-hydroxychrysene	6-CHRY
24	Benzo[a]pyrene	3-hydroxybenzo[a]pyrene	3-BAP
25	Benzo[a]pyrene	7-hydroxybenzo[a]pyrene	7-BAP

2. Safety Precautions

a. Reagent toxicity or carcinogenicity

Some of the reagents necessary to perform this procedure are toxic. Special care must be taken to avoid inhalation or dermal exposure to the reagents necessary to carry out the procedure.

b. Radioactive hazards

There are no radioactive hazards associated with this procedure.

c. Microbiological hazards

Although urine is generally regarded as less infectious than serum, the possibility of being exposed to various microbiological hazards exists. Appropriate measures must be taken to avoid any direct contact with the specimen (See Section 2.e.). CDC recommends a Hepatitis B vaccination series and a baseline test for health care and laboratory workers who are exposed to human fluids and tissues. Observe Universal Precautions.

d. Mechanical hazards

There are only minimal mechanical hazards when performing this procedure using standard safety practices. Laboratorians must read and follow the manufacturer's information regarding safe operation of the equipment. Avoid direct contact with the mechanical and electronic components of the mass spectrometer unless all power to the instrument is off. Generally, mechanical and electronic maintenance and repair must only be performed by qualified technicians. The autosampler and the mass spectrometer contain a number of areas which are hot enough to cause burns. Precautions must be used when working in these areas.

e. Protective equipment

Standard safety precautions must be followed when performing this procedure, including the use of a lab coat/disposable gown, safety glasses, appropriate gloves, and chemical fume hood. Refer to the laboratory Chemical Hygiene Plan and CDC Division of Laboratory Sciences safety policies and procedures for details related to specific activities, reagents, or agents.

f. Training

Formal training in the use of a high resolution mass spectrometer is necessary. Users are required to read the operation manuals and must demonstrate safe techniques in performing the method. Anyone involved in sample preparation must be trained in all sample preparation equipment, chemical handling, and have basic chemistry laboratory skills.

g. Personal hygiene

Follow Universal Precautions. Care must be taken when handling chemicals or any biological specimen. Routine use of gloves and proper hand washing must be practiced. Refer to the laboratory Chemical Hygiene Plan and CDC Division of Laboratory Sciences safety policies and procedures for details related to specific activities, reagents, or agents.

h. Disposal of wastes

Waste materials must be disposed of in compliance with laboratory, federal, state, and local regulations. Solvents and reagents must always be disposed of in an appropriate container clearly marked for waste products and temporarily stored in a chemical fume hood. All disposable items that come in direct contact with the biological specimens are to be placed in a biohazard autoclave bag that must be kept in appropriate containers until sealed and autoclaved. The unshielded needles, pipette tips and disposable syringes must be placed immediately into a sharps container and autoclaved when this container becomes full. Wipe down all surfaces with a freshly prepared bleach solution (a 10% dilution of commercial sodium hypochlorite (bleach) or equivalent) when work is finished. Any non-disposable glassware or equipment that comes in contact with biological samples must be

washed with bleach solution before reuse or disposal. Any other non-disposable glassware must be washed and recycled or disposed in an appropriate manner.

***Observe Universal Precautions.** Dispose of all biological samples and diluted specimens in a brown glass bottle; disinfect the bio-hazardous material with bleach (10% in final volume), and dispose according to CDC/DLS guidelines for disposal of hazardous waste. Dispose all used disposable laboratory supplies (tubes, pipette tips, etc.) in an autoclave bag at the end of the analysis according to CDC/DLS guidelines for disposal of hazardous waste.*

3. Computerization; Data-System Management

a. Software and knowledge requirements

This method has been validated using the Thermo Finnigan GC/HRMS system controlled by Xcalibur™ Software 1.3. Analyte peaks are integrated by Quan Browser under Xcalibur™. Results are exported from Quan Browser result files to Microsoft Excel files that are subsequently used for calculations. Final results are stored in Excel format. Knowledge of and experience with these software packages (or their equivalent) is required to utilize and maintain the data management structure.

b. Sample information

Information pertaining to particular specimens is entered into the database either manually or electronically transferred. The result file is transferred electronically into the database. No personal identifiers are used, and all samples are referenced to a blind coded sample identifier.

c. Data maintenance

All sample and analytical data are checked prior to being entered into the MS Excel for transcription errors and overall validity. The database is routinely backed up locally through the standard practices of the NCEH network. The local area network manager must be contacted for emergency assistance.

d. Information security

Information security is managed at multiple levels. The information management systems that contain the final reportable results are restricted through user ID and password security access. The computers and instrument systems that contain the raw and processed data files require specific knowledge of software manipulation techniques and physical location. Site security is provided at multiple levels through restricted access to the individual laboratories, buildings, and site. Confidentiality of results is protected by referencing results to blind coded sample IDs (no names or personal identifiers have used).

4. Procedures for Collecting, Storing, and Handling Specimens; Criteria for Specimen Rejection

a. Special instructions

No special instructions such as fasting or special diets are required.

b. Sample collection

Urine specimens are collected from subjects in standard urine collection cups. Samples must be refrigerated as soon as possible, and must be transferred to specimen vials within 24 hours of collection. A minimum of 5 milliliters of urine is collected and poured into sterile vials with screw-cap tops. The specimens are then labeled, frozen immediately to $-20\text{ }^{\circ}\text{C}$, and stored on dry ice for shipping. Special care must be taken in packing to protect vials from breakage during shipment. All samples in long-term storage must be kept at $-70\text{ }^{\circ}\text{C}$ until analysis.

c. Sample handling

In general, urine specimens must be transported and stored at frozen ($< -10\text{ }^{\circ}\text{C}$). Once received, they can be frozen at $-70 \pm 10\text{ }^{\circ}\text{C}$ until time for analysis. Portions of the sample that remain after analytical aliquots are withdrawn must be refrozen at $-70 \pm 10\text{ }^{\circ}\text{C}$. Samples are not compromised by repeated freeze and thaw cycles.

d. Sample quantity

The minimum amount of specimen required for analysis is 0.5 mL, with the optimal amount being 2.0 mL.

e. Unacceptable specimens

Specimens must be frozen at a minimum of $-20 \pm 5\text{ }^{\circ}\text{C}$ when delivered to the lab. The minimum volume required is 0.5 mL. If either of these criteria is violated, then specimen must be rejected. Specimens are also rejected if suspected of contamination due to improper collection procedures or devices. Specimen characteristics that may compromise test results include contamination of urine by contact with dust, dirt, etc. from improper handling. Samples with visible microbiological growth (e.g. mold, bacteria) must also be rejected. In all cases, request a second urine specimen if available. A description of reasons for each rejected sample must be recorded on the sample transfer sheet, such as low sample volume, leaking or damaged container.

5. Procedures for Microscopic Examinations; Criteria for Rejecting Inadequately Prepared Slides

Not applicable for this procedure.

6. Preparation of Reagents, Calibration Materials, Control Materials, and all Other Materials; Equipment and Instrumentation

a. Reagents and sources

See Table 2.

Table 2. Reagents and the Respective Manufacturers

Reagent	Manufacturers*
organic de-ionized water (D.I. H ₂ O)	Prepared in house, CDC (Aqua Solutions, Inc.)
β-glucuronidase/arylsulfatase, H-1, powder enzyme	Sigma Chemical, St. Louis, MO
pentane, hexane, acetonitrile, toluene, and methanol (ABSOLV grade)	Tedia Company, Fairfield, OH
glacial acetic acid	Sigma Chemical, St. Louis, MO
sodium acetate	Sigma Chemical, St. Louis, MO
N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA)	Sigma Chemical, St. Louis, MO
argon, nitrogen	Air Products and Chemicals, Allentown, PA
¹³ C ₆ 1-NAP	Synthesized in house, CDC
¹³ C ₆ 1-PYR, ¹³ C ₆ 3-FLRAN,	TerraChem, Merriam, KS
¹³ C ₆ 3-PHEN, ¹³ C ₆ 6-CHRY, ¹³ C ₆ 1-BAA, ¹³ C ₆ 3-BAP, ¹³ C ₆ 4-OH-PCB79	Cambridge Isotope Laboratories, Andover, MA
Reagent	Manufacturers*
¹³ C ₆ 2-FLUO, ¹³ C ₆ 3-BCP, ¹³ C ₆ 3-CHRY	ChemSyn, Lenexa, KS
¹³ C ₆ 3-FLUO, ¹³ C ₆ 9-FLUO, ¹³ C ₆ 2-PHEN	Los Alamos National Laboratory, Los Alamos, NM
1-NAP, 2-NAP, 2-FLUO, 3-FLUO, 9-FLUO, 1-PYR	Sigma-Aldrich Chemicals, St. Louis, MO
1-PHEN, 2-PHEN, 3-PHEN, 4-PHEN	Promochem, Wesel, Germany

3-FLRAN, 1-BCP, 2-BCP, 3-BCP, 1-BAA,
3-BAA, 9-BAA, 1-CHRY, 2-CHRY, 3-
CHRY, 4-CHRY, 6-CHRY, 3-BAP, 7-BAP

Midwest Research Institute, NCI, Kansas
City, MO

* Equivalent products from other manufacturers may be used.

b. Preparation of Reagents

1) 1 mol/L Sodium Acetate Buffer

Place 41 g sodium acetate in a 500-mL volumetric flask and add about 300 mL de-ionized water (D.I. H₂O). Swirl solution to dissolve sodium acetate. Fill flask to the 500 mL line with D.I. H₂O. Adjust the pH to 5.5 with glacial acetic acid.

2) N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA)

Open the sealed vial containing MSTFA from the vendor and place the MSTFA solution in an amber screw-cap vial (2-mL). Displace the air over the MSTFA with a gentle stream of argon. The MSTFA can be stored in the microreaction vessel for up to 1 month. To add MSTFA to multiple samples, use an Eppendorf repeater pipette with a 100- μ L pipette tip, set the pipette volume at 10 μ L, and then withdraw 100 μ L of MSTFA. Discard the first two aliquots and the last aliquot of MSTFA (10 μ L per aliquot), aliquot 10 μ L into each of the sample vials.

c. Preparation of Calibration Materials

All OH-PAH are light sensitive and precautions to minimize exposure to light must be taken, such as use of UV-filtered yellow light in lab areas where samples

1) Stock Solutions of Individual Analytes (100 ng/ μ L, 400 ng/ μ L for 1- and 2-NAP)

Approximately 5-10 mg of neat standard is weighed into a silanized screw cap amber vial. Add 3 mL of acetonitrile into the vial and record the exact weight of the solvent. Allow the OH-PAH to dissolve by gentle swirling or placing in an ultrasonic bath. Dilute the individual standards using toluene to 100 ng/ μ L (400 ng/ μ L for 1- and 2-NAP) with a final volume of 3.0 mL. All solutions are stored in a -70 °C freezer with an inert argon atmosphere in the vials.

2) Working Standard Solution of 24 native OH-PAH mix (W.S.A)

Combine 400 μ L from each of the 24 native OH-PAH standard stock solutions in a silanized screw cap amber vial to generate the working standard solution (W.S.A, 4 ng/ μ L for each native OH-PAH concentration, except for 1- and 2-NAP at 16 ng/ μ L). Homogenize the mixture by gentle swirling and vortexing.

Solutions of other concentrations may also be prepared, if needed. Displace air in the vial with argon, cap the vials, and stored them at -70 °C until needed.

3) Working Standard Solution of 13 C13-labeled OH-PAH mix (W.S.I)

Combine 3.76 mL of acetonitrile and 480 µL of each of the 13 ¹³C-labeled OH-PAH standard stock solutions (90 ng/µL) in a silanized screw cap amber vial to generate the working internal standard solution (W.S.I, 4 ng/µL for each of the 13 ¹³C-labeled OH-PAHs). Homogenize the mixture by gentle swirling and vortexing. Displace air in the vial with argon, cap the vials, and stored them at -70 °C until needed.

4) External Calibration Standards (E.C.S)

External calibration standards (E.C.S.) are prepared as presented in Table 3 below. Concentrations for 1- and 2-NAP in all standards are 4 times higher than the rest of the native compounds, because these two compounds are present in urine samples at high concentrations. Concentrations for ¹³C-labeled internal standards are 100 pg/µL in all calibration standards.

Table 3. Preparation of external calibration standards (ECS)

Standard No.	Analytes, excluding 1- & 2-NAP		1- and 2- NAP	
	Concentration (pg/µL)	Equivalent concentration in urine(pg/mL)	Concentration (pg/µL)	Equivalent concentration in urine (pg/mL)
0	0.5	5	2	20
1	1	10	4	40
2	2	20	8	80
3	5	50	20	200
Standard No.	Analytes, excluding 1- & 2-NAP		1- and 2- NAP	
	Concentration (pg/µL)	Equivalent concentration in urine (pg/mL)	Concentration (pg/µL)	Equivalent concentration in urine (pg/mL)
4	10	100	40	400
5	50	500	200	2,000
6	100	1,000	400	4,000
7	500	5,000	2,000	20,000
8	1000	10,000	4,000	40,000
9	2000	20,000	8,000	80,000
10	4000	40,000	16,000	160,000

5) Internal Qualification Standards (I.Q.S)

Measure 5.0 mL of W.S.I into a 200-mL silanized volumetric flask. Dilute the solution with toluene to the 200-mL line to get the internal qualification standard (I.Q.S). The concentration of each ^{13}C -labeled OH-PAH is 100 pg/ μL . Aliquot 1.7 mL of I.Q.S. into amber 2-mL standard vials, cap and seal the vials with Argon. Store all I.Q.S. vials in the fridge until use.

6) Mass Spectrometric Check Solution

This solution is used daily to monitor the operating performance of the GC column and the mass spectrometer. A solution of 20 fg/ μL of a tetra-chlorinated dioxin is used as the check solution. For a 1 μL injection, a minimum signal-to-noise of 4 on the m/z 321.894 peak must be obtained.

d. Preparation of Control Materials

1) Quality Control (QC) materials

Prepare quality control materials by spiking a known amount of native OH-PAH mixture (in acetonitrile) into 2000 mL of an anonymous filtered urine pool (500 pg/mL urine). Homogenize the QC solutions overnight for equilibration. On the next day aliquot the QC solutions into 16 x 100 mm test tubes (3 mL in each tube) and store them at -70 ± 10 °C until use.

2) Recovery Spiking Solution (R.S.S)

Approximately 3 mg of neat $^{13}\text{C}_6$ 4-OH-PCB79 is weighed into a silanized screw cap amber vial. Add 3 mL of toluene into the vial and record the exact weight of the solvent. Allow the standard to dissolve by gentle swirling or placing in an ultrasonic bath. Methylate the standard using diazomethane to obtain its methylated derivative $^{13}\text{C}_6$ 4-MeH-PCB79. Dilute the methylated standard solution using toluene to 200 pg/ μL . This will be used as the recovery spiking solution (R.S.S.). Aliquot 1.7 mL of E.R.S. into amber 2-mL standard vials, cap and seal the vials with Argon. Store all E.R.S. vials at 4 ± 5 °C until use.

3) Proficiency Test Material (PT)

Prepare quality control materials by spiking a known amount of W.S.A (at a different level as the QC) into 100 mL of an anonymous urine pool (filtered) to achieve the target concentration. Prepare the urine pools at levels within the linear range of the method. After spiking the urine pool with a known amount of W.S.A, homogenize the PT solutions overnight for equilibration. On the next day aliquot the PT solutions into 16 x 100 mm test tubes (3 mL in each tube) and store them at -70 ± 10 °C until use.

e. Other equipment, materials, and supplies

Materials / supplies and sources used during the development, validation, and application of this method are listed below. Materials / supplies procured from other sources must meet or exceed these specifications.

- Gilson 215 liquid handler (Gilson Inc., Middleton, WI)
- 818 Automix mixer (Gilson Inc., Middleton, WI)
- Water bath/sonicator (Branson Ultrasonics, Danbury, CT)
- TurboVap LV evaporator (Caliper LifeSciences, Hopkinton, MA)
- Incubator ovens (Fisher Scientific)
- pH meter (Thermo)
- Microbalance (Mettler-Toledo)
- Stirring/heating plates (Corning)
- Miscellaneous glassware (Pyrex, Kimax, Wheaton or Corning)
- Eppendorf Repeater Plus Pipette (Brinkmann Instruments Inc., Westbury, NY).
- Rainin Electronic Pipettes (Rainin, California)
- Pasteur pipettes and bulbs (VWR).
- Maxi-mix Vortex mixer (Barnstead International)
- Allegra-6 centrifuge (Beckman-Coulter)
- Amber screw top vials with various volume (Supelco, Inc., Bellefonte, PA)
- Clear autosampler vials, 0.5 mL (SunSri)

f. Instrumentation

The analyses are performed on a ThermoFinnigan MAT-95XL high resolution mass spectrometer (Finnigan MAT, San Jose, CA) equipped with an electron impact ionization and interfaced to an Agilent Technologies 6890 gas chromatograph (GC) system (Agilent Technologies, Palo Alto, CA)

1) Gas chromatograph configuration

Chromatographic separation is performed on an Agilent 6890 gas chromatograph fitted with a J&W DB-5 30-m fused silica capillary column. The column ID is 0.25-mm and the film thickness is 0.25-micron (J&W, # 22-5022 or

equivalent). The temperature program lasts a total of 23 minutes. (See Table 4 for GC configuration and Table 5 for the GC temperature program.)

Table 4. GC Configuration

GC Parameter	Setting
Carrier gas	Helium
Constant flow rate	1 mL/minute
GC purge flow rate	70 mL/minute
GC saver time	5 minutes
GC save flow rate	15 mL/minute
Injection mode	Splitless
Injector purge delay	2 minutes
Injector temperature	270 °C

Table 5. GC Temperature Program

Time (min.)	Temperature (°C)
0	95
2	95
6	160
Time (min.)	Temperature (°C)
20	295
21	320
25	320

2) High Resolution Mass spectrometer (HRMS) configuration

Thermo Finnigan MAT 95XL HRMS configuration is presented in Table 6. The mass spectrometer is operated under Multiple Ion Detection (MID) mode. The masses used to quantify analytes are presented in Table 7.

Table 6. Thermo Finnigan MAT 95XL HRMS Configuration

HRMS Parameter	Setting
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HRMS Parameter	Setting
Scan mode	Multiple ion detection
Ionization type	Electron impact
Ion polarity mode	Positive
Electron energy	45 eV
Resolution	10,000
Ion source	250 °C
Conversion dynode voltage	Positive
Electron multiplier voltage	1.45 – 2.25 kV (or 10 ⁶ gain)

Table 7. Analyte Masses

Analyte	Molecular Ion [M ⁺]	Confirmation Ion [M ⁺ -15]
1-NAP, 2-NAP	216.0970	201
2-FLUO, 3-FLUO, 9-FLUO	254.1127	239
1-PHEN, 2-PHEN, 3-PHEN, 4-PHEN	266.1127	251
3-FLRAN, 1-PYR	290.1127	275
3-BAP	340.1283	325
Analyte	Molecular Ion [M ⁺]	Confirmation Ion [M ⁺ -15]
1-BCP, 2-BCP, 3-BCP, 1-BAA, 3-BAA, 9-BAA, 1-CHRY, 2-CHRY, 3-CHRY, 4-CHRY, 6-CHRY	316.1283	301
¹³ C ₆ 1-NAP	222.1172	207
¹³ C ₆ 2-FLUO, ¹³ C ₆ 3-FLUO, ¹³ C ₆ 9-FLUO	260.1328	245
¹³ C ₆ 3-PHEN, ¹³ C ₆ 2-PHEN	272.1328	257
¹³ C ₆ 3-FLRAN, ¹³ C ₆ 1-PYR	272.1328	257
¹³ C ₆ 3-CHRY, ¹³ C ₆ 6-CHRY, ¹³ C ₆ 1-BAA, ¹³ C ₆ 3-BCP	296.1328	281

7. Calibration and Calibration Verification

a. Calibration of Mass Spectrometer

Calibrate and tune the Finnigan MAT 95XL mass spectrometer using FC43 (perfluorotributylamine) according to the instructions in the operator's manual located next to the instrument. After calibrated with 10,000 resolution and maximum sensitivity, the instrument is prepared for the analysis of OH-PAHs as described in Section 8.

b. Creation of calibration curve

1) Calculation data

A linear calibration curve, using eight ECS with concentration ranging from 1 to 1000 pg/ μ L, is generated using the ratio of the peak area of the analyte to the labeled internal standard. The concentrations in ECS correspond to 10 - 10,000 pg/mL (40-40,000 pg/mL) levels in 2 mL urine. For urine samples with concentrations higher than the calibration curve, the highest two standards (2000 & 4000 pg/ μ L, 8,000 and 16,000 pg/ μ L for 1-&2-NAP) are prepared to extend the calibration curve and to accurately quantify those samples.

2) Evaluation of curve statistics

The R-squared value of the curve must be equal to or greater than 0.990. Linearity of the standard curve must extend over the entire standard range.

3) Use of the calibration curve

The lowest point on the calibration curve is the lowest reportable level and the highest point is above the expected range of results. The remainders of the points are distributed between these two extremes, with the majority of points in the concentration range where most unknowns fall.

c. Calibration verification

In order to verify that this calibration of this test system is accurate and stable throughout reportable range, three E.C.S from a different batch are analyzed every six months. Calculated concentration must be within 10% deviation from expected concentration.

8. Procedure Operation Instructions; Calculations; Interpretation of Results

An analytical run consists of two blanks, two QCs, and 16 unknown urine samples.

a. Sample Preparation

All samples were prepared in a laboratory with non-UV yellow fluorescent lights.

1) Hydrolysis

Allow urine samples and QCs to thaw and reach room temperature. Aliquot 2 mL of urine sample (or 2 mL of D.I. H₂O as blank) into a 16x100 mm test tube. Add 10 µL of I.Q.S, 1 mL of 1 M sodium acetate buffer (pH = 5.5) containing β-Glucuronidase/ arylsulfatase enzyme from *Helix pomatia* (10 mg enzyme/1 mL buffer) into the test tube. Turn the test tube gently several times to mix well. Place the caps on the tube and incubate the sample at 37 °C overnight.

2) Automated Liquid-Liquid Extraction

Add D.I. water (2 mL) to the sample, and extract the sample twice with pentane using the Gilson 215 liquid handler (Gilson Inc., Middleton, WI) for automation. Place the sample in the automix tray and initiate the extraction procedure. The automated procedure starts with adding pentane (5 mL) to the sample and mixing the sample by rotating it in the automix for 5 minutes (20 rpm). Then the procedure is paused and the sample is centrifuged at 2800 rpm on an Allegra™ 6 centrifuge (Beckman Coulter Inc., Fullerton, CA) for 20 minutes. After returning the samples to the automix tray, resume the procedure and a probe transfers the organic phase to clean collection tubes. This extraction procedure is repeated again with additional pentane (5 mL).

3) Evaporation

Sample extract is spiked with 5 µL dodecane and placed in a TurboVap LV evaporator. Under a stream of N₂ and in a 40 °C water bath, the sample is evaporated to ~5 µL within 10-15 minutes. Spike 20 µL toluene and 5 µL R.S.S into the sample tube, vortex the sample, and transfer the contents to clear autosampler vial.

4) Derivatization

Add 10 µL of MSTFA into the GC vial and then displace the air in the vial with a gentle stream of argon. Quickly screw a cap onto the vial. Place the vials in an incubator or oven set at 60 °C for 30 minutes. The samples are then ready for analysis on the mass spectrometer. The samples may also be stored in a refrigerator for up to 1 week.

b. Instrument and software setup for the GC/HRMS

1) Preliminary MAT 95 system setup and performance check

Turn on the MAT 95, inject 2 µL FC-43 into the reference inlet. In the TUNE window, adjust resolution between 9900 and 10100. Perform a GC/HRMS

analysis of the mass spectrometer check solution (2, 3, 7, 8-TCDD, 10 fg/μL, 1 μL injection) and verify chromatographic resolution and peak intensity. In the lab note book, record the signal to noise of the check compound.

2) Final setup and operation

a) *Create the run sequence*

In the Xcalibur Sequence Setup window, create a sequence for the run using the template. Make sure that the appropriate number of samples is loaded, the appropriate filenames are assigned, and the appropriate sample positions on the autosampler tray are included in the run sequence. Make sure that the correct process method and instrument method are selected. The latter defines GC, MID, Autosampler, and ICL methods. The methods used are listed in Table 8.

Table 8. GC/HRMS Methods

Program	Method
Instrument	NHANES0304.met
GC	pah_deriv.mcr
MID	pah_deriv.mid
Autosampler	pah_1ul.mcs
Program	Method
ICL	Sleep90.icl 5
Process	NHANES0304.pmd

Filenames conform to the following format: PMYYNNN or KMYNNN where P = PAH samples run on the MAT 95 instrument named as CASSI, K = PAH samples run on the MAT 95 instrument named as MIKE; M = month (A = January, B = February, C = March, etc.); YY = year (04 = year 2004), and NNN = run number for the month. For example, the filename PB01012 corresponds to PAH-February-2001-sample #12 run on MAT 95 – CASSI, and KD02267 would correspond to PAH-April-2002-sample #267 run on MAT 95 – MIKE.

b) *Start the sequence*

Click **Run Sequence** in the main menu under Action. Verify the sequence set up and make sure the whole sequence instead of one single sample is selected to be run. Click **OK**; the system will immediately start by turning green on the first sample to run.

3) System standby

To place the MAT 95 in standby mode, enter the command **.bye** at the ICL prompt. This command turns off the accelerator, multiplier and dynode voltage, and vents the reference compound (FC-43). To reactivate the MAT 95, enter **.run 0.50** at the command prompt. Add the reference compound and tune.

c. Processing of data

After the run sequence finishes, process all raw data files in the whole sequence using the "NHANES0304.pmd" process method after which the analyte peaks in data files are automatically integrated using the process method. Visually review and manually correct if needed the integration of each peak in the QuanBrowser window of the Xcalibur software. Save the reviewed result file, export it as a MS Excel file, and save the Excel file on the Q:\ share drive. Perform all further calculations such as standard curve generation, QC analysis, blank analysis, limit-of-detection determination, unknown sample calculations, data distribution, etc. in MS Excel and in SAS. Import final results and all supporting information into an RBase database located on the Q:\ share drive.

3-BAA and 9-BAA co-elute in the GC chromatograms, so they are reported together as 3_9-BAA. 3-FLRAN had an interfering peak in the GC chromatograms; therefore, 3-FLRAN is not reported.

d. Replacement and Periodic Maintenance of Key Components

1) MAT 95 XL Mass Spectrometer

- Check cooling water level and temperature monthly
- Clean the ion volume or replace it monthly
- Clean the ion source or replace it annually
- Replace the calibration gas septum monthly
- Trained Thermo Finnigan technicians perform all other maintenance based on an annual schedule, or as needed

2) Agilent 6890 GC

- Change the injection port liner and septum daily
- Clean the injection port, clean or replace the gold seal monthly
- Cut the GC column for ~10" monthly or as needed
- Replace the GC column at 1000 analyses or sooner
- Replace Helium tank when the pressure is below 500 psi.

Note: Accelerate the above maintenance schedules if necessary.

9. Reportable Range of Results

The linear range of the standard calibration curves determines the highest and lowest analytical values of an analyte that are reportable. The calibration verification of the method encompasses this reportable range. However, urine samples with analytical data values exceeding the highest reportable limit may be re-extracted using a smaller volume and re-analyzed so that the result is in the reportable range.

a. Linearity Limits

Analytical standards were linear for all analytes through the range of concentrations evaluated. The linear range for all analytes except 1-NAPH and 2-NAPH is 5 pg/mL to 40,000 pg/mL. Calibration curves for 1-NAPH and 2-NAPH are extended to 160,000 ng/L, because their high concentrations detected from unknown samples. Therefore, the linear range for 1-NAPH and 2-NAPH were 20 pg/mL to 160,000 pg/mL. Urine samples whose concentrations exceed these ranges must be diluted and re-analyzed using a smaller aliquot.

b. Limit of Detection

The limit of detection (LOD) for this method is defined as the higher LOD calculated by two methods: (i) in direct relation to method blanks prepared in parallel with the unknown samples, as 3 times the standard deviation of the method blanks, and (ii) according to the instrumental detection limit defined as the lowest point in the calibration curve (0.5 pg/uL, or 5 pg/mL in 2-mL urine samples) verified to give a signal with the S/N equal to or greater than 3. The detection limits determined for the analytes are presented in Table 9.

Table 9. Limits of Detection (LODs)

Analyte	LOD (pg/mL)
1-NAP	18
2-NAP	12
9-FLUO	3.9
2-FLUO	4.5
3-FLUO	6.9
1-PHEN	2.6
2-PHEN	3.8
3-PHEN	2.6
4-PHEN	3.7
1-PYR	4.9
1-BCP	2.8
1-BAA	2.6

3_9-BAA	5.2
1-CHRY	2.6
2-CHRY	2.6
3-CHRY	2.6
4-CHRY	2.6
6-CHRY	2.6
3-BAP	2.6
7-BAP	2.6

c. Precision

The precision of the method is reflected in the variance of quality control samples analyzed over time. The coefficients of variation (CV) of the method over analyses of six batches of QC samples (N=42) are listed in Table 10 below. These QC samples were prepared over 3 weeks by four different analysts using two automated liquid handlers and two GC/HRMS instruments.

Table 10. Mean Concentration and CV for 42 QC samples

Analyte	Mean (pptr)	CV (%)
1-NAP	1584	2.9
2-NAP	2912	3.3
9-FLUO	596	2.9
2-FLUO	559	3.0
3-FLUO	485	2.8
1-PHEN	391	4.4
2-PHEN	606	3.5
3-PHEN	533	3.2
4-PHEN	531	3.9
1-PYR	542	3.4
1-BCP	925	8.0
2-BCP	559	3.3
3-BCP	522	3.0

1-BAA	528	2.7
3_9-BAA	843	10
1-CHRY	496	11
2-CHRY	565	4.4
3-CHRY	510	3.3
4-CHRY	468	6.7
6-CHRY	509	4.5
3-BAP	686	6.6
7-BAP	772	8.5

d. Analytical specificity

The HRMS system provides excellent analytical specificity. The analyte peaks are located in well defined regions of the chromatogram with no visible interferences and low background. The observation of confirmation ions (M-15 ions) confirms the presence of analytes of interest. In addition, the retention time for the analytes relative to the isotope internal standards give additional confirmation of the presence of analytes in the sample.

e. Accuracy

Presently no established standard reference material (SRM) exists for OH-PAHs in human urine. Therefore, we cannot evaluate the accuracy of this method by analyzing an SRM and compare to its certified concentrations. Nonetheless, the usage of isotopically labeled internal standards can adjust for any deviates occurred on the OH-PAH analytes during sample preparation and instrumental analysis, and thus provide assurance on the accuracy of this method.

10. Quality Assessment and Proficiency Testing

a. Quality Assessment

Quality assessment procedures follow standard practices⁶. Daily experimental checks are made on the stability of the analytical system. Blanks and standards, as well as QC materials, are added to each day's run sequence. The blank and standard are analyzed at the beginning of each run to check the system for possible contamination or in the spiking solutions and/or reagents. Two QCs are prepared and analyzed with each run; their concentrations are compared with acceptance criteria to assure the proper operation of the analysis. Relative retention times are examined for the internal standard to ensure the choice of the correct chromatographic peak.

b. Quality Control Procedures

1) Establishing QC limits

Quality control limits are established by characterizing assay precision with repetitive analyses of the QC pool. Different variables are included in the analysis (e.g. different analysts and instruments) to capture realistic assay variation over time. The mean, standard deviation, coefficient of variation, and confidence limits are calculated from this QC characterization data set. Individual quality control charts for the characterization runs are created, examined, and quality control limits are used to verify assay precision and accuracy on a daily basis. Typical QC characterization statistics for OH-PAH analytes are listed in Table 11.

Table 11. QC Characterization Statistics

Analyte	Mean - 3 σ (pptr)	Mean - 2 σ (pptr)	Mean (pptr)	Mean + 2 σ (pptr)	Mean + 3 σ (pptr)
1-NAP	2950	3146	3536	3927	4123
2-NAP	2201	2359	2677	2995	3153
2-FLUOR	563	583	624	664	684
3-FLUOR	444	460	493	527	543
9-FLUOR	575	599	647	695	719
1-PHEN	336	357	398	439	459
2-PHEN	489	507	542	577	595
3-PHEN	492	515	560	606	628
4-PHEN	433	468	537	606	641
1-PYR	489	507	544	581	599
2-BCP	420	458	534	611	649
3-BCP	460	476	508	540	557
1-BAA	451	473	517	561	583
3-9-BAA	446	592	885	1178	1324
2-CHRY	359	399	479	559	599
3-CHRY	457	477	517	557	577
4-CHRY	399	435	506	577	613
6-CHRY	402	433	497	560	592
3-BAP	228	328	528	727	827
7-BAP	99	291	676	1060	1253

2) Quality Control evaluation

After the completion of a run, the quality control limits are consulted to determine if the run is “in control”. The quality control rules apply to the average of the beginning and ending analyses of each of the QC pools. The quality control results are evaluated according to Westgard rules (6):

- a) If both of the QCs are within the 2σ limits, then accept the run.
- b) If one of two QC results is outside the 2σ limits, then apply the rules below and reject the run if any condition is met.
 - i. Extreme outlier: the result is outside the characterization mean by more than 4σ limit.
 - ii. $1_{3\sigma}$ – Average of both QCs is outside of a 3σ limit.
 - iii. $2_{2\sigma}$ –QC results from two consecutive runs are outside of 2σ limit on the same side of the mean.
 - iv. **$R_{4\sigma}$ sequential** –QC results from two consecutive runs are outside of 2σ limit on opposite sides of the mean.
 - v. **10_x sequential** – QC results from ten consecutive runs are on the same side of the mean.

If the QC result for an analyte is declared “out of control”, the results of that analyte for all patient samples analyzed during that run are invalid for reporting.

c. Proficiency Testing (PT)

1) Scope of PT

There are no established PT materials; currently we are the only laboratory running this essay. Therefore, the proficiency testing (PT) scheme for this method is administered by an in-house PT coordinator. Because no standard reference materials exist for urinary analysis of hydroxy-PAH levels, PT samples are prepared in-house by spiking a known amount of standard into a well characterized urine pool, and blink-coded by in-house PT coordinator.

2) Frequency of PT

Four samples of unknown PT concentrations are analyzed twice a year using the same method described for unknown samples. Proficiency testing must be performed a minimum of once every 6 months. The PT administrator will randomly select five of the PT materials for analysis. A passing score is obtained if at least four of the five samples fall within the prescribed limits established beforehand.

3) Documentation of PT

Analytical PT results are reviewed by the analyst and laboratory supervisor, and then submitted to the in-house PT Coordinator. The PT results are evaluated by the PT Coordinator; the analysis passes proficiency testing if at least four of the five sample results deviate $\leq 20\%$ from the known value. All proficiency results shall be appropriately documented. If the assay fails proficiency testing then the sample preparation and instrumentation are thoroughly examined to identify and correct the source of assay error.

11. Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria

If the calibration or QC systems fail to meet acceptable criteria, suspend all operations until the source or cause of failure is identified and corrected. If the source of failure is easily identifiable, for instance, failure of the mass spectrometer or a pipeting error, the problem is immediately corrected. Otherwise, prepare fresh reagents and clean the mass spectrometer system. Before beginning another analytical run, re-analyze several QC materials (in the case of QC failure) or calibration standards (in the case of calibration failure). After re-establishing calibration or quality control, resume analytical runs. Document the QC failures, review the cases with supervisor to determine source(s) of problem, and take measures to prevent re-occurrence of the same problem.

12. Limitations of Method, Interfering Substances and Conditions

This method is an isotope dilution mass spectrometry method, widely regarded as the definitive method for the measurement of organic toxicants in human body fluids. By using high resolution mass spectrometry, most interference is eliminated. Due to the matrix used in this procedure, occasional unknown interfering substances have been encountered. If chromatographic interference with the internal standards occurs, reject that analysis. If repeat analysis still results in an interference with the internal standard, the results for that analyte are not reportable.

13. Reference Ranges (Normal Values)

Population-based reference ranges for 23 OH-PAH analytes were determined from a subset of National Health and Nutritional Survey (NHANES) 2001-2002 urine samples. The reference values are presented in Table 12.

Table 12. Reference values from NHANES 2001-2002 (95% confidence interval in parentheses)

Analyte	N	Urine Concentration (ng/L)		Creatinine Adjusted Concentration (ng/g crea.)	
		Median	95 th	Median	95 th

**OH-PAH in Urine
NHANES 2003-2004**

1-NAP	2748	2050 (1790-2340)	22300 (18000-26600)	1910 (1700-2160)	17800 (14500-20800)
2-NAP	2748	2470 (2110-2890)	26000 (22500-29700)	2310 (1980-2300)	16700 (14100-19200)
2-FLUO	2745	318 (277-366)	2820 (2340-3450)	298 (361-340)	1890 (1590-2290)
3-FLUO	2745	134 (115-155)	1620 (1390-1900)	125 (108-144)	1060 (909-1290)
9-FLUO	2745	219 (191-251)	1090 (950-1300)	205 (183-229)	852 (736-1120)
Analyte	N	Urine Concentration (ng/L)		Creatinine Adjusted Concentration (ng/g crea.)	
		Median	95 th	Median	95 th
1-PHEN	2741	140 (125-158)	684 (581-763)	132 (118-147)	464 (404-539)
2-PHEN	2742	54.0 (46.0-63.5)	332 (299-377)	50.6 (43.3-59.2)	231 (206-275)
3-PHEN	2741	105 (92.5-118)	649 (542-747)	98.0 (87.4-110)	428 (360-621)
4-PHEN	2741	41.9 (31.3-56.0)	280 (250-339)	39.2 (29.6-52.0)	347 (293-411)
1-PYR	2747	49.6 (43.4-56.7)	349 (290-400)	46.4 (40.9-52.8)	243 (206-269)
1-BCP	2732	<LOD	34.0 (16.0-54.0)	<LOD	41.3 (21.0-70.8)
2-BCP	2748	<LOD	13.0 (6.00-20.0)	<LOD	20.3 (15.9-23.3)
3-BCP	2748	<LOD	11.0 (8.00-16.0)	<LOD	15.9 (14.9-16.7)
1-BAA	2748	<LOD	30.0 (22.0-36.0)	<LOD	27.0 (19.6-32.4)
3_9-BAA	2748	<LOD	24.0 (19.0-31.0)	<LOD	31.8 (28.4-35.5)
1-CHRY	2748	<LOD	105 (85.0-138)	<LOD	89.2 (71.4-104)
2-CHRY	2748	<LOD	31.0 (14.0-45.0)	<LOD	27.8 (20.9-35.0)
3-CHRY	2748	<LOD	42.0 (36.0-46.0)	<LOD	56.8 (47.4-62.5)
4-CHRY	2748	<LOD	<LOD	<LOD	<LOD
6-CHRY	2736	<LOD	77.0 (57.0-101)	<LOD	61.5 (47.7-76.4)
3-BAP	2748	<LOD	179 (139-251)	<LOD	184 (147-248)

14. Critical Call Results (“Panic Values”)

It is unlikely that any result would be a "critical call", which would only be observed in acute poisonings. There are no established “critical call” values. Application of this method to NHANES studies will assist in determining levels of OH-PAH normally found in healthy US populations. Test results in this laboratory are reported in support of epidemiological studies, not clinical assessments. Data will help determine critical exposures.

15. Specimen Storage and Handling During Testing

Urine specimens may reach and maintain ambient temperature during analysis. The urine extracts are stored in GC vials in a -70 °C freezer after analysis. Current studies indicate (CDC data) that the extracts are stable for three weeks.

16. Alternate Methods for Performing Test or Storing Specimens if Test System Fails

Alternate validated methods have not been evaluated for measuring OH-PAH in urine. If the analytical system fails, then samples must be refrigerated (at 4 ± 3 °C) until the analytical system is restored to functionality. If long-term interruption (greater than 4 weeks) is anticipated, then store urine specimens at -70 ± 10 °C.

The method is designed to run on a GC/HRMS instrument, and is not generally transferable to other instrumentation. If the system fails, then samples must be refrigerated (at 4 ± 3 °C) until the analytical system is restored to functionality. If long-term interruption (greater than 4 weeks) is anticipated, then store urine specimens at -70 ± 10 °C. Sample extracts in GC vials may be refrigerated for as long as three weeks. If long-term interruption is anticipated, store sample extracts at -70 ± 10 °C.

17. Test Result Reporting System; Protocol for Reporting Critical Calls (if Applicable)

Study subject data is reported in both concentration units (ng/L) and adjusted based on creatinine excretion ($\mu\text{g/g}$ creatinine).

Once the validity of the data is established by the QC/QA system outlined above, these results are verified by a DLS statistician, and the data reported in both hard copy and electronic copy. This data, a cover letter, and a table of method specifications and reference range values will be routed through the appropriate channels for approval (i.e. supervisor, branch chief, division director) as outlined in the DLS policy and procedure manual. After approval at the division level, the report will be sent to the contact person who requested the analyses.

18. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

If greater than 1 mL of sample remains following successful completion of analysis, this material must be returned to storage at -70 ± 10 °C in case reanalysis is required. These samples shall be retained until valid results have been obtained and reported and sufficient time has passed for review of the results.

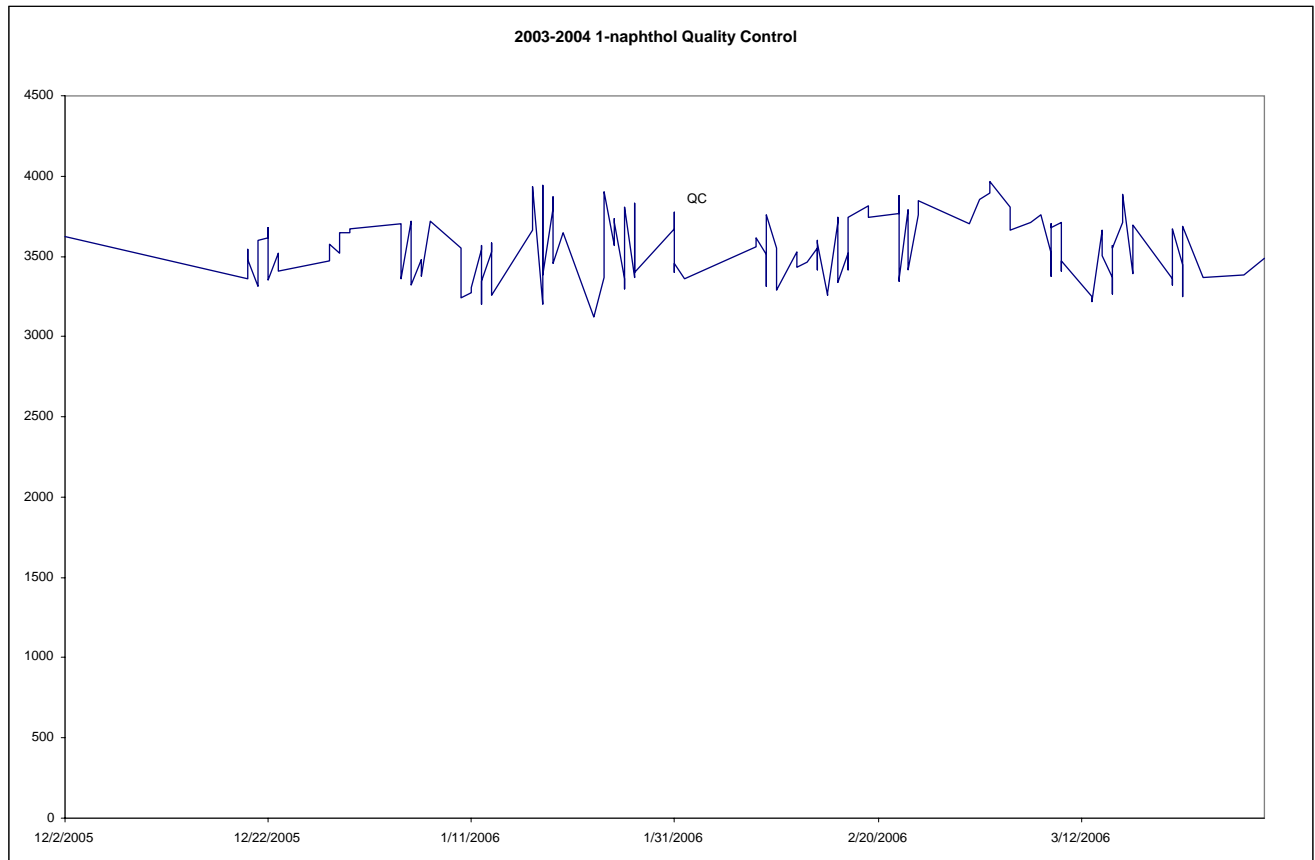
Standard record keeping (e.g., database, notebooks, and data files) is used to track specimens. Specimens will only be transferred or referred to other DLS Branch laboratories or, if required, to CLIA certified laboratories. Transfer is carried out through the DLS Samples Logistic Group. Specimens may be stored at CDC specimen handling and storage facility (CASPIR).

19. Summary Statistics and QC Graphs

A. 1-naphthol

Summary Statistics for 1-naphthol by Lot

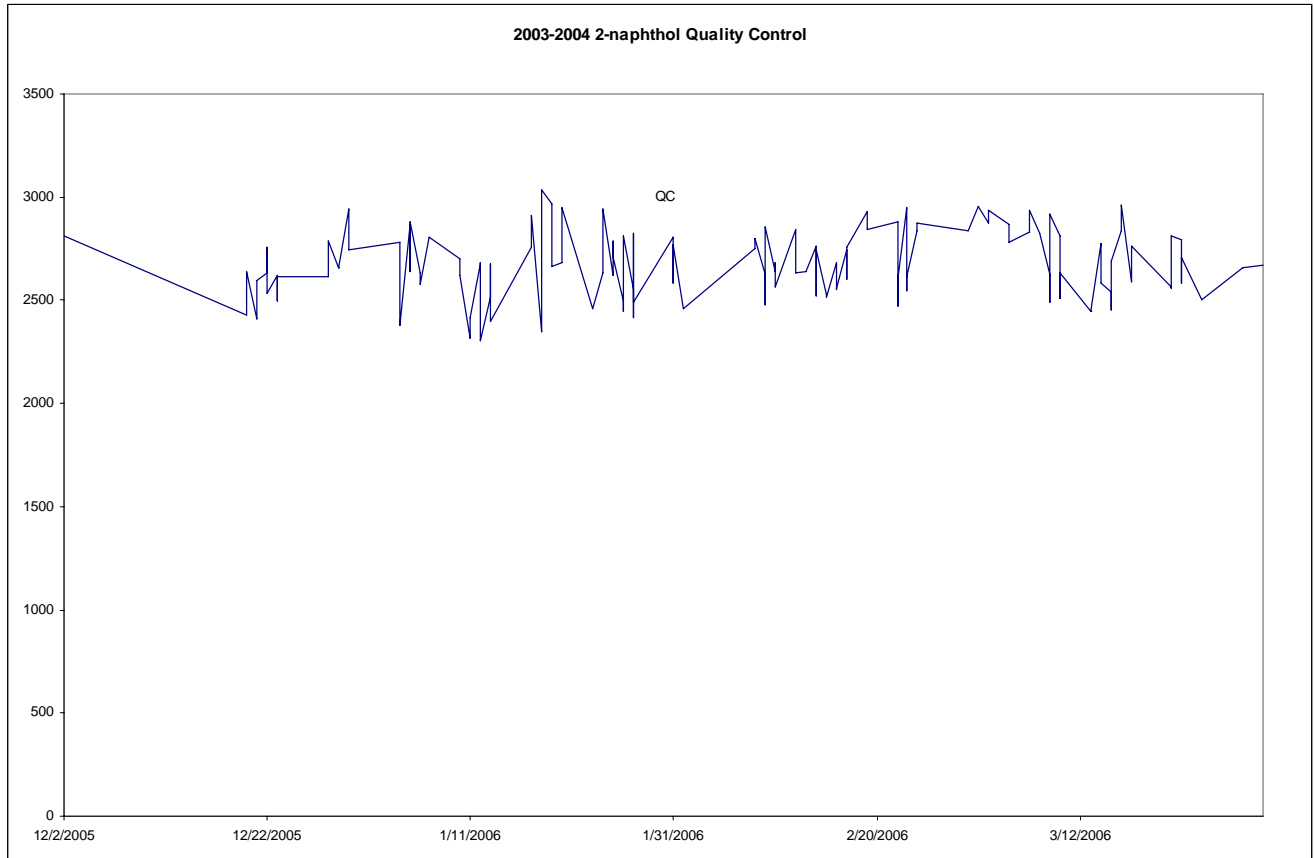
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	157	12/2/2005	3/30/2006	3540.24	188.83	5.3



B. 2-naphthol

Summary Statistics for 2-naphthol by Lot

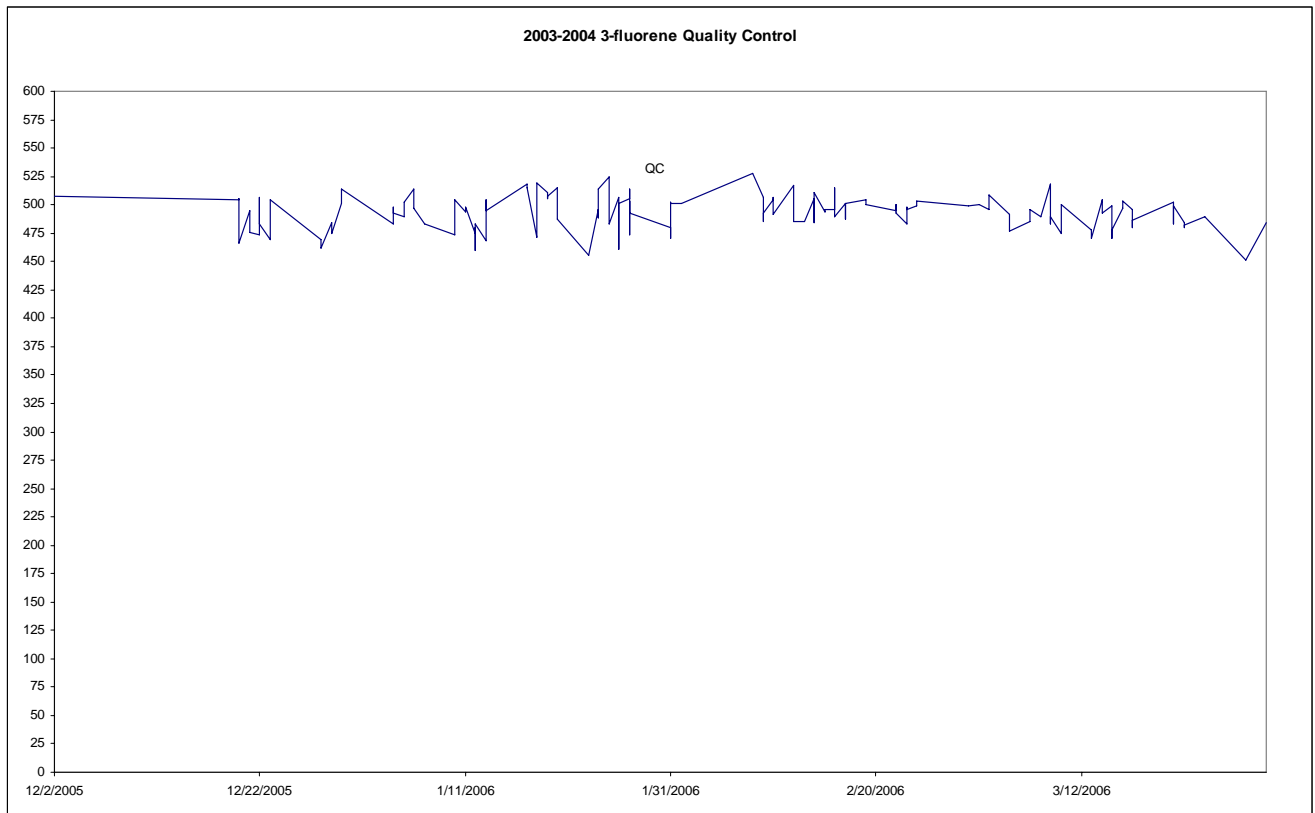
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	156	12/2/2005	3/30/2006	2676.16	157.34	5.9



C. 3-fluorene

Summary Statistics for 3-fluorene by Lot

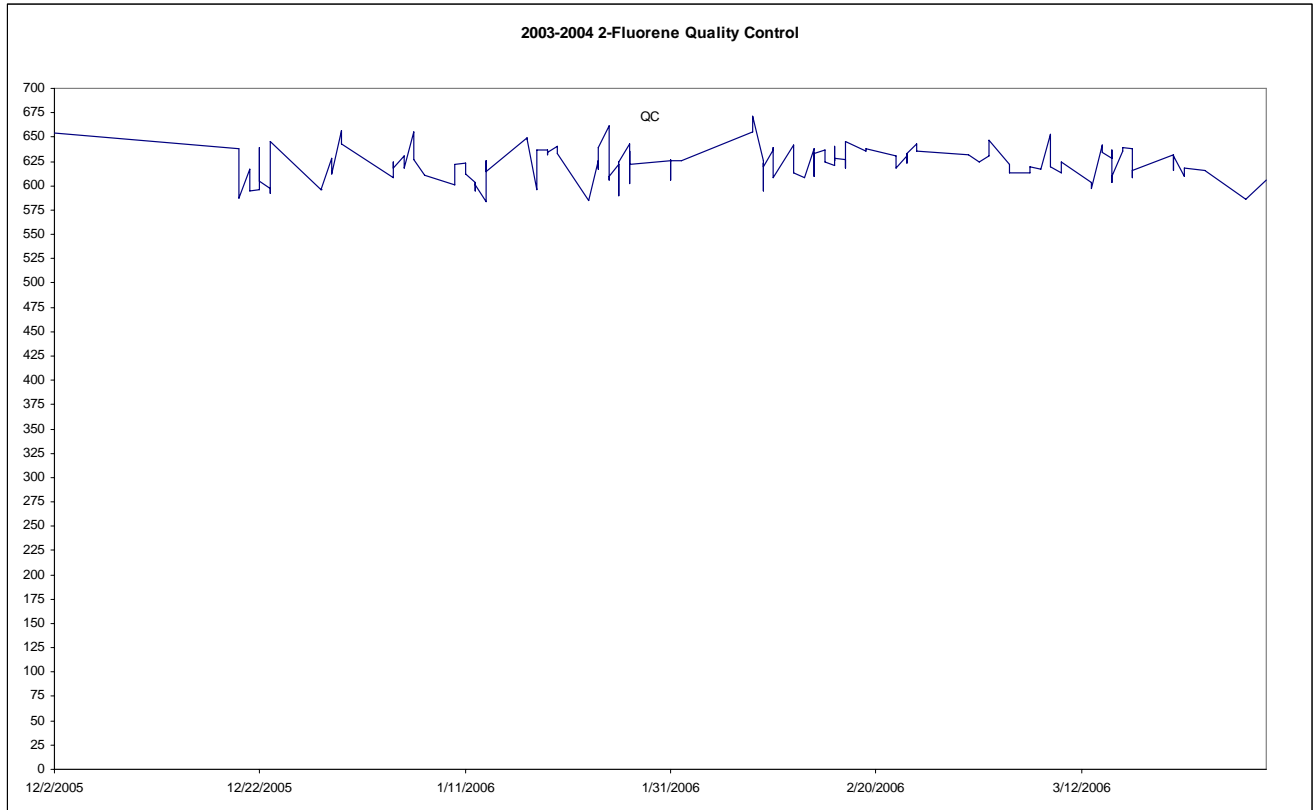
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	153	12/2/2005	3/30/2006	492.08	14.53	3.0



D. 2-fluorene

Summary Statistics for 2-fluorene by Lot

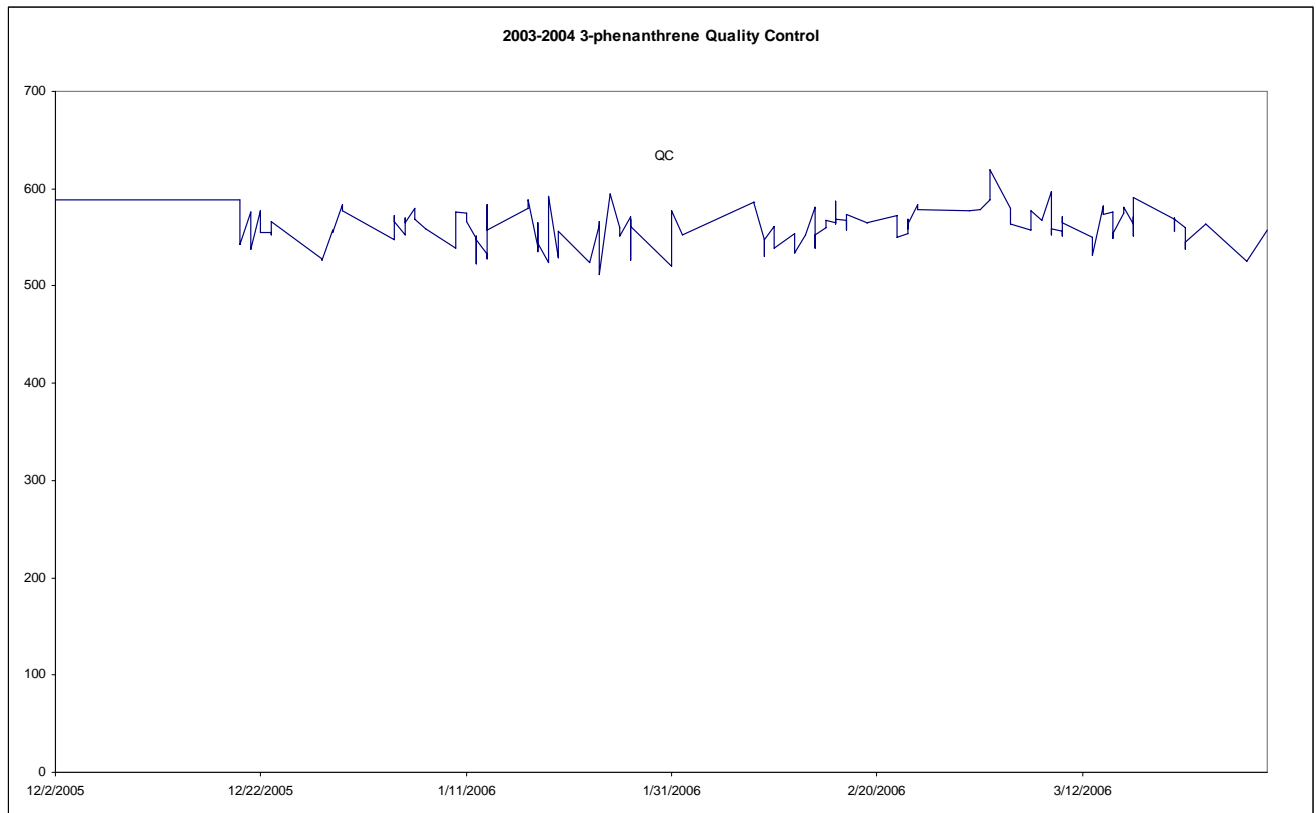
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	153	12/2/2005	3/30/2006	621.75	17.1	2.7



E. 3-phenanthrene

Summary Statistics for 3-phenanthrene by Lot

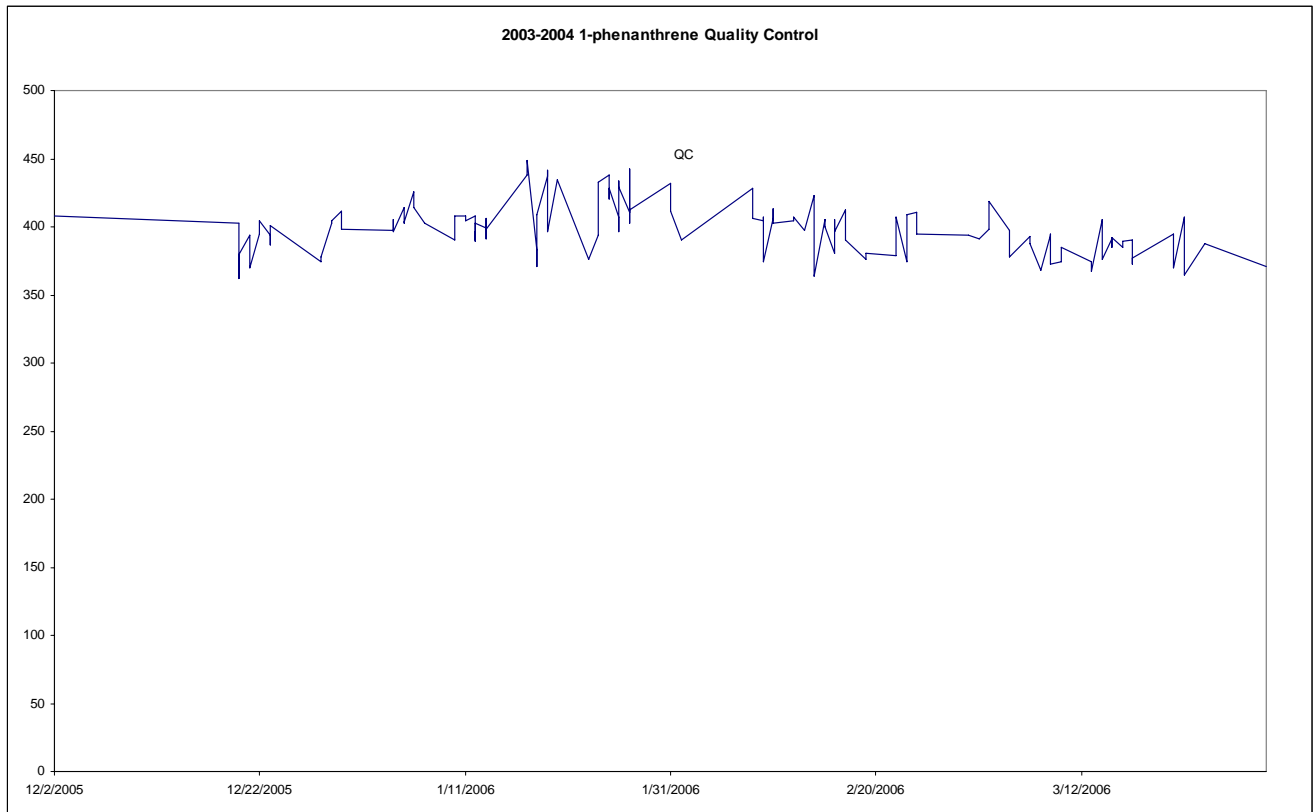
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	148	12/2/2005	3/30/2006	560.38	18.91	3.4



F. 1-phenanthrene

Summary Statistics for 1-phenanthrene by Lot

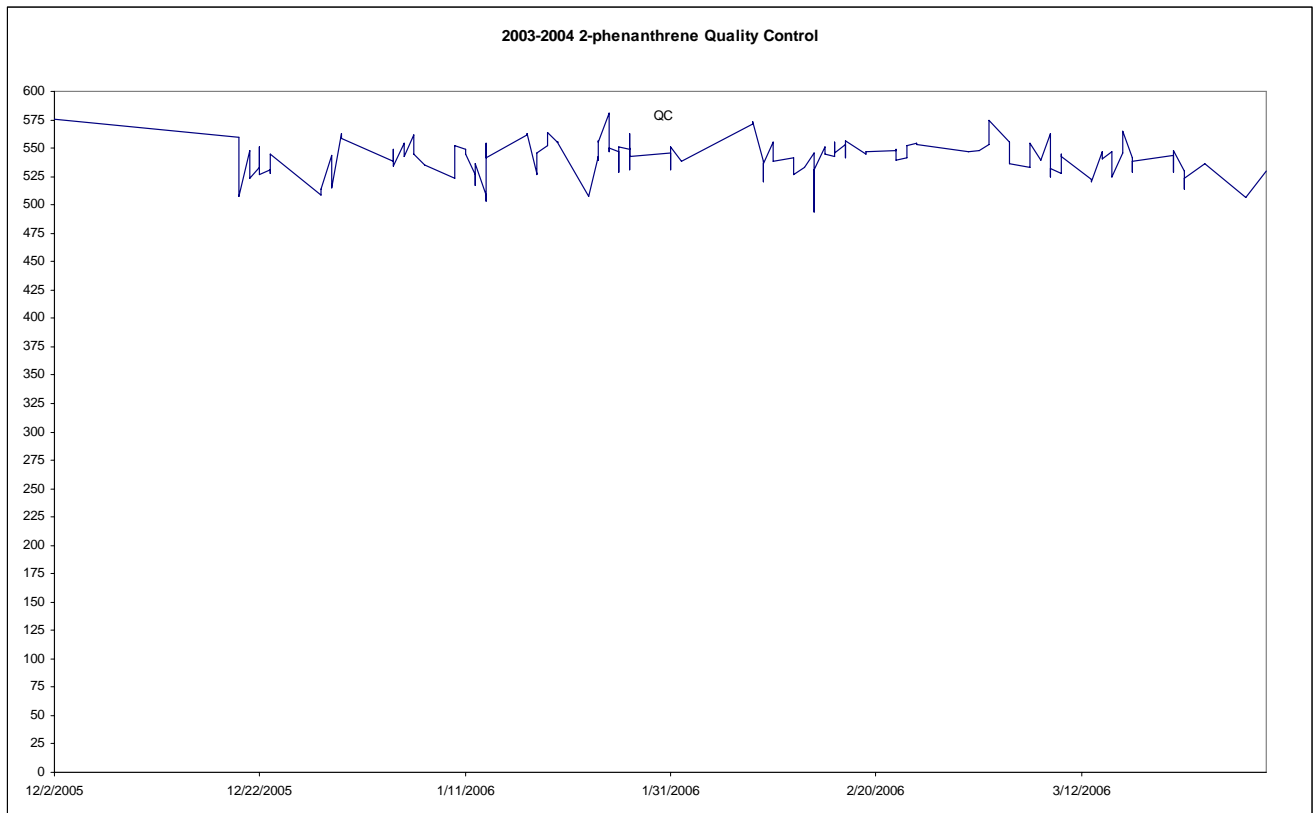
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	150	12/2/2005	3/30/2006	397.58	18.16	4.6



G. 2-phenanthrene

Summary Statistics for 2-phenanthrene by Lot

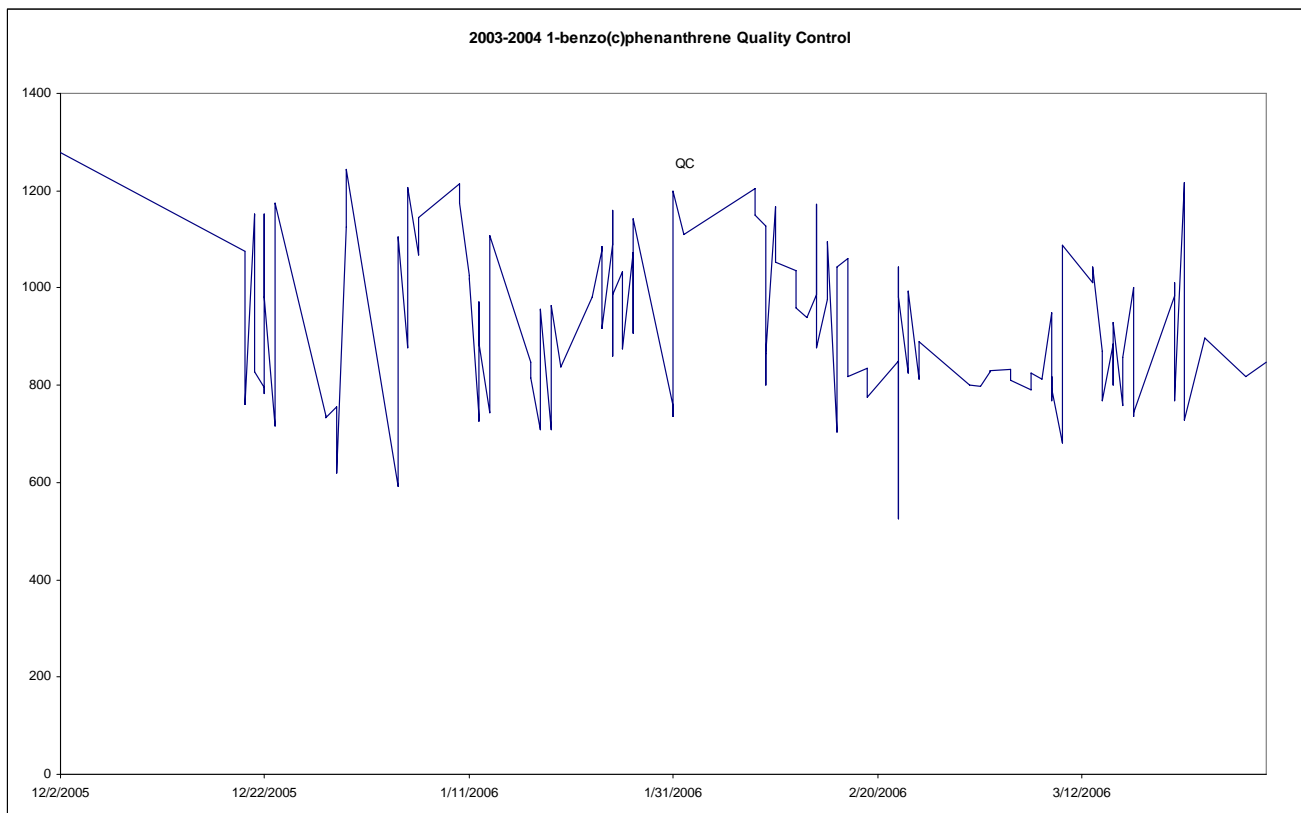
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	152	12/2/2005	3/30/2006	541.17	15.22	2.8



H. 1-benzo(c)phenanthrene

Summary Statistics for 1-benzo(c)phenanthrene by Lot

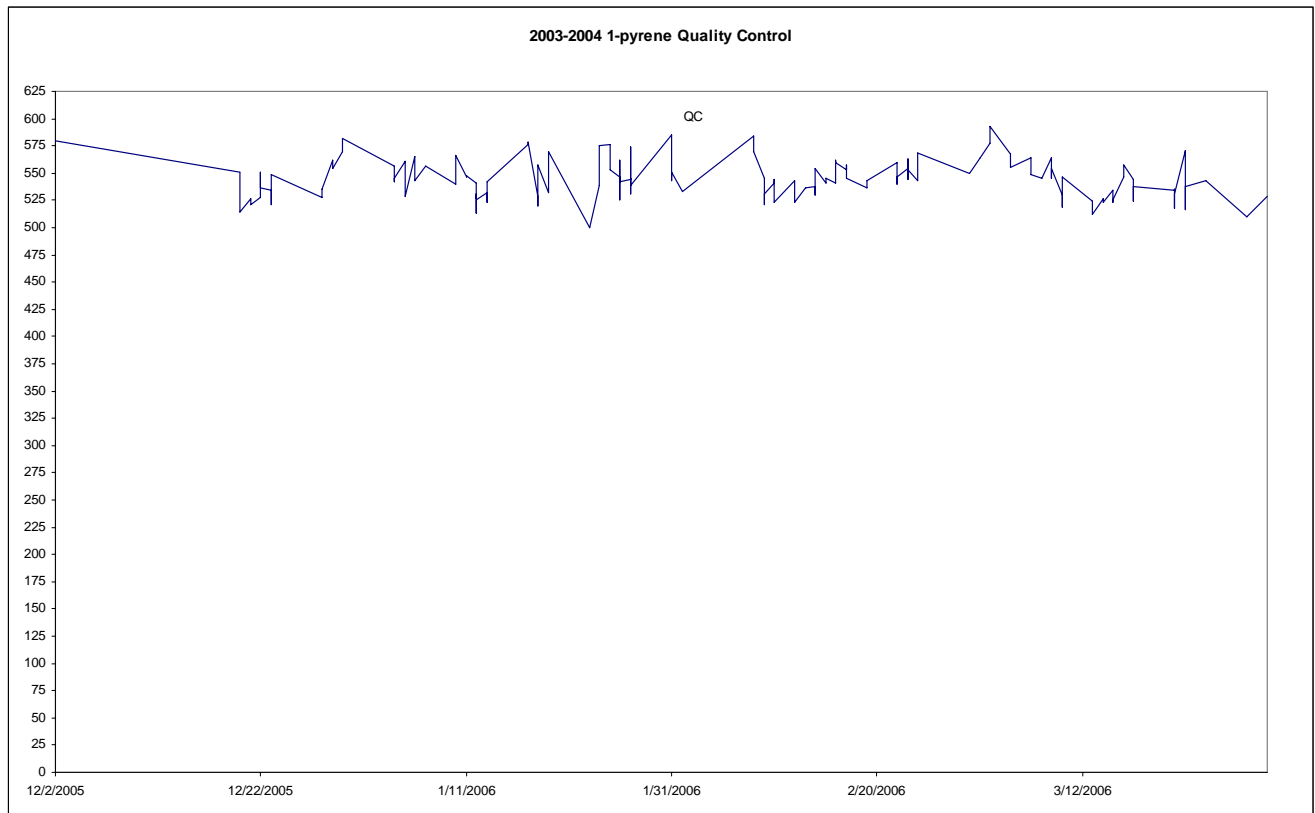
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	151	12/2/2005	3/30/2006	929.08	154.06	16.6



I. 1-pyrene

Summary Statistics for 1-pyrene by Lot

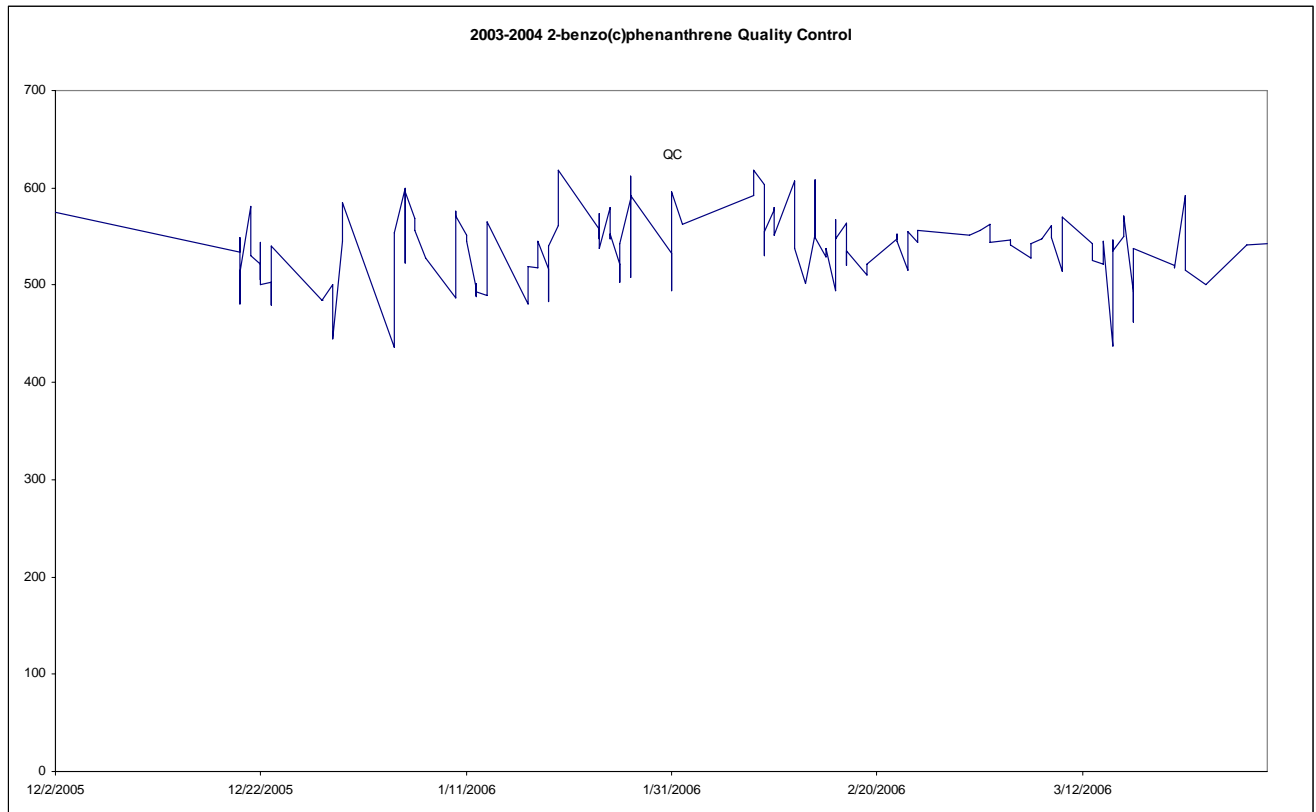
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	152	12/2/2005	3/30/2006	544.21	17.79	3.3



J. 2-benzo(c)phenanthrene

Summary Statistics for 2-benzo(c)phenanthrene by Lot

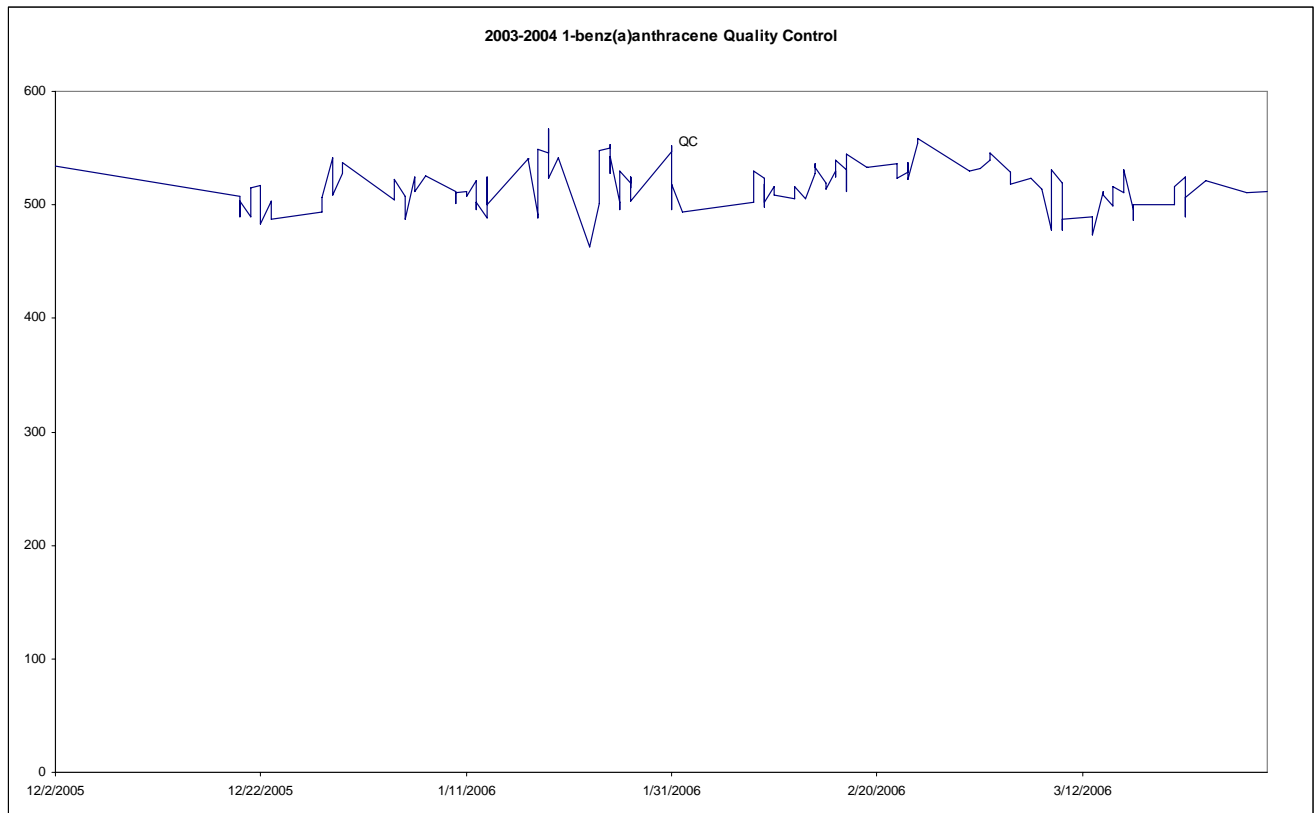
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	151	12/2/2005	3/30/2006	538.02	35.33	6.6



K. 1-benz(a)anthracene

Summary Statistics for 1-benz(a)anthracene by Lot

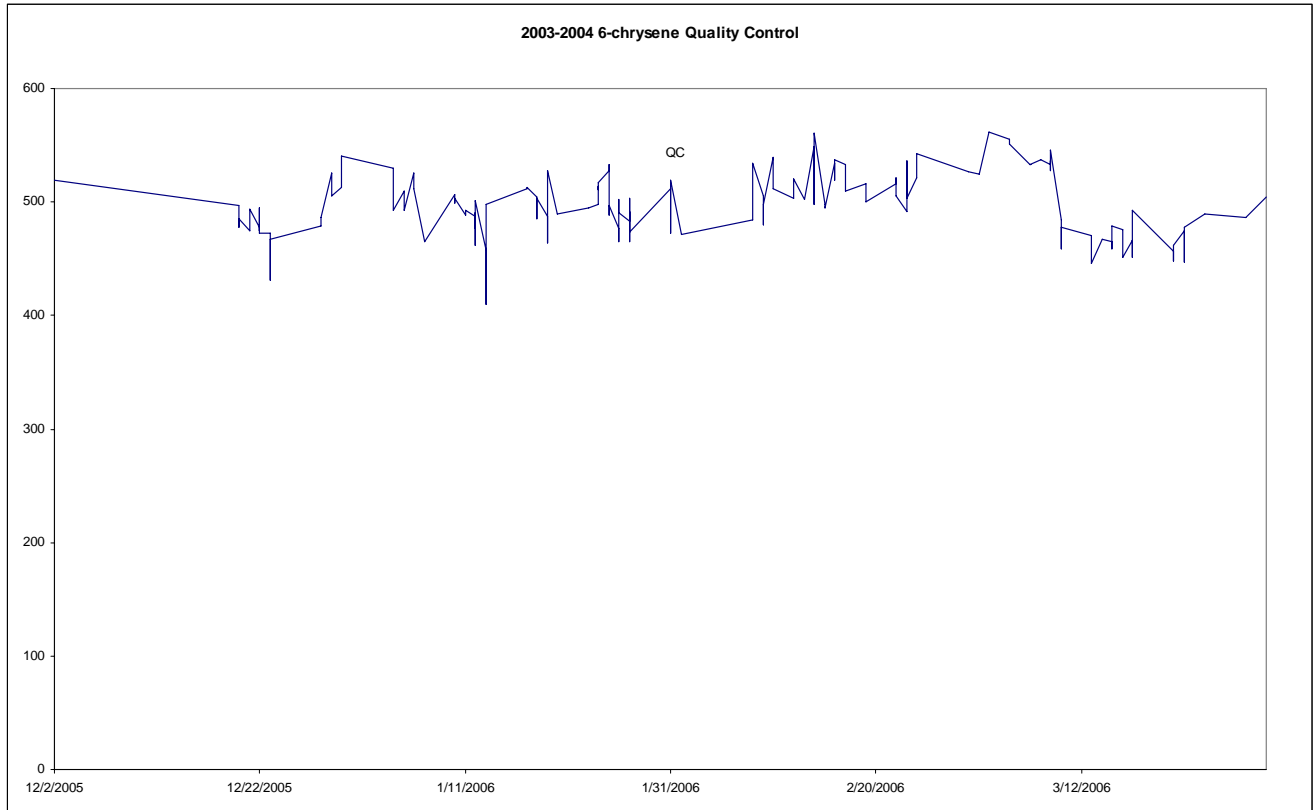
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	152	12/2/2005	3/30/2006	515.77	19.05	3.7



L. 6-chrysene

Summary Statistics for 6-chrysene by Lot

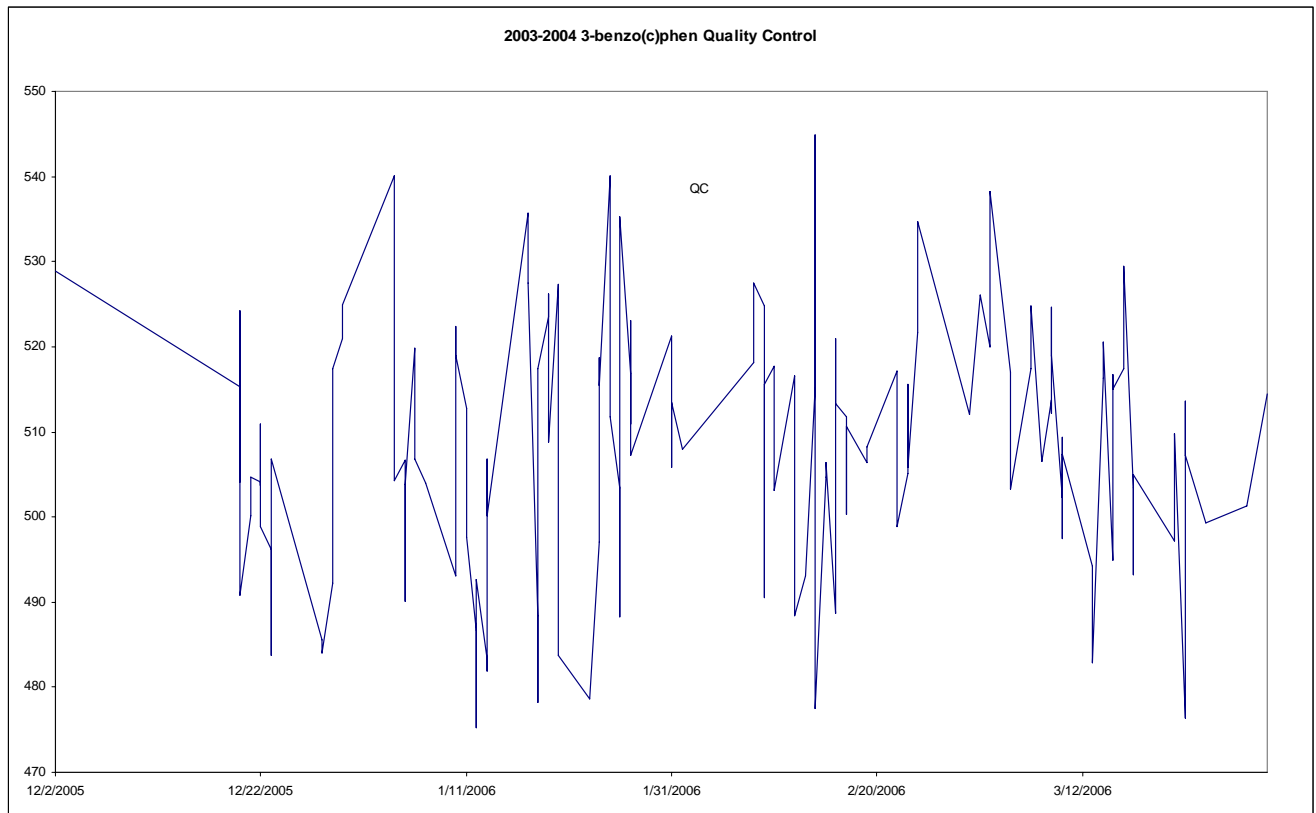
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	153	12/2/2005	3/30/2006	497.4	27.75	5.6



M. 3-benzo(c)phen

Summary Statistics for 3-benzo(c)phen by Lot

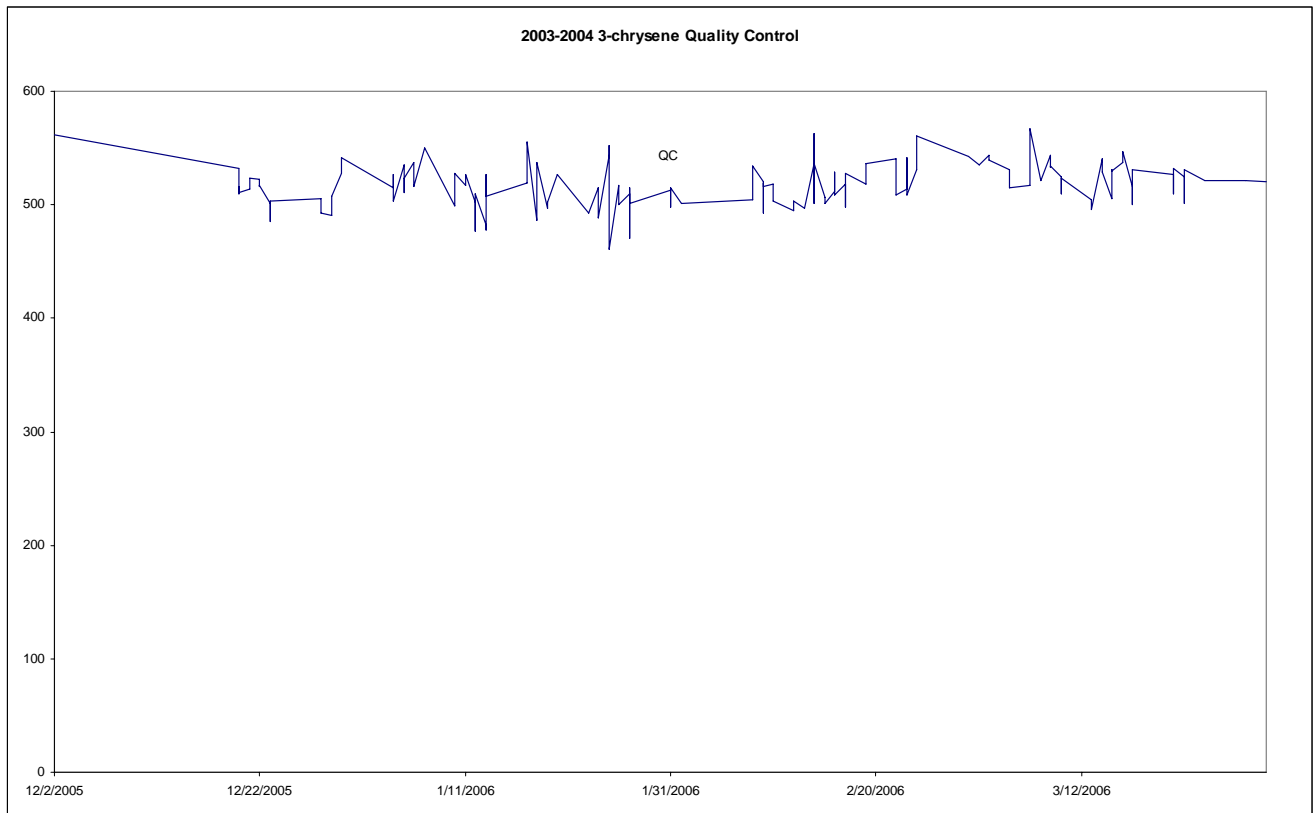
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	152	12/2/2005	3/30/2006	508.02	14.3	2.8



N. 3-chrysene

Summary Statistics for 3-chrysene by Lot

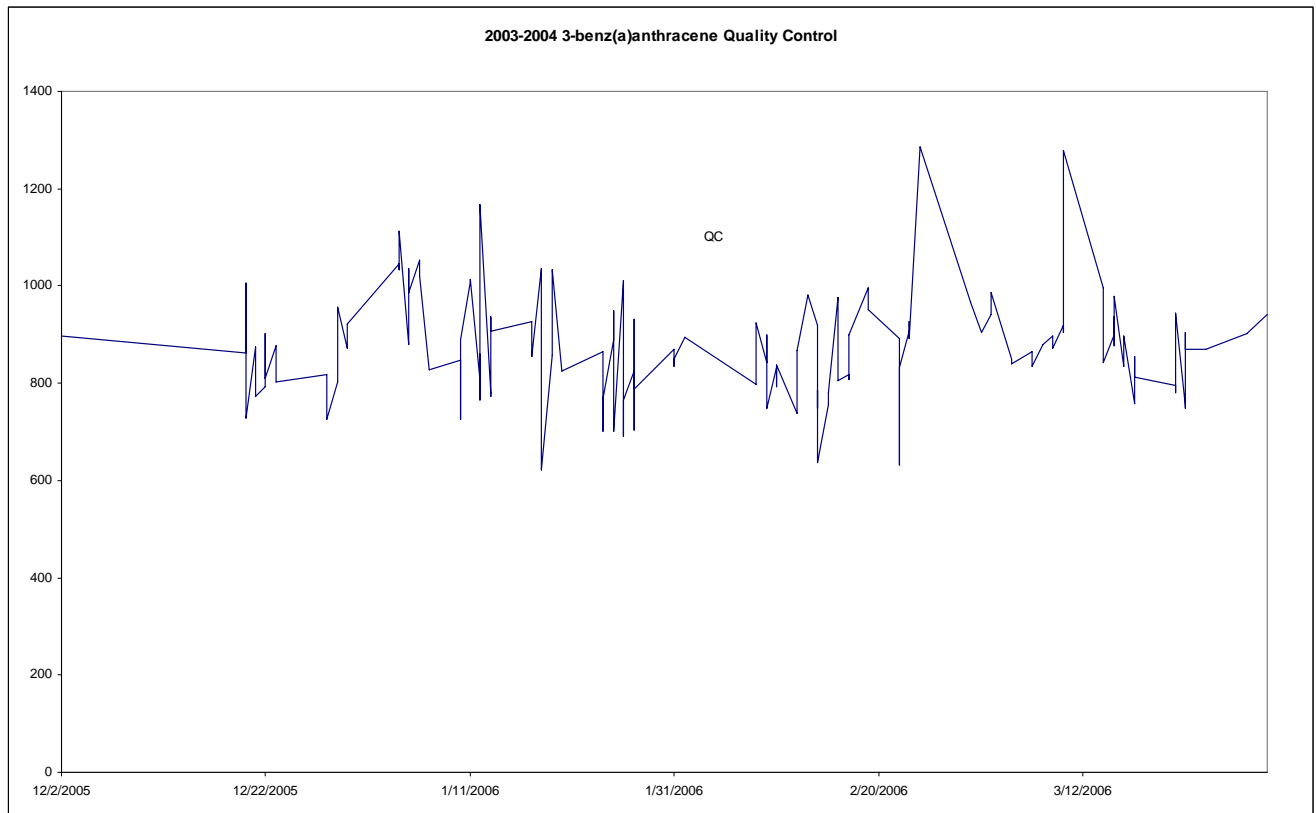
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	150	12/2/2005	3/30/2006	516.51	18.67	3.6



O. 3-benz(a)anthracene

Summary Statistics for 3-benz(a)anthracene by Lot

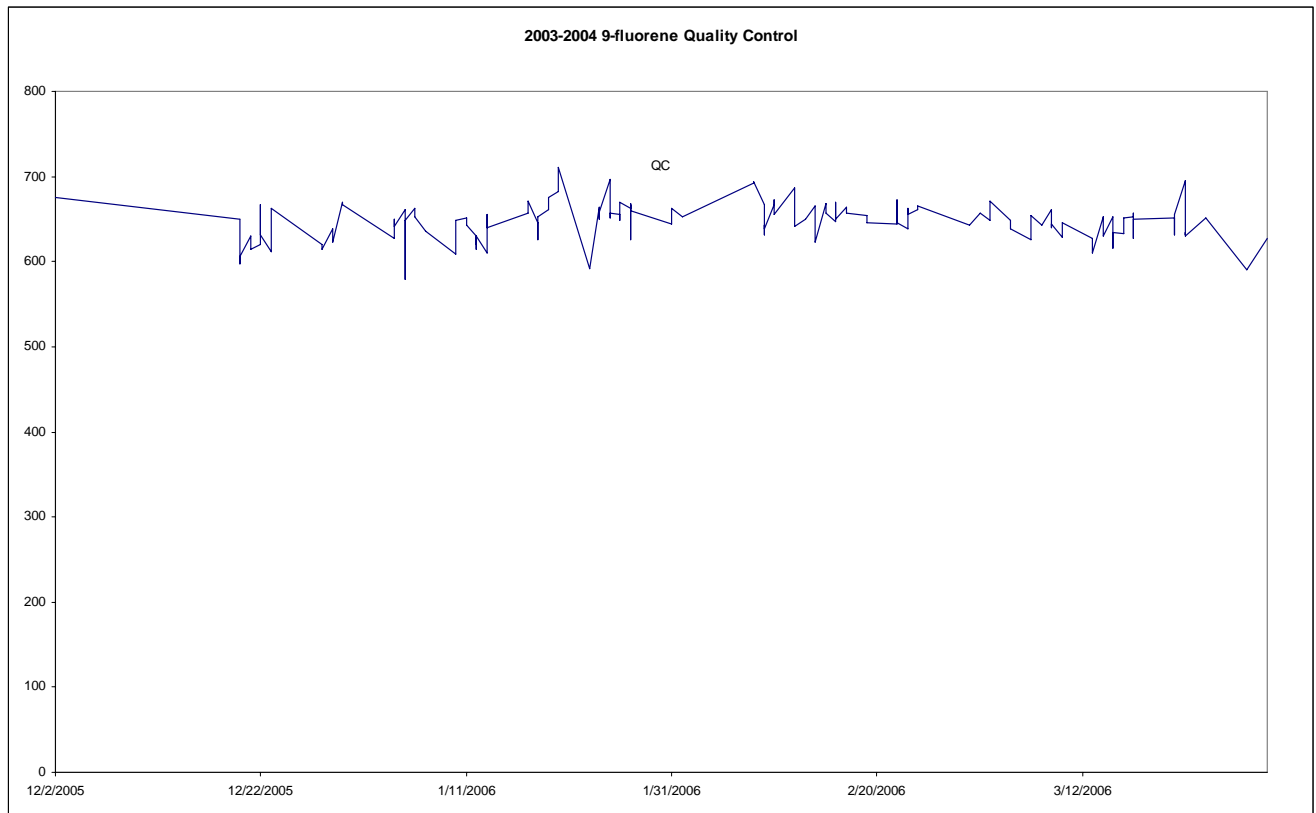
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	150	12/2/2005	3/30/2006	871.37	107.97	12.4



P. 9-fluorene

Summary Statistics for 9-fluorene by Lot

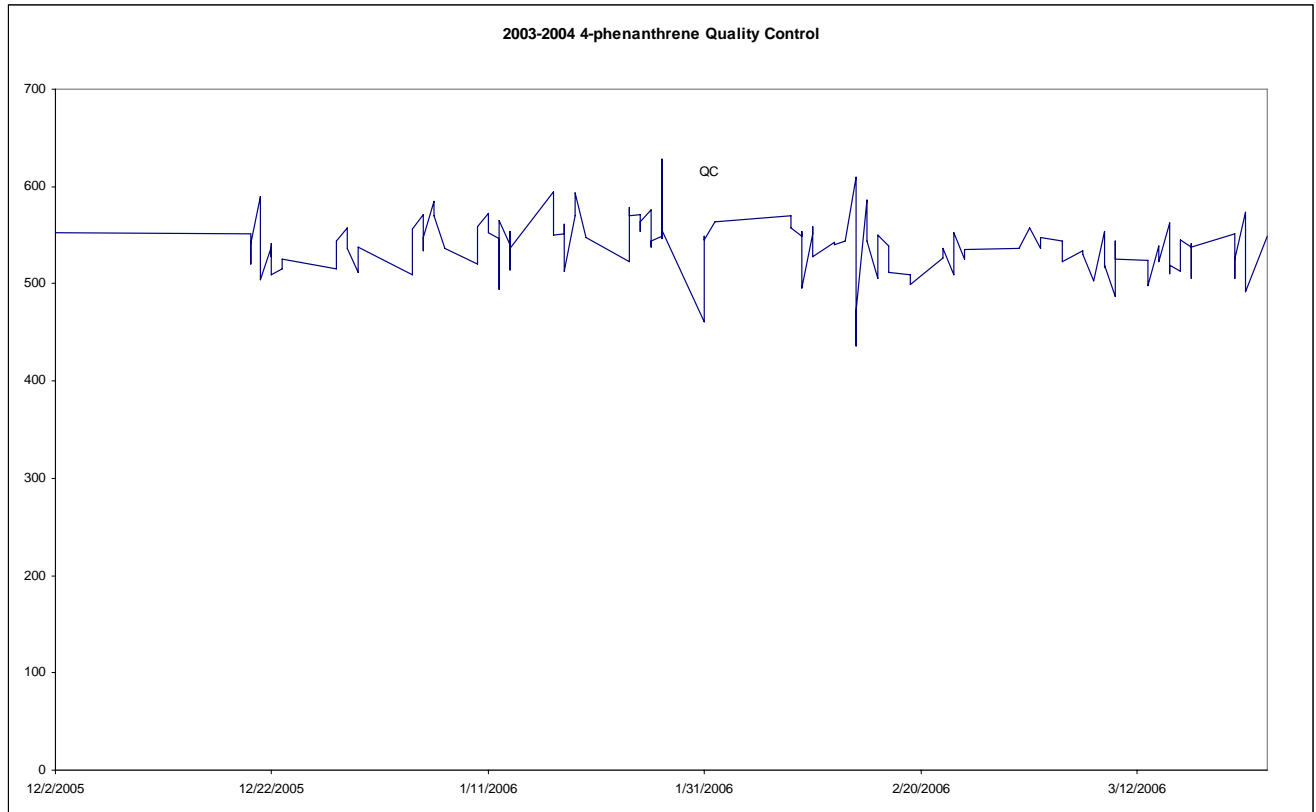
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	152	12/2/2005	3/30/2006	646.52	21.14	3.3



R. 4-phenanthrene

Summary Statistics for 4-phenanthrene by Lot

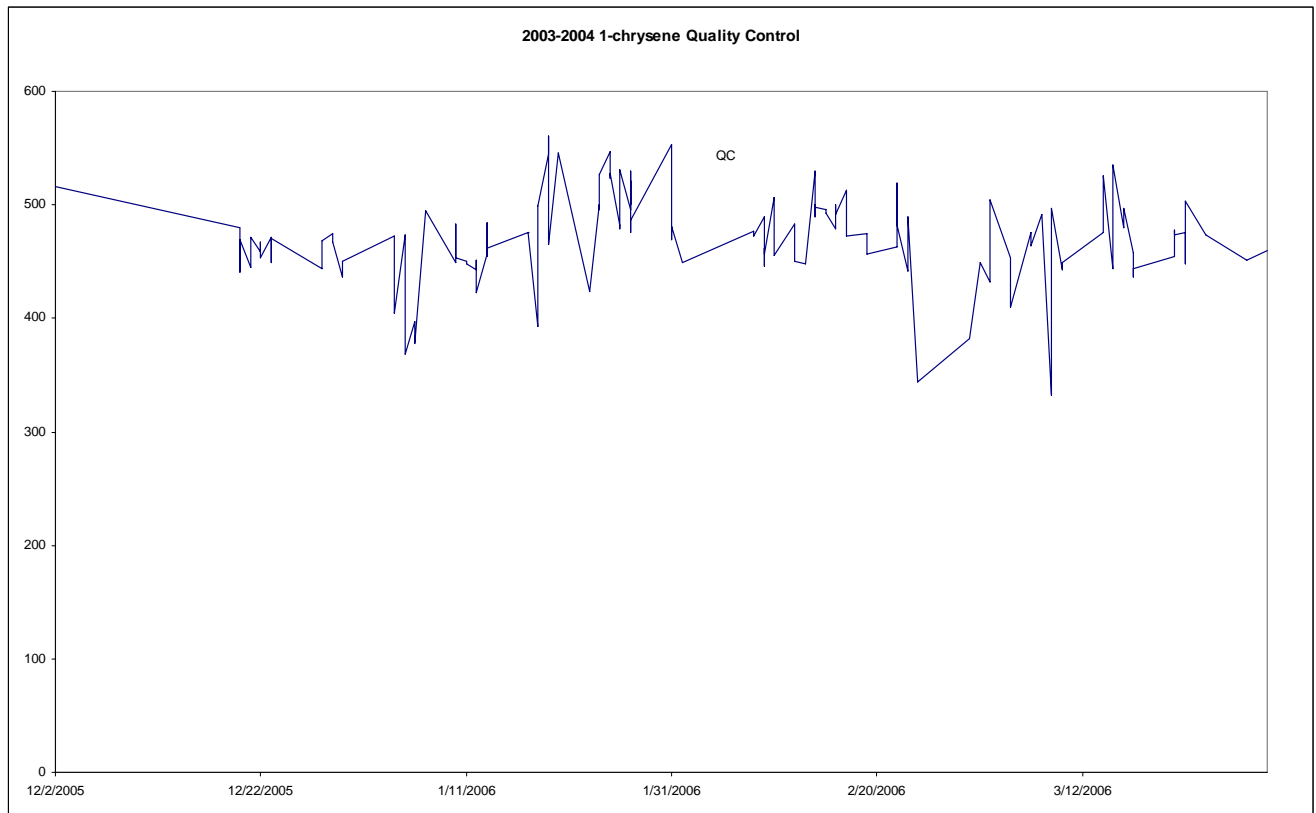
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	149	12/2/2005	3/24/2006	538.52	27.18	5.0



S. 1-chrysene

Summary Statistics for 1-chrysene by Lot

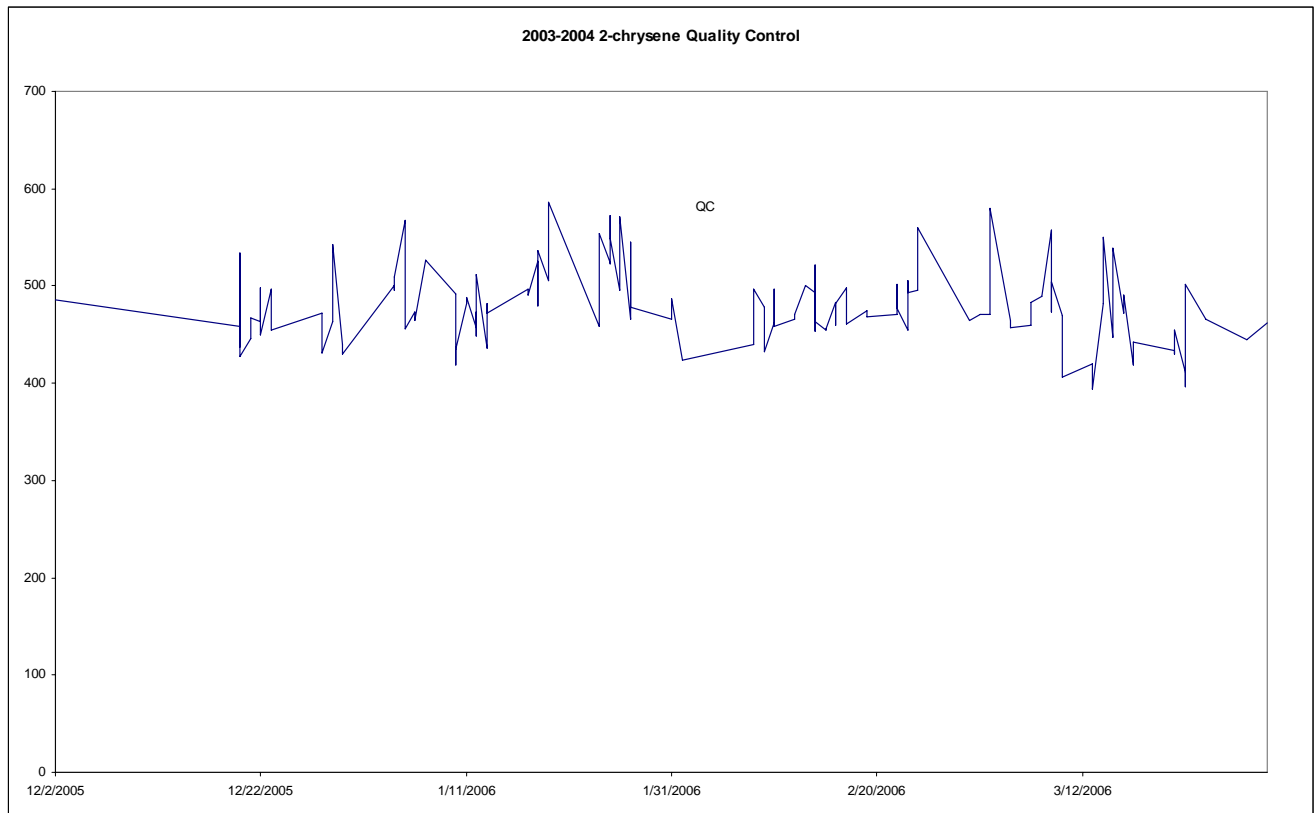
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	148	12/2/2005	3/30/2006	469.75	38.82	8.3



T. 2-chrysene

Summary Statistics for 2-chrysene by Lot

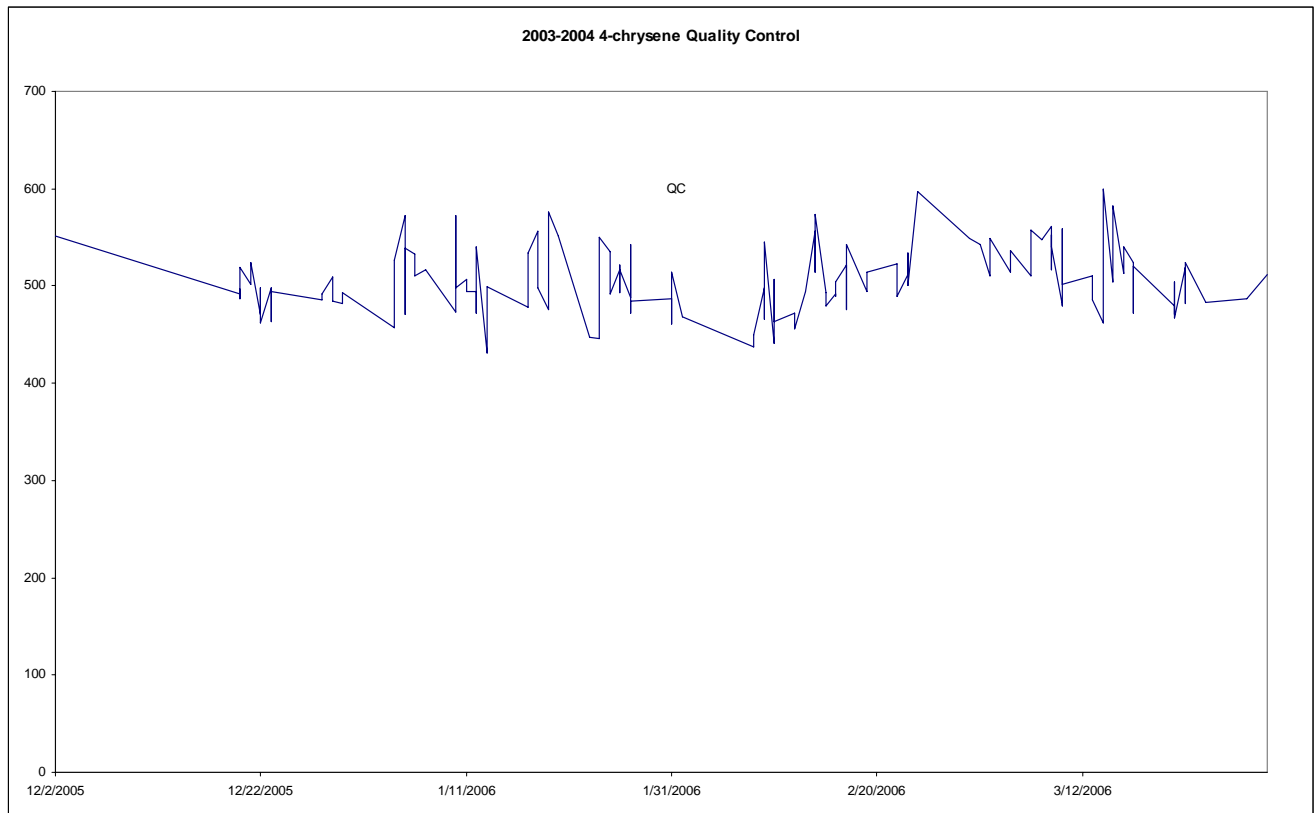
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	149	12/2/2005	3/30/2006	478.72	37.87	7.9



U. 4-chrysene

Summary Statistics for 4-chrysene by Lot

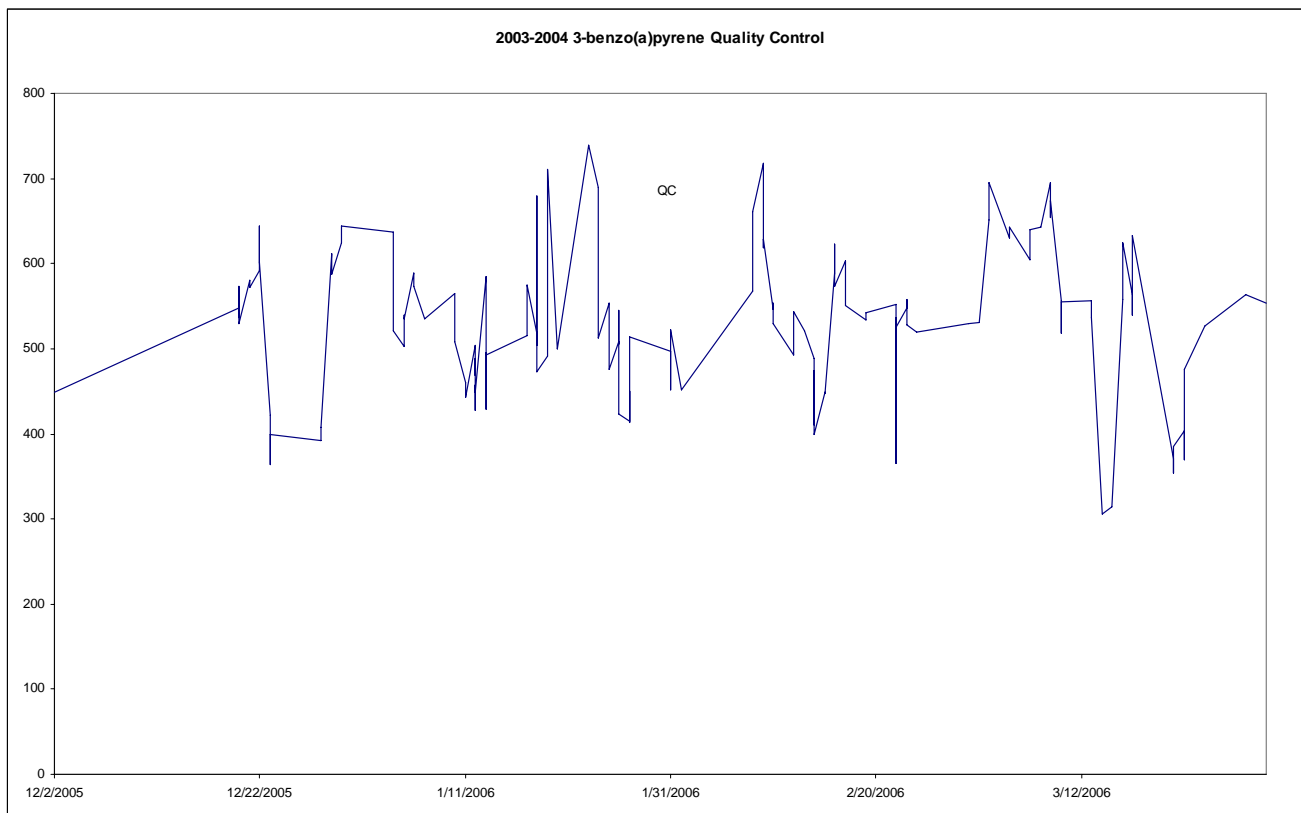
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	154	12/2/2005	3/30/2006	505.36	32.7	6.5



V. 3-benzo(a)pyrene

Summary Statistics for 3-benzo(a)pyrene by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QC	151	12/2/2005	3/30/2006	530.64	86.16	16.2



20. References

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