Laboratory Procedure Manual

Analyte: **PCBs and Persistent Pesticides**

Matrix: **Serum**

Method: **HRGC/ID-HRMS**

Method No.: **DLS 6502.02 – NHANES LAB 28**

Revised: ***August 10, 2012 [NHANES 2005-2006]***

as performed by: ***Organic Analytical Toxicology Branch***
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Important Information for Users

CDC periodically revises 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.

Public Release Data Set Information

This document details the Lab Protocol for NHANES 2001-2001 data.
A tabular list of the released analytes for LAB 28 follows:

IUPAC Number	CAS Number	SAS Label	Chlorine Substitution
PCB 28	7012-37-5	LBX028	2,4,4'
PCB 44	41464-39-5	LBX044	2,2',3,5'
PCB 49	41464-40-8	LBX049	2,2',4,5'
PCB 52	35693-99-3	LBX052	2,2',5,5'
PCB 66	32698-10-0	LBX066	2,3',4,4'
PCB 74	32690-93-0	LBX074	2,4,4',5
PCB 87	38380-02-8	LBX087	2,2',4,4',5
PCB 99	38380-01-7	LBX099	2,2',4,4',5
PCB 101	37680-73-2	LBX101	2,2',4,5,5'
PCB 105	32598-14-4	LBX105	2,3,3',4,4'
PCB 110	38380-03-9	LBX110	2,3,3',4',6
PCB 118	31508-00-6	LBX118	2,3',4,4',5
PCB 128	38380-07-3	LBX128	2,2',3,3',4,4'
PCB 138/158	35065-28-2 / 74472-42-7	LBX138	2,2',3,4,4',5'/ 2,3,3',4,4',6
PCB 146	51908-16-8	LBX146	2,2',3,4',5,5'
PCB 149	38380-04-0	LBX149	2,2',3,4',5',6
PCB 151	52663-63-5	LBX151	2,2',3,5,5',6
PCB 153	35065-27-1	LBX153	2,2',4,4',5,5'
PCB 156	38380-08-4	LBX156	2,3,3',4,4',5
PCB 157	69782-90-7	LBX157	2,3,3',4,4',5'
PCB 167	52663-72-6	LBX167	2,3',4,4',5,5'
PCB 170	35065-30-6	LBX170	2,2',3,3',4,4',5
PCB 172	52663-74-8	LBX172	2,2',3,3',4,5,5'
PCB 177	52663-70-4	LBX177	2,2',3,3',4,5',6'
PCB 178	52663-67-9	LBX178	2,2',3,3',5,5',6
PCB 180	35065-29-3	LBX180	2,2',3,4,4',5,5'
PCB 183	52663-69-1	LBX183	2,2',3,4,4',5',6
PCB 187	52663-68-0	LBX187	2,2',3,4',5,5',6
PCB 189	39635-31-9	LBX189	2,3,3',4,4',5,5'
PCB 194	35694-08-7	LBX194	2,2',3,3',4,4',5,5'
PCB 195	52663-78-2	LBX195	2,2',3,3',4,4',5,6
PCB 196/203	42740-50-1 / 52663-76-0	LBX196	2,2',3,3',4,4',5,6' /2,2',3,4,4',5,5',6
PCB 206	40186-72-9	LBX206	2,2',3,3',4,4',5,5',6
PCB 209	2051-24-3	LBX209	2,2',3,3',4,4',5,5',6,6'
PCB114	74472-37-0	LBX114	2,3,4,4’,5
PCB123	65510-44-3	LBX123	2’,3,4,4’,5

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

1.1 Summary of Test Principle

Forty ortho-substituted polychlorinated biphenyls (PCBs), 9 persistent chlorinated pesticides and selected pesticide metabolites are measured in serum by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry (HRGC/ID-HRMS). All serum specimens are handled using *Universal Precautions*.

Serum specimens (1-2 mL) to be analyzed for PCBs and persistent pesticides are spiked with $^{13}C_{12}$-labeled internal standards and the analytes of interest are isolated in dichloromethane (DCM) using an OASIS HLB solid phase extraction (SPE) column followed by extraction through a SPE column containing neutral silica and acid silica. PCBs and pesticides are eluted from the acid silica column with 5% dichloromethane /hexane. For PCBs and pesticides, each analytical run consists of 24 unknown specimens, 3 method blanks, and 3 quality control samples. Before quantification, the vials are reconstituted with 10µL ^{13}C-labeled external standard. Sample extracts are then analyzed simultaneously for PCBs and pesticides by HRGC/ID-HRMS where 1 µL is injected, using a GC Pal (Leap Technology) auto sampler, into a Hewlett-Packard 7890 gas chromatograph operated in the splitless injection mode with a flow of 1 mL/min helium through a Restek Rxi-5 sil ms capillary column (30m x 0.25 mm x 0.25 µm film thickness) where analytes are separated prior to entering a Thermo Electron DFS (5kV) magnetic sector mass spectrometer operated in EI mode at 40 eV, using selected ion monitoring (SIM) at 10,000 resolving power (10% valley). Two ion current responses corresponding to two masses are monitored for each native (carbon-12) compound and it corresponding ^{13}C-internal standard. The instrumental response factor for each analyte is calculated as the sum of the two carbon-12 isomers divided by the sum of two ^{13}C-isomers

Calibration of mass spectrometer response factor vs. concentration is performed using calibration standards containing known concentrations of each native ($^{12}C_{12}$) compound and its corresponding $^{13}C_{12}$-internal standard. The concentration of each analyte is derived by interpolation from individual linear calibration curves and is adjusted for sample weight. The validity of all mass spectrometry data are evaluated using a variety of established criteria, such as signal-to-noise ratio ≥ 3 for the smallest native ion mass, instrument resolving power ≥ 10,000, chromatographic isomer specificity index with 95% limits, relative retention time ratio of native to isotopically labeled analyte within 4 parts-per-thousand compared to a standard, response ratios of the two $^{12}C_{12}$ and $^{13}C_{12}$ ions must be within ± 20 % of their theoretical values and analyte recovery ≥10 % and ≤ 120%. In addition, the calculated mean and range of each analyte in the quality control sample must be within their respective confidence intervals. The method detection limit (MDL) for each analyte is calculated correcting for sample weight. The total lipid content of each specimen is estimated from its total cholesterol and triglycerides values using a “summation” method. Analytical results for PCBs and pesticides are reported on a whole-weight [ng/g or parts-per-billion (ppb)] and lipid-adjusted basis [ng/g or ppb]. International toxicity equivalents (I-TEQs) are also reported for PCDDs, PCDFs, cPCBs and other mono-ortho or “dioxin-like” PCBs, based on the WHO-TEF system. Prior to reporting results, all quality control (QC) data undergo a final review by a Division of Laboratory Science quality control officer.

2. SAFETY PRECAUTIONS

All serum specimens are handled using Universal Precautions. Specimens received for analysis must be considered potentially positive for infectious agents including HIV and hepatitis B viruses. Universal Precautions must be observed; laboratory coats, safety glasses and protective gloves should be worn during all steps of this method. The Hepatitis B vaccination series is recommended for all

analysts working with whole blood and/or serum samples. Laboratory personnel should abide by common safety practices: no eating, drinking, or smoking in the laboratory. Protective clothing should not be worn out of the laboratory; and hands should be washed with soap and water before leaving the area. When organic solvents are being used, all operations should be performed under a fume hood. As an added precaution, laboratory staff should also wear solvent-resistant nitrile gloves during all phases of the sample enrichment procedure, including glassware washing. The laboratory should have formal written policies for handling PCB and pesticide standards, potentially infectious biological samples and disposal of waste solvents and reagents. Spill kits for solvents, acids and bases, as well as a disinfectant for biological spills (such as 70% ethanol or 5% sodium hypochlorite) should be available in the laboratory. Standard solutions containing more than 1 µg of TCDD toxic equivalents should not be stored in sample preparation or GC/MS laboratories.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

Relational databases have been set up on PC network using R:BASE version 9.1 (R:BASE Technologies, Murryville, PA). The databases are used for storage, retrieval, and analysis of data from projects of the Dioxin and Persistent Organic Pollutants Laboratory. Data entries are made into four tables containing: 1) Demographic information; 2) Information from the clean-up section; 3) Mass spec data; and 4) Lipid results. Each section has access only to the information that it entered. However, after the information from each section has been entered, the data sets can be merged for a complete report on each sample. Data sets can be sent to SAS, Statistical Analysis System, on the PC network. Entry forms and reports can be changed to fit the needs of each section.

The statistical analysis of the results are performed using the software package SAS, Statistical Analysis System. The data from the each of the sections is brought together by specimen identification number, the notebook number of the clean-up section, and the mass spec run number. Only the project supervisor and the database manager will have access to the whole database. Exposure codes will be broken only after all valid results have been reported to appropriate project coordinator by memo, thus, insuring that no data will be changed.

After entering R:BASE, menus are used to guide the user through the various steps. The MASTER menu displays the following options: 1) demographic information processing; 2) cleanup sample processing; 3) mass spec result processing; 4) Lipid analysis; 5) supervisory functions; and 6) exit. The demographic table contains the specimen identification numbers, the study number and any additional information received about the sample, such as collection date. The cleanup table contains the specimen identification number, the weight of sample used in the analysis, the analyst’s initials, and the notebook number where the cleanup information is recorded, the cleanup date and the lot numbers of adsorbents used. In the cleanup table, specimens are identified as unknowns, quality control samples, blanks or standards. The lipid table contains the specimen identification number and lipid results. The mass spec table contains the data from the mass spectrometer, retention times and area counts for each congener, as well as the notebook number assigned in cleanup and a run number assigned by the mass spectrometer operator. When the data is imported into R:BASE from the mass spectrometer, log transformed regression parameters from the daily calibration curve are used to calculate the concentrations of each congener in each specimen and this concentration is stored in the mass table.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; AND CRITERIA FOR SPECIMEN REJECTION

Fasting prior to sample collection is not necessary since the lipid adjustment normalizes the serum levels. Individuals providing a large amount of blood may have a low-fat meal such as toast (no butter) with jelly and black coffee.

The specimen type is serum, processed by the procedures outlined in this section.

The larger the serum volume, the lower the detection limits are. With more sensitive mass spectrometers, the volumes of serum are now routinely between 1 mL and 2 mL. The minimum amount is about 0.5 mL of serum.

Specimen Collection Materials for Each Participant. Up to 10 g Serum sample.

1. Gauze sponges, sterile, individually wrapped 2"x2" (2 ea).
2. Alcohol wipe
3. Band-aid
4. Red-top Vacutainers(size depends on volume to be collected)
5. 21 gauge multi sample needle, sterile
6. Pre-printed labels
7. Tourniquet
8. Vacutainer holder
9. Freezer
10. Pasteur pipette (1 each*)
11. Qorpak bottle (1 each*)
12. Teflon-lined stoppers for above Qorpak bottle (2 ea.*)
13. Aluminum seals (2 each)
14. Pre-printed labels
15. Pipette bulb
16. Racks
17. Centrifuge
18. Freezer (-20 °C)

*These items are to be rinsed with acetone, toluene, hexane, and acetone.

Collection of 1-10 g serum sample

Blood is collected in red top Vacutainers. For collection, loosen the tourniquet immediately after blood flow is established and release entirely as the last tube fills. Completely fill all the Vacutainer tubes and then withdraw the needle with a slow but firm motion. Red-top tubes should not be inverted or mixed. Label all tubes. Place the red-top tubes upright in a rack and allow them to clot at room temperature for 20-30 minutes. Centrifuge the red-top tubes for 10 minutes at the RPM necessary to attain a force of 1000 x g. Using a transfer pipette, pipette the serum from each participant's red-top tubes into the Wheaton Bottle and cap. Check to make sure that the numbers on the labels are the same. DO NOT ALLOW SERUM TO REMAIN IN CONTACT WITH THE CLOT FOR LONGER THAN 1 HOUR AFTER THE SPECIMEN IS COLLECTED. Mix the serum gently, cap each bottle and place upright in a -20 NC freezer and store at the same temperature until shipment to CDC. The time between collecting blood and freezing serum should not be more than 1 1/2 hours. Note on the sample log if a sample is turbid or hemolyzed, or if the serum was left in contact with red cells for more than 1 hour or left at room temperature for more than 90 minutes before freezing.

Sample Shipment supplies

(a) 1 Styrofoam shipper
(b) 3-4 lbs. dry ice
(c) 4 bubble-pack bags 4"x7"
(d) Safety glasses or eye shield
(e) Strapping tape
(f) Gloves for handling dry ice and frozen specimens
(g) Sheets of bubble-pack packing material
(h) CDC "Specimen Shipping List" filled out
(i) Zip-lock bag

For all shipments, do not pack shippers with frozen specimens and dry ice until

just before shipment. Telephone the laboratory at CDC the day the shipment is transported. For each shipment, fill out a blank Specimen Shipping List provided by CDC. When packing the shippers, use gloves to handle the dry ice to avoid burning the hands. Glasses or an eye shield should also be worn if the dry ice cakes are to be broken into small pieces. Place the frozen serum specimens from each participant in one 4"x7" bubble bag and seal. Pack 1 set of filled bubble bags upright in the bottom of the shipper. If necessary, use sheets of bubble-pack, packing material to ensure the specimens are in a vertical position. Fill the shipper with dry ice. Insert the completed "Specimen Shipping List" in a 12x12" zip-lock bag and secure to the top of the Polyfoam lid with filament tape. Secure the outer carton lid on the shipper with EPA seal tape and complete the appropriate information. Attach pre-addressed “FEDERAL EXPRESS" shipping label, the HUMAN BLOOD - THIS SIDE UP label, and the DRY ICE label.

Specimen Stability has been demonstrated for analytes measured by this method for at least 10 years at -30 ^{0}C or below. However, due to the chemical inertness of these compounds, they can be assumed to be stabile indefinitely if specimens are maintained in a frozen state.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. PREPARATION OF REAGENTS, CALIBRATORS (STANDARDS), CONTROLS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION

6.1 Reagents and consumables

The method has been validated using the chemicals, solvents and expendables listed in Table 1 and 2. Other manufacturer’s products of equivalent purity can be used after verification of chemicals purity.

Table 1. Solvents and chemicals used for development of current methodology, equivalent products from other manufacturer may be used with exception to the SPE sorbent.

Chemical/Solvent	Manufacturer	Grade
Acids		
Formic acid	EM Science	ACS, 88%
Hydrochloric acid	Sigma-Aldrich	37%
Sulfuric acid	Sigma-Aldrich	99.999%
Solvents		
Dichloromethane	TEDIA	Pesticide
Dodecane	Sigma-Aldrich	min 99%
Hexane	TEDIA	Pesticide
Methanol	TEDIA	Pesticide
n-Nonane	Sigma-Aldrich	99%
Water	TEDIA	Pesticide
Ethanol	TEDIA	Anhydrous
Acetone	TEDIA	
DI Water	Culligan	deionized
SPE sorbents		
OASIS HLB®	Waters	n/a
Silica gel	Sigma-Aldrich	63-230 µm
Micro detergent	Cole-Parmer	Liquid cleaner

Nitrogen gas		High purity

Table 2. Expendables used for development of current methodology, equivalent products from other manufacturer may be used.

Item	Manufacturer/Source
Glassware and caps	
Test tube 16 x 100 mm	Fisher Scientific
Septum for test tube	Wheaton
Open top cap for test tube	Wheaton
Pasteur pipet	Fisher Scientific
Boston Round (amber glass bottle)	Qorpak
V-vial (3 mL) with septum-cap	Wheaton
GC vials and caps	Waters
Others	
Label printer (Brady TLS PClink)	Brady
Magnetic stirrer (heavy duty, large)	Fisher Scientific
Pipet dispenser	VWR
Balance	Sartorius BP310S
Vortex mixer	Fisher Scientific
TurboVap II	Biotage
TurboVap LV	Biotage
Eppendorf pipet	Brinkman
Microman M50, M25, M250	Rainin
EDP and EDP Plus pipet	Rainin
Rxi 30m x 0.25mm x 0.25 µm GC column	Restek
GC syringe	SGE

6.1.1 Preparation of rinse solution used at extraction

The rinse solution is eluted though the SPE cartridge, after the serum, during the extraction step and contains 0.1M hydrochloric acid and 5% methanol by volume. This solution is prepared by adding specified amounts, to an amber 4 L solvent bottle according to below.

Table 3. Preparation of 0.1M hydrochloric acid in 5% (v/v) methanol solution.

Volume to prepare (mL)	Water (mL)	Hydrochloric acid (mL)	Methanol (mL)
500	470	5	25
1000	940	10	50
2000	1880	20	100
3000	2820	30	150
4000	3760	40	200

6.2 Calibration Standards.

All PCB and chlorinated pesticide calibration standards were purchased from Cambridge Isotopes Laboratory (CIL, Woburn, MA). They were prepared in nonane according to CDC specifications and contain 40 PCBs found in humans and 11 chlorinated pesticides. Standards were prepared from individual stock solutions of labeled $^{13}C_{12}$-PCBs, $^{13}C_n$-pesticides, and native $^{12}C_{12}$-PCBs, and $^{12}C_n$-pesticides that are certified to be at least 99% pure. All of these compounds are suspected carcinogens. Lab coats and gloves should be worn when handling them, but their concentrations in these standards are very low. Tables 4A and 4B list the components of the isotope dilution standards. Carbon-13 labeled PCBs are not commercially available for all of the PCBs measured. In those cases, another $^{13}C_{12}$-labeled PCB is used as its internal standard. Tables 4A and 4B list the internal standards used for each of the 40 PCB measured. The concentrations of each of the PCB congeners in each of the IDMS standards are shown in Table 5A and the concentrations of each pesticide are shown in Table 5B.

Diluent for sample extract reconstitution was also purchased from Cambridge Isotopes, (CIL Woburn, MA). It is a standard containing 25 pg/mL of $^{13}C_6$-1,2,3,4-TCDD in nonane. This standard is used to reconstitute sample extracts before mass spectral analysis of PCBs and chlorinated pesticides. The quantification standards (Table 5) also contain 25 pg/mL of $^{13}C_6$-1,2,3,4-TCDD and therefore a comparison between the ratio of the internal standards ($^{13}C_{12}$-PCBs or $^{13}C_{n-}$Pesticides) and the recovery standard ($^{13}C_6$-1,2,3,4-TCDD) can be used to calculate the absolute percent recovery of the ^{13}C -labeled internal standards during sample analysis. This recovery standard also allows researchers to show that the mass spectrometer remained at 10,000 resolving power during the analysis of each sample. The $^{13}C_6$-1,2,3,4-TCDD in each sample extract can also demonstrate capillary column isomer specificity on the basis of its separation from $^{13}C_{12}$-2,3,7,8-TCDD.

Analytical standards, isotopically labeled internal standards, and reconstitution standards are dispensed in equal volumes into silanized ampoules and are flame sealed. The sealed ampoules are stored at room temperature.

Table 4A Standard Materials for Ortho-Substituted PCBs

Compound	Formula	PCB BZ Number	Native $^{12}C_{12}$	Label $^{13}C_{12}$
2,2',5-Trichloro biphenyl	$C_{12}H_7Cl_3$	PCB18	Yes	PCB28
2,4,4'-Trichloro biphenyl	$C_{12}H_7Cl_3$	PCB28	Yes	Yes
2,2',5,5'-Tetrachloro biphenyl	$C_{12}H_6Cl_4$	PCB52	Yes	Yes
2,2',4,5'-Tetrachloro biphenyl	$C_{12}H_6Cl_4$	PCB49	Yes	PCB52
2,2'3,5'-Tetrachloro biphenyl	$C_{12}H_6Cl_4$	PCB44	Yes	PCB52
2,4,4',5-Tetrachloro biphenyl	$C_{12}H_6Cl_4$	PCB74	Yes	PCB101
2,3',4,4'-Tetrachloro biphenyl	$C_{12}H_6Cl_4$	PCB66	Yes	PCB101
2,2',4,5,5'-Pentachloro biphenyl	$C_{12}H_5Cl_5$	PCB101	Yes	Yes
2,2',4,4',5-Pentachloro biphenyl	$C_{12}H_5Cl_5$	PCB99	Yes	PCB101
2,2',3,4,5'-Pentachloro biphenyl	$C_{12}H_5Cl_5$	PCB87	Yes	PCB123
2,3,3',4',6-Pentachloro biphenyl	$C_{12}H_5Cl_5$	PCB110	Yes	PCB123
2,3',4,4',5-Pentachloro biphenyl	$C_{12}H_5Cl_5$	PCB118	Yes	Yes
2,3,3',4,4'-Pentachloro biphenyl	$C_{12}H_5Cl_5$	PCB105	Yes	Yes
2,2',3,5,5',6-Hexachloro biphenyl	$C_{12}H_4Cl_6$	PCB151	Yes	PCB123
2,2',3,4',5',6-Hexachloro biphenyl	$C_{12}H_4Cl_6$	PCB149	Yes	PCB123
2,2',3,4',5,5-Hexachloro biphenyl	$C_{12}H_4Cl_6$	PCB146	Yes	PCB153
2,2',4,4',5,5'-Hexachloro biphenyl	$C_{12}H_4Cl_6$	PCB153	Yes	Yes
2,2',3,4,4',5' and 2,3,3',4,4',6-Hexachloro biphenyl	$C_{12}H_4Cl_6$	PCB138 PCB158	Yes	Yes
2,2',3,3',4,4'-Hexachloro biphenyl	$C_{12}H_4Cl_6$	PCB128	Yes	Yes
2,3',4,4',5,5'-Hexachloro biphenyl	$C_{12}H_4Cl_6$	PCB167	Yes	Yes
2,3,3',4,4',5-Hexachloro biphenyl	$C_{12}H_4Cl_6$	PCB156	Yes	Yes

Compound	Formula	IUPAC Number	Native $^{12}C_{12}$	Label $^{13}C_{12}$
2,3,3',4,4',5'-Hexachloro biphenyl	$C_{12}H_4Cl_6$	PCB157	Yes	Yes
2,2,3,3',5',5',6-Heptachloro biphenyl	$C_{12}H_3Cl_7$	PCB178	Yes	Yes
2,2',3,4',5,5',6-Heptachloro biphenyl	$C_{12}H_3Cl_7$	PCB187	Yes	PCB178
2,2',3,4,4',5',6-Heptachloro biphenyl	$C_{12}H_3Cl_7$	PCB183	Yes	PCB178
2,2',3,3',4,5′,6′-Heptachloro biphenyl	$C_{12}H_3Cl_7$	PCB177	Yes	PCB156
2,2',3,3',4,5,5'-Heptachloro biphenyl	$C_{12}H_3Cl_7$	PCB172	Yes	PCB180
2,2',3,4,4',5,5'-Heptachloro biphenyl	$C_{12}H_3Cl_7$	PCB180	Yes	Yes
2,2',3,3',4,4',5-Heptachloro biphenyl	$C_{12}H_3Cl_7$	PCB170	Yes	Yes
2,3,3',4,4',5,5' –Heptachloro biphenyl	$C_{12}H_3Cl_7$	PCB189	Yes	Yes
2,2',3,3',4,5,5',6'-Octachloro biphenyl	$C_{12}H_2Cl_8$	PCB199	Yes	PCB170
2,2',3,3,4,4',5,6'- and 2,2',3,4,4',5,5'6-Octachloro biphenyl	$C_{12}H_2Cl_8$	PCB196 PCB203	Yes	PCB170
2,2'3,3',4,4',5,6-Octchloro biphenyl	$C_{12}H_2Cl_8$	PCB195	Yes	PCB194
2,2',3,3',4,4',5,5'-Octachloro biphenyl	$C_{12}H_2Cl_8$	PCB194	Yes	Yes
2,2',3,3',4,4',5,5,6'-Nonachloro biphenyl	$C_{12}H_1Cl_9$	PCB206	Yes	Yes
2,2',3,3',4,4',5,5',6,6'-Decachloro biphenyl	$C_{12}Cl_{10}$	PCB209	Yes	Yes
2,3,4,4′,5-Pentachloro biphenyl	$C_{12}Cl_4$	PCB114	Yes	Yes
2′,3,4,4′,5-Pentachloro biphenyl	$C_{12}Cl_5$	PCB123	Yes	Yes
$^{13}C_6$ 1,2,3,4-TCDD	Recovery standard			

Table 4B Standard materials for Chlorinated Pesticides

Compound	Formula	Native $^{12}C_n$	Label $^{13}C_n$
Hexachlorobenzene	C_6Cl_6	Yes	Yes
β-Hexachlorocyclohexane	$C_6H_6Cl_6$	Yes	Yes
γ-Hexachlorocyclohexane	$C_6H_6Cl_6$	Yes	Yes
Heptachlor epoxide	$C_{10}H_5O_2Cl_7$	Yes	Yes
Oxychlordane	$C_{10}H_4OCl_8$	Yes	Yes
trans-Nonachlor	$C_{12}H_5Cl_9$	Yes	Yes
p,p'- DDE	$C_{14}H_8Cl_4$	Yes	Yes
Dieldrin	$C_{12}H_8OCl_6$	Yes	Yes
o,p'- DDT	$C_{14}H_9Cl_5$	Yes	Yes
p,p'- DDT	$C_{14}H_9Cl_5$	Yes	Yes
Mirex	$C_{10}Cl_{12}$	Yes	Yes
$^{13}C_6$ 1,2,3,4-TCDD	Recovery Standard		

Table 5A. High Resolution IDMS Calibration Solutions for ortho-substituted PCBs in Human Serum

STD NAME	$^{12}C_{12}$ PCB (pg/µL)	$^{13}C_{12}$ PCB (pg/µL)	$^{12}C_6$ 1234-TCDD (pg/µL)
P01	0.5	75.0	25
P02	1.0	75.0	25
P03	5.0	75.0	25
P04	10.0	75.0	25
P05	25.0	75.0	25
P06	50.0	75.0	25
P07	75.0	75.0	25
P08	100.0	75.0	25
P09	500.0	75.0	25
P10	1000.0	75.0	25

Table 5B. High Resolution IDMS Calibration Solutions for Chlorinated Pesticides in Human Serum

STD NAME	$^{12}C_n$ Pest. (pg/µL)	$^{13}C_n$ Pest. (pg/µL)*	$^{12}C_6$ 1234-TCDD (pg/µL)
T03	5.0	100.0	25
T04	10.0	100.0	25
T05	25.0	100.0	25
T06	50.0	100.0	25
T07	75.0	100.0	25
T08	100.0	100.0	25
P09	500.0	100.0	25
T10	1000.0	100.0	25

* The concentration of the p,p'-DDE label in the standards and the spiking solution is 250 pg/µL.

6.3 Instrumentation

6.3.1 Gilson 215 liquid handler: Liquid handling is automated using the Gilson 215 Liquid handler (Figure 1). Place the samples in the auto-mix to the far right in Figure 1. The probe (moving arm) picks up and dispenses reagents (internal standards, formic acid and water) to the samples according to a predefined sequence with mixing in-between each type of addition.

Recovery of the internal standards, as a percentage, is an important quality measurement of the analytical run. In order to enable recovery measurements, in this automated procedure, recovery standard will be added to empty GC vials located in a rack at the far left in Figure 1. These GC vials will be stored capped until the last step of the sample preparation method in which the purified extract will be transferred to the GC vials and mixed with the recovery standard.

6.3.2 Rapid Trace®, SPE work station: The Rapid Trace® SPE workstation) includes (A) syringe pump for drawing and dispensing solvents and sample (B) mixing chamber (not used in this method), (C) plunger, compressing SPE cartage and dispensing liquids through cartridge, (D) cannula used for drawing serum sample from test tube and (F) rack containing serum samples and collected fractions. The Rapid Trace® instrument processes the samples in sequence. Up to 5 samples can be loaded in any one module for unattended extraction, and 10 samples per module for unattended cleanup. Six modules are used for the default batch size of 30 samples, resulting in simultaneous processing of six samples at any one time.

Instrumental operation and steps in automated extraction method

Load serum samples into positions 1, 3, 5, 7 and 9 on the right-hand side of the rack. Load collection tubes into positions 2, 4, 6, 8, and 10 on the left-hand side. Load tubes for collecting serum waste into position 1, 3, 5, 7 and 9 on the left-hand side of the rack. Load SPE cartridges containing OASIS HLB sorbent (540 mg, Waters) into the cartridge carousel at positions 1, 3, 5, 7 and 9.

Process the first sample run on each module with a method that includes purge steps (Method: EXT1ONLY.spe) (see below for instructions on setting-up

the software), including a complete purge of all solvent lines as well as the extraction of the sample. Purge the system sequentially by drawing 6 mL of each: water, rinse solution (0.1M hydrochloric acid in 5% methanol), methanol, dichloromethane and methanol and rinse solution. Dispensing these liquids through the cannula seated in the rinse port. (After this purge, no air remains in the instruments solvent lines) The method used for remaining samples (EXT3TO9.SPE) is identical to EXT1ONLY.spe except for the initial purge steps included only in the method EXT1ONLY.spe.

The extraction includes the following steps:

- Rinse the SPE cartridge with 3 mL each of methanol, dichloromethane, methanol and rinse solution at a flow rate of 3 mL/min.
- Draw the serum from the test tube through the cannula into the syringe pump and dispensed through the plunger and the SPE cartridge into the serum waste tube on the left hand side adjacent to the original serum tube.
- Rinse the SPE cartridge first with 1.5 mL of the rinse solution into the same waste tube located in the rack. After this initial rinse removing almost all serum residues from the SPE cartridge, send an additional 1.5mL of rinse solution through the cartridge into the biological waste line. (This procedure is used to avoid the biological waste line clogging-up with time. In the current method, only clean water is passed through this waste line)
- Initiate the drying step with the SPE cartridge still pressed into the biological waste port, forcing compressed nitrogen (40 psi ± 10) through the cartridge.
- Elute the sample into the collection tube located behind the serum waste tube with dichloromethane.
- Processing of the next sample in the sequence is then automatically initiated.

6.3.3 High-resolution gas chromatography/high-resolution mass spectrometry systems: Thermo Electron DFS Mass Spectrometer (5kv), with X-caliber data systems (Thermo Electron, San Jose, CA) and Agilent Technologies 7890 Gas Chromatograph (Agilent Technologies, Palo Alto, CA) and a GC-Pal autosampler (Leap Technologies, Carrboro, NC). Sample extracts are analyzed for PCBs and pesticides by HRGC/ID-HRMS. One microliter of extract is injected, using an auto sampler, into the gas chromatograph operated in the splitless injection mode with a flow of 1 mL/min helium through a Rxi-5 sil ms capillary column (30m x 0.25 mm x 0.25 µm film thickness) where analytes are separated prior to entering the magnetic sector mass spectrometer operated in EI mode at 45 eV, using selected ion monitoring (SIM) at 10,000 resolving power (10% valley).

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

7.1 Isotope-Dilution Calibration

7.1.1 Slope and Intercept.

Calibration of mass spectrometer response factor vs. concentration is performed using quantitative analytical standards containing known concentrations of each native (^{12}C-) compound and its corresponding ^{13}C-internal standard. The quantitative analytical standards are listed in Table 6A for PCBs and Table 6B for pesticides. The standards are analyzed in ascending order at the beginning of each analytical run. The log transformed regression model ($y = a*b^X$) is used. The log transformed slope

ranges from 0.97 to 1.03 and the log transformed intercepts range from -0.1 to +0.25.

7.1.2 Blank Correction and Background Correction for PCBs and Pesticides.

Blank Corrections and background corrections for all analytes are made using the average blank over the course of the study. The average concentration of the blank is subtracted from the apparent concentration of the analyte in an unknown sample or QC sample to obtain the actual concentration of the analyte in the sample. The first, fifteenth and twenty-fifth samples in every clean-up run are the analytical blanks. It consists of ^{13}C labeled internal standard (spiking solution) that is carried through the entire analytical procedure, including clean-up and GC/MS analysis. It represents the amount of contamination or interference in the solvents and adsorbents, and in the laboratory equipment and in the environment (i.e. air). Since the percent coefficient of variation for measurements of the blank is about 50%, using the average blank minimizes the problem of over-correcting or under-correcting that can occur when the blank for a given clean-up run is used to correct all of the analytical results for that run.

7.1.3 Isotope ratios.

When performing calibration, calculate the average isotope ratios (see Tables 6A and 6B) for the two native ions and the two primary-labeled internal standard ions in the calibration standards for each analyte. Determine the 95% and 99% confidence intervals for each analyte based upon their theoretical ion ratios as follows: 95% confidence limit [2 standard deviations (SD)] for the isotope ratio for the two native ions is defined to be 20% of their theoretical ion ratio. The 99% confidence interval (3 SD) is calculated by dividing the 95% confidence limit by 1.96 to get 1 SD and multiplying 1 SD by 2.58 to get 3 SD. The 95% and 99% confidence intervals for the isotope ratio of the two internal standard ions are computed similarly to the intervals for the ratio of the native ions except that their limits are based upon 15% of their average ratios.

Table 6A. Ion Ratios for Analysis of PCBs

Compound	Ions Monitored	Theoretical Ratio	Acceptable Range[1] 99% Confidence
Tri-CB	255.9613/257.9584	1.03	(0.76-1.30)
$^{13}C_{12}$-Tri-CB	268.0016/269.9986	1.03	(0.83-1.23)
Tetra-CB	289.9224/291.9194	0.77	(0.57-0.97)
$^{13}C_{12}$-Tetra-CB	301.9626/303.9597	0.77	(0.62-0.92)
Penta-CB	323.8834/325.8804	0.62	(0.46-0.78)
$^{13}C_{12}$-Penta-CB	335.9237/337.9207	0.62	(0.50-0.74)
Hexa-CB	289.9037/291.9008	2.09	(1.54-2.64)
$^{13}C_{12}$-Hexa-CB	301.944/303.9441	2.09	(1.68-2.50)
Hepta-CB	323.8834/325.8804	1.57	(1.16-1.98)
$^{13}C_{12}$-Hepta-CB	335.9237/337.9207	1.57	(1.26-1.88)
Octa-CB	357.8258/359.8229	1.25	(0.92-1.58)
$^{13}C_{12}$-Octa-CB	369.8661/371.8631	1.25	(1.00-1.50)
Nona-CB	463.7216/465.7187	1.35	(0.99-1.70)
$^{13}C_{12}$-Nona-CB	475.7619/477.7589	1.35	(1.08-1.64)
Deca-CB	497.6826/499.6797	1.17	(0.86-1.48)
$^{13}C_{12}$-Deca-CB	509.7229/511.7199	1.17	(0.85-1.40)

[1] Each congener has its own confidence intervals. These ranges are the minimum and maximum value within each group (e.g., within Hexa-CBs).

Table 6b. Ion Ratios for Analysis of Chlorinated Pesticides

Compound	Ions Monitored	TheoreticalRatio	Acceptable Range[1] 99% Confidence
Hexachlorobenzene	283.8102/285.8072	1.26	(0.93-1.59)
$^{13}C_6$-HCB	289.8303/291.8273	1.26	(1.01-1.51)
β-HCCH	218.9115/220.9085	2.09	(1.54-2.64)
$^{13}C_6$-β-HCCH	224.9347/226.9287	2.09	(1.68-2.50)
γ-HCCH	218.9115/220.9085	2.09	(1.54-2.64)
$^{13}C_6$-γ-HCCH	224.9347/226.9287	2.09	(1.68-2.50)
Heptachlor epoxide	352.8442/354.8413	1.26	(0.93-1.59)
$^{13}C_{10}$ Heptachlor epoxide	362.878/364.8748	1.26	(1.01-1.51)
Oxychlordane	386.8052/388.8023	1.03	(0.76-1.30)
$^{13}C_{10}$ Oxychlordane	396.8388/398.8358	1.03	(.83-1.23)
trans-Nonachlor	406.787/408.784	0.89	(0.66-1.12)
$^{13}C_{10}$ trans-Nonachlor	416.8205/418.8176	0.89	(0.71-1.07)
p,p’ DDE	246.0003/247.9974	1.57	(1.16-1.98)
$^{13}C_{12}$-p,p’ DDE	258.0406/260.0376	1.57	(1.26-1.88)
Dieldrin	260.8859/262.8570	0.64	(0.47-0.81)
$^{13}C_{12}$-Dieldrin	267.8834/269.8805	0.64	(0.51-0.77)
o,p’- DDT	235.0081/237.0052	1.57	(1.16-1.98)
$^{13}C_{12}$-o,p’-DDT	247.0484/249.0454	1.57	(1.26-1.88)
p,p’- DDT	235.0081/237.0052	1.57	(1.16-1.98)
$^{13}C_{12}$-p,p’ DDT	247.0484/249.0454	1.57	(1.26-1.88)
Mirex	271.8102/2 73.8072	1.26	(0.93-1.59)
$^{13}C_8$-Mirex	276.8269/278.824	1.26	(1.01-1.51)

[1] Each congener has its own confidence intervals. These ranges are the minimum and maximum value within each group (e.g., within Mirex).

7.1.4 Instrument resolving power

At the beginning of each run, analyze a 2378 TCDD sensitivity check standard. Calculate the ratio of the peak areas for $^{13}C_{12}$-2,3,7,8-TCDD and $^{13}C_6$-1,2,3,4-TCDD in the m/z 331.9078 channel. The daily calculations of resolving power may be displayed for visual purposes as a quality control chart

7.1.5 Column isomer specificity.

Calculate the retention time ratio of $^{13}C_6$-1,2,3,4-TCDD relative to the retention time of $^{13}C_{12}$-2,3,7,8-TCDD for the sensitivity check standard. For PCB and pesticide standards, the retention time ratio can be calculated for

every standard. Determine the 95% and 99% confidence intervals which may be displayed for visual purposes as a quality control chart with upper and lower 95% and 99% confidence intervals for this ratio. Calculate for each standard the retention time ratio of the native analyte (ion 1) relative to the retention time of its $^{13}C_{12}$ labeled ion (ion 3). This variable is called RT_13 and is used to insure that the proper ions are used in the native/label ion ratio. When the RT_13 for an unknown sample or QC sample is divided by RT_13 for the standard, the ratio must be within 1.000 +0.004 in order for the data to be reportable.

Table 7 contains a list of all the mass ions used for the determination of the 38 PCBs and the 9 chlorinated pesticides and their relative order of elution. Figure 4 and 5 show reconstructed ion chromatograms of PCBs and pesticides showing peak identities and retention times.

Table 7 Ions Monitored for High-Resolution Mass Spectrometric Analysis of PCBs and Pesticides on a DFS Mass Spectrometer with a Rxi-5 sil ms 30m x 0.25mm id x 0.25 µm thickness.

WINDOW 1	**Start Time**	4:30	**Label**	**Lock Mass**	213.9903	
	End Time	7:52	**IDMS Std**			
				Cali Mass	264.9905	
	Low Mass	213.9903				
	High Mass	291.8273		**Cycletime**	0.65sec	
	Ratio	1.36				
	Analytes			**Mass**		**Fragment**
	Tri-PCB	-18	28	255.9613		M
		-28	28	257.9584		M+2
				268.0016		
				269.9986		
	HCB			283.8102		M+2
				285.8072		M+4
				289.8303		
				291.8273		
	HCH	beta	C13	218.9115		M
		gamma	C13	220.9085		M+2
				224.9317		
				226.9287		
WINDOW 2	**Start Time**	7:52		**Lock Mass**	264.9005	
	End Time	10:58				
				Cali Mass	413.977	
	Low Mass	260.8596				
	High Mass	413.977		**Cycletime**	0.64sec	
	Ratio	1.58				
	Analytes			**Mass**		**Fragment**
	Tetra-PCB	-52	52	289.9224		M
		-49	52	291.9194		M+2
		-44	52	301.9626		
		-74	101	303.9597		
		-66	101			

	HeptaEpoxide		C13	352.8442		M+2-Cl
				354.8413		M+4-Cl
				362.8777		
				364.8748		
	Oxychlordane		C13	386.8052		M+2-Cl
				388.8023		M+4-Cl
				396.8388		
				398.8358		
WINDOW 3	**Start Time**	10:58		**Lock Mass**	264.9905	
	End Time	13:05				
				Cali Mass	413.977	
	Low Mass	246.0003				
	High Mass	418.8176		**Cycletime**	0.63sec	
	Ratio	1.7				
	Analytes			**Mass**		**Fragment**
	DDE		C13	246.0003		M-Cl2
				247.9973		M+2-Cl2
				258.0406		
				260.0376		
			C13	260.8599		M+2-Cl
	Dieldrin			262.857		M+4-Cl
				267.8834		
				269.8805		
	Penta-PCB	-101	101	323.8834		M
		-99	101	325.8804		M+2
				335.9237		
				337.9207		
	t_Nonachlor		C13	406.787		
				408.784		
				416.8205		
				418.8176		
WINDOW 4	**Start Time**	13:05		**Lock Mass**	213.9903	
	End Time	15:28				
				Cali Mass	313.9839	

	Low Mass	213.9903				
	High Mass	337.9207		**Cycletime**	0.83sec	
	Ratio	1.58				
	Analytes			**Mass**		**Fragment**
	op-DDT		C13	235.0081		M-CCl3
				237.0052		M+2-CCl3
				247.0484		
				249.0454		
	dieldrin		C13	260.8599		M-C5H6ClO
	endrin			262.857		M+2C5H6ClO
				267.8834		
				269.8805		
	Penta-PCB					
		-87	123	323.8834		M
		-110	123	325.8804		M+2
		-123	123			
		-118	118			
		-114	114	335.9237		
		-105	105	337.9207		
	Hexa-PCB	-151	123	289.9037		M+2-Cl2
		-149	123	291.9008		M+4-Cl2
		-146	153	335.9237		
		-153	153	337.9207		
WINDOW 5	**Start Time**	15:28		**Lock Mass**	213.9903	
	End Time	17:38				
				Cali Mass	313.9839	
	Low Mass	213.9903				
	High Mass	337.9021		**Cycletime**	0.73sec	
	Ratio	1.58				
	Analytes			**Mass**		**Fragment**
	pp-DDT		C13	235.0081		M-CCl3
				237.0052		M+2-CCl3
				247.0484		
				249.0454		

	Hexa-PCB	-138	138	289.9037		M+2-Cl2
		-158	138	291.9008		M+4-Cl2
		-128	128	301.994		
		-167	167	303.9441		
	Hepta-PCB	-178	178	323.8834		M+2-Cl2
		-187	178	325.8804		M+4-Cl2
		-183	178	335.9237		
				337.9207		
	1234D			327.9137		
				327.9465		
	2378D	Label		331.9368		
				333.9338		
WINDOW 6	**Start Time**	13:38		**Lock Mass**	264.9905	
	End Time	19:34				
				Cali Mass	313.9839	
	Low Mass	264.9905				
	High Mass	337.9021		**Cycletime**	0.52sec	
	Ratio	1.28				
	Analytes			**Mass**		**Fragment**
	Hexa-PCB	-156	156	289.9037		M+2-Cl2
		-157	157	291.9008		M+4-Cl2
				301.994		
				303.9441		
	Hepta-PCB	-177	156	323.8648		M+2-Cl2
		-172	180	325.8618		M+4-Cl2
		-180	180	335.905		
				337.9021		
WINDOW 7	**Start Time**	19:34		**Lock Mass**	264.9905	
	End Time	22:43				
				Cali Mass	413.977	
	Low Mass	264.9905				
	High Mass	413.977		**Cycletime**	0.69sec	

	Ratio	1.56				
	Analytes			**Mass**		**Fragment**
	Mirex		C13	271.8102		M+2-C5Cl6
				273.8072		M+4-C5Cl6
				276.8269		
				278.824		
	Hepta-PCB	-170	170	323.8648		M+2-Cl2
		-189	189	325.8618		M+4-Cl2
				335.905		
				337.9021		
	Octa-PCB	-199	170	357.8258		M+2-Cl2
		-196	170	359.8229		M+4-Cl2
		-203	170	369.8661		
		-195	194	371.8631		
		-194	194			
WINDOW 8	**Start Time**	22:43		**Lock Mass**	463.7241	
	End Time	23:53				
				Cali Mass	502.9745	
	Low Mass	463.7214				
	High Mass	511.7199		**Cycletime**	0.52sec	
	Ratio	1.1				
	Analytes			**Mass**		**Fragment**
	Nona-PCB	-206	206	463.7216		M+4
				465.7187		M+6
				475.7619		
				477.7589		
	Deca-PCB	-209	209	497.6826		M+4
				499.6797		M+6
				509.7229		
				511.7199		

7.2 Calibration Verification

Calibration is performed at the beginning of each analytical run. Calibration of mass spectrometer response factor vs. concentration is performed using quantitative analytical standards containing known concentrations of each native (^{12}C-) compound and its corresponding ^{13}C-internal standard. The quantitative analytical standards are listed in Table 6A for PCBs and Table 6B for pesticides. The standards are analyzed in ascending order at the beginning of each analytical run. The log transformed regression model ($y = a*b^X$) is used. The log transformed slope ranges from 0.97 to 1.03 and the log transformed intercepts range from -0.1 to +0.25.

8. Procedure Operation Instructions; Calculations; Interpretation of Results

Formal training in the use of a high resolution mass spectrometer is necessary for all GC/HRMS operators. Users are required to read the operation manuals and must demonstrate safe techniques in performing the method. New operators must be evaluated after 6 months of initial training by the supervisor to certify that they are appropriately qualified to perform the assay.

Anyone involved in sample preparation must be trained in for all sample preparation equipment, chemical handling, and have basic chemistry laboratory skills. The training may be delegated to more experienced analyst.

8.1 Sending aliquot of serum for lipid determination

Serum lipid concentration in serum is determined in an aliquot of the sample (250 µl) using enzymatic methods by the Clinical Chemistry Branch (CCB). Aliquot 250 µl of each sample into polypropylene vials after mixing the thawed serum samples; use a new pipette tip for every sample to avoid cross contamination. Label vials for lipid weight determination with Study name, Study Number and notebook number. An lipid aliquot may have been drawn upon arrival of the samples to CDC and prior to the samples being sent to the CPPBL laboratory in which case no lipid aliquot needs to drawn prior to analysis

8.2. Thawing and weighing samples

Store samples awaiting analysis in –70 ^{o}C freezer. Samples are taken out of the freezer to thaw completely; this can be done the day before analysis and the samples placed in a refrigerator overnight. Thoroughly mix the samples by vortexing. For each batch of 30 samples, complete a run sheet. On the run sheet, enter ALL requested information under heading “Contact Information”, e.g., analyst’s name or initials, the date and run number.

To ensure optimum performance of the balance (Sartorius BP310S) used for weighing serum samples, verify the balance calibration using NIST calibration weights (1.000 g and 10.000 g) before weighing each batch of samples. Calibration weights are placed on the balance after taring, and the reading is recorded on the run sheet. The difference from true value may not exceed +/- 0.01 g. If this limit is exceeded, any problems must be resolved, such as cleaning the balance tray, recalibration of balance and/or calling for service of balance. After verifying the balance calibration, weigh serum samples into 16 x 100 mm glass test tubes. Record all sample weights on the run sheet. Add septum-equipped open-top screw caps.

8.3. Sample pretreatment, using Gilson 215 - Liquid handler

Procedure

A. Place new internal standards vials in the rack containing the internal standards on the Gilson 215.

B. Place the serum samples (weighed into 16x100mm test tubes fitted with septum-equipped screw caps) in the auto-mix (far right in Figure 1). The locations for the tubes in the auto-mix. Place the lid for the auto-mix on the auto-mix. Make certain that all locks in the four corners of the lid snapped into place. (Failure to secure lid properly will adversely affect the function of the auto-mix)

C. Refill formic acid and water containers in rack (Boston-round flasks).

D. Go to the application screen in the 735 Software (Gilson), select application “Complete 30 sample run including references” and press the green play bottom.

E. During the procedure All samples are spiked with the internal standards (Equilibrate approximately 20 minutes). After completion of this part of the sequence, the samples will be mixed and fortified with formic acid and water with mixing between each addition. During this time, prepare the Rapid Trace uints for extraction.

8.4. Solid Phase Extraction, using Biotage, Rapid Trace SPE workstation

The extraction procedure is automated using the Rapid Trace® modular SPE system, cf. section 6.3.2.

The software controlling the workstation is initiated by the Rapid Trace Development icon located on the desktop. After launching the software, the main menu is displayed (Figure 6). For setting up the software for extraction, click on “Setup Racks”, the menu given in Figure 7 is displayed. Select the modules to be used in lower left corner in this menu, and transfer method "EXT1ONLY.spe" to position “one” and transfer method "EXT3to9.spe" to positions 3, 5, 7 and 9, cf. Figure 8. Exit this menu by pressing “OK”. Enter the “Run Monitor Menu”, and launch the modules to be used for extraction, cf. Figure 8.

Check List - Extraction

A. Ensure that sufficient quantities of all solvents and reagents are present in containers under the Rapid Trace instrument and that all solvent lines are kept at the bottom of each container by an attached weight at the end of the solvent line.

B. If necessary, empty waste containers by replacing the container with an empty one.

C. Place the SPE cartridges in position 1, 3, 5, 7 and 9 in the cartridge carousel for each module used.

D. Place samples in racks (one rack per module) in position 1, 3, 5, 7 and 9 on the right hand side of the racks and remove screw cap fitted with septum.

E. Place waste tubes in position 1, 3, 5, 7 and 9 on the left hand side of the racks.

F. Place collection tubes in position 2, 4, 6, 8 and 10 on the left-hand side of the racks.

G. Place racks in moving tray at the bottom of each module.

H. Assign method to each module by clicking “Setup racks” in the main menu of the Rapid Trace software and placing method EXT1ONLY.spe as sample one for each module used and method EXT3to9.spe for the samples 3, 5, 7 and 9.

I. Exit the setup racks menu by pressing OK.

J. Enter the Run Monitor Screen. Wait a few seconds after entering the Run Monitor Screen to allow the software time to detect all modules present. Press start on modules to be run.
K. Watch the instrument for a few minutes to ensure that all modules has been initiated and inspect the modules running during the initial purge to ensure that all solvents lines are connected properly.

8.5. Cleanup, using Biotage, Rapid Trace SPE workstation

The cleanup procedure is automated using the Rapid Trace® modular SPE system, (cf. section 6.3.2).

Preparation of Silica gel / Silica gel:Sulfuric acid and packing of SPE cartridges

The SPE cartridges packed a with Silica and Silica:Sulfuric acid have a shelf life of 2 days and hence must be prepared directly prior to use.

Procedure for preparation of cartridges

A. See section 6.1 for Manufacturer, grade and brand for all chemicals used
B. Activate silica gel in oven at 210 ^{o}C overnight
C. Using laboratory balance add 13.333g Silica gel to 50-mL glass tube fitted with Teflon lined cap and add 6.667g of concentrated sulfuric acid to the tube with. After adding the acid, vigorously shake mixture to break up large lumps. Standard laboratory Personal Protective Equipment must be used, such as lab coat, safety glasses and gloved.
D. Allow the mixture to rotate overnight using rotating mixer. After overnight rotation, no lumps should be present in mixture.
E. Press frit to bottom of empty 3-mL SPE
F. Add 1.0 Silica/Sulfuric acid mixture to the cartridge, and place another frit on top
G. Add 0.1 g activated Silica gel (250 ^{o}C) overnight and place another frit on top of the silica
H. Store packed cartridges in resealable plastic bag in desicator until just prior to use

Setting up the Equipment for Processing Samples (Cleanup)

The software controlling the workstation is launched by the Rapid Trace™ Development Icon on the desk top. After launching the software the main menu is displayed (Figure 6). For setting up the software for cleanup click on “Setup Racks”, the menu given in Figure 7 is displayed. Select the modules to be used in lower left corner in this menu and transfer method CL#1ONLY.spe to position “one”. Transfer method CL2to10.spe to positions 2-9, if needed no skipping of any holes in the black rack are required. Exit this menu by pressing “OK”. Enter the “Run Monitor Menu” and launch the modules to be used for cleanup, cf. Figure 8.

Check List - Cleanup

A. Evaporate samples from extraction step to dryness by placing samples in the Caliper TurboVap evaporator and starting the evaporization with the following settings as a guide: 40deg C water bath temperature and ~5psi line pressure.
B. Make certain that sufficient quantities of the 5% DCM in Hexane solution are present in the solvent bottle under the Rapid Trace™ instrument and that all solvent lines are kept at the bottom of the container by an attached weight at the end of the solvent line.

C. If necessary, empty waste containers by replacing the container with an empty one.
D. Place extracts in racks (one rack per module) on the right hand side of the racks, and remove screw caps.
E. Place collection tubes on the left hand side of the racks.
F. Place racks in tray at the bottom of each module.
G. Assign method to each module by clicking “Setup racks” in the main menu of the Rapid Trace™ software and placing method "CL#1ONLY.spe" as sample one for each module used and method "CL2to10.spe" for remaining positions.
H. Exit the setup racks menu by pressing OK.
I. Enter the Run Monitor Screen. Wait a few seconds after entering the Run Monitor Screen to allow the software time to detect all modules present. Press start on modules to be run.
J. Watch the instrument for a few minutes to ensure that all modules has been initiated and inspect the modules running during the initial purge to ensure that all solvents lines are connected properly.

8.6. Evaporation and transfer to final GC-vial

A. Conduct all evaporations and sample transfers in a fume hood or BSC or at the Caliper TurboVap evaporator.
B. Samples from cleanup step are evaporated to approximately 0.5 mL using the the Caliper TurboVap evaporator and starting the evaporization with the following settings as a guide: 40deg C water bath temperature and ~5psi line pressure. It is essential that the samples are not evaporated to dryness at this step, since all volatile analytes would be lost.
C. Transfer the sample to the GC vial that was spiked with recovery standard in section 6.2. MAKE CERTAIN THAT THE SAMPLES ARE TRANSFERRED TO THE CORRECT VIAL !!!
D. Rinse the sample test tube with ~0.5mL of hexane and transfer to the GC-vial
E. Evaporate samples until ~10uL remains using the the Caliper TurboVap evaporator and starting the evaporation with the following settings as a guide: ~5-10psi line pressure.
F. Complete any lab notes, and bring samples to HR-MS operator.

8.3 HIGH RESOLUTION MASS SPECTROMETRY ANALYSIS OF PCBs

8.3.1 GC Conditions (Agilent 7890 GC)

30 m X 0.25mm i.d. X 0.25µm thickness Rxi-5 sil ms

	Splitless injection
Injection port temperature	275°C
Oven temperature program	120°C, hold 0.65 min; 4°C/min to 200°C, hold 5 min; 4°C/min to 250°C; 45 °C /min to 320°C, hold 1 min
Carrier gas	Helium flow rate 1 cc/min

Constant flow mode; vacuum correct off; pressure correct off
Restek splitless liner (4mm x 6.5 x 78.5) IP deactivated with deactivated glass wool.

8.3.2 MASS SPECTROMETRY CONDITIONS:

Ion Source	High Sensitivity
Ionizing electron energy	45 eV
Accelerating Voltage	4800 V
Trap Current	900 µA
Source temperature	275°C
Transfer line temperature	275°C;
Mass Resolution	10,000

8.3.3 Spectrometer Tuning and Mass Calibration

Calibrate and tune the mass spectrometer to 10,000 resolving power (RP) (defined by a 10% overlap when using the peak match unit) according to the protocol outlined below. Multi-group analyses for 40 PCBs and 9 pesticides on the Thermo Fisher DFS mass spectrometers consist of eight groups. Table 7 lists all the calibration masses. The GC and MS analyzers are operated by computer to calibrate, acquire raw data, detect and integrate peaks, and print chromatograms and output ASCII files that are transferred to R:BASE for data storage. The analyses are conducted in an isomer-specific mode, with a 30-m, 0.25-mm i.d., 0.25-µm film thickness Rxi sil ms capillary column. Seven channels are monitored for each analyte: one channel for $^{13}C_6$-1,2,3,4-TCDD, which is added to each sample to assess the instrument resolving power; two channels for the two lock masses (one to centroid, the other to actually measure the response); and four channels to monitor the native and ^{13}C-labeled internal standards

8.3.4 GC/MS Identification of PCBs and Pesticides

After installation of a new type of GC column, inject a PCB and a pesticide calibration standard and determine the retention time windows for all the congeners. Verify the GC column specificity for each compound. For each congener, determine the retention times relative to the ^{13}C-labeled isomer present for each congener group.

Daily Instrument Function Checks

8.3.5 Daily Signal-to-noise (S/N) ratio Function Check.

Inject 2µL of a 0.25 pg/µL 2378-TCDD S/N ratio check standard. Begin the run by programming the 30m Rxi sil ms capillary column: after an initial 1 min at 150 °C, increase temperature to 270 °C at 40 °C/min, hold 4 minutes, then increase temperature to 310 °C at 50 °C/min. The column temperature is held at 310 °C for 3 min. Check the sensitivity of the instrument by verifying that the S/N ratio for the unlabeled 2,3,7,8-TCDD (m/z 319.8965)is at least 100:1 before analyzing specimen. If the S/N ratio is less than 100:1, check the tuning (retune if necessary), cut 1-2 inches from the GC end of the capillary column, replace the GC injector liner if it is dirty, replace the GC injector septum if it is leaking, replace the ion volume if it is dirty, bake out the source if it is dirty, or replace a bad filament.

8.3.6 Daily Slope Function Check.

Inject 2 µL of a each calibration standard for the PCBs and pesticides (Table 5) and compute the Slope and Intercept for each compound

Slope Check = R_factor of Std/Conc of Std (pg/µL)
and
R_factor = (Ion_1 + Ion_2)/Ion_3 + Ion_4)

The ratio of the peak areas for $^{13}C_{12}$-2,3,7,8-TCDD and $^{13}C_6$-1,2,3,4-TCDD in

the m/z 331.9078 channel (RPI) will be calculated in R:BASE and the ratio compared with the previously determined 99% confidence intervals or a QC chart to verify that the instrument resolution was greater than 10,000. If outside the 99% confidence intervals, a repeat MS analysis will be conducted.

The sum of the area responses for the two C-13 labeled ions [ion3 + ion 4] (Tables 3) of the primary internal standard for each analyte and the area response for the recovery standard [ion 6] ($^{13}C_6$-1,2,3,4-TCDD, m/z 331.9078) are determined. These area counts are used to calculate in R:BASE the absolute recovery of the primary internal standards for each sample in the analytical run.

The retention time ratio of $^{13}C_6$-1,2,3,4-TCDD relative to the retention time of $^{13}C_{12}$-2,3,7,8-TCDD will be calculated in R:BASE and this ratio compared with the previously determined 99% confidence intervals or quality control to verify that the capillary column is isomer specific for 2,3,7,8-TCDD [within the 99% confidence interval. If it is outside the 99% confidence intervals, the capillary column will be replaced and the analysis repeated.

The retention time of each analyte peak relative to its associated ^{13}C -labeled isomer is determined. This ratio is used in R:BASE as a QC parameter for peak identification.

8.4 Mass spectral Analysis of processed specimen

Reconstitute samples from cleanup with diluent and analyze. To minimize the possibility of carry-over or cross-contamination of samples and analytical standards, the analysts use a separate syringe to for each analytical standard. In addition, a glass syringe used in reconstituting an unknown or QC sample is not reused. After each injection by the auto sampler, the syringe is rinsed 5 times with nonane. Before the next injection, the syringe is rinsed 10 times with toluene and 10 times with nonane.

The 30 samples in the cleanup run are analyzed as an analytical run. Samples with notebook numbers ending in 10, 20 and 30 are usually the QC samples in the analytical run. The area counts and retention times for each ion in Tables 6 are measured and sent to the mass spec table in R:BASE. The sum of the area responses for each ion (Table 7) in the unlabeled, the labeled primary internal, and the recovery standards will be determined in the appropriate R:BASE database. For each sample, the resolving power ratio, and the retention time ratio will be determined in R:BASE. Calibration is performed with each analytical run, and the daily slopes and intercepts are calculated in SAS and imported into R:BASE where they are used to compute the concentrations of each analyte in each sample.

For each congener, the following will also be calculated in R:BASE: the mass fraction, the absolute recovery of the primary internal standard ,the isotope ratio (Tables 6) for the two native ions and the two primary-labeled internal standard ions, and the retention time of each analyte peak relative to its associated ^{13}C-labeled isomer .

8.4 Recording of Mass Spectral Data

All raw data files are processed using the QUAN DESK application of the XCALIBER software which allows manual peak selection and area integration. The integrated values and retention times are transferred into a MSPEC table in R:BASE. Data is exported from R:BASE and imported into SAS. SAS programs for calibration, QC analysis, the evaluation of sample results, and data reporting have been created and are executed in SAS when this information is needed.

8.5 Replacement and periodic maintenance of key components

Note: The instrumentation used is serviced according to the manufacturer's guidance included in the instrument manuals or based on the recommendation of experienced analysts/operators after following appropriate procedures to determine that the instrument performs adequately for the intended purposes of the method.

Daily, check the sensitivity of the instrument by verifying that the S/N ratio for the unlabeled 2,3,7,8-TCDD (m/z 319.8965) is greater than 100:1. If the S/N ratio is unsatisfactory, check the tuning (retune if necessary), cut 1-2 inches from the GC end of the capillary column, replace the GC injector liner if it is dirty, replace the GC injector septum if it is leaking, replace reference inlet septum if leaking, replace the ion volume if it is dirty, bake out the source if it is dirty, or replace a bad filament.

The ion volume is cleaned and replaced as needed. The multiplier is changed every 6-12 months, or as needed once the setting is greater than 2400. The outer source is replaced every 6 months, or as needed. GC column is replaced as needed usually every two months. Reference inlet septum and autosampler syringe are replaced after each run. Magnetic calibration (MCAL) is performed monthly. Electric calibration (ECALIB) is performed weekly. Multiplier gain check id performed monthly, or as needed. Instrument preventive maintenance (changing vacuum pump oil, etc.) is performed by service technician twice annually.

Check the pressure in the helium and nitrogen tanks. If the pressure is below 500 psi, replace the tank with a full one.

8.6 Calculations

All computations and statistical analyses were carried out using the SAS v.9.3 statistical software package (SAS Institute 2005).

8.6.1 Using the $\log_{10}$ transformation of the regression equation Y = A* B**x, the concentration of the Analyte 'x', for which an internal standard 'xi' was added is given by:

(1) LOG_CONC= ((L_FACTOR - L_INTERCEPT) / L_SLOPE) / SWEIGHT

(2) $CONC = 10^{LOG_CONC}$

where L_FACTOR = $\log (A_x / A_{xi})$

A_x = the sum of the area responses for the two native ions of Analyte 'x' ;
A_{xi}= the sum of the area responses for the two ions of the primary internal standard;

L_INTERCEPT = the log intercept established by the linear regression equation for Analyte 'x';

L_SLOPE = the log slope established by the linear regression equation for Analyte 'x'; and

SWEIGHT = weight of the test portion

CONC = concentration of an analyte in a sample as weight per gram of sample. For PCBs and chlorinated pesticides, the units are ppb(ng/g).

8.6.2 The absolute recovery, R_{xj}(%) of the primary internal $^{13}C_{12}$-x standard, is given by:

$$(3) \quad R_{xi} = \frac{A_{xi} / A_{RSj}}{A_{RSi} / A_{xj}} \times 100$$

A_x = the sum of the area responses for the two native ions of Analyte'x' ;

A_{RSi} = the area of the external standard in the sample;

A_{xj} = the sum of the area responses for the two ions of the primary internal standard in the recovery standard; and

A_{RSj} = the area of the external standard in the recovery standard.

8.6.3 The lipid adjusted concentration (C_{SAMPLE}) of an analyte is given by

$$(4) \quad C_{SAMPLE} = \frac{CONC}{TL} \times 102.6$$

Where, C_{SAMPLE} = the lipid adjusted concentration of an analyte;

TL (total lipid) = (2.27 x TCHOL + TRIG + 62.3); TCHOL=total cholesterol mg/dL and TRIG=triglycerides mg/dL

CONC = the concentration of the analyte as defined in equations (1) and (2)

102.6 = the average density of serum in g/dL.

8.6.4 Calculation of Detection and Quantification Levels

The standard deviation at any concentration level is an estimate of the expected precision at that level. Long-term standard deviations, estimated from multiple measurements of low-level standards, are plotted as a function of observed concentrations, and a straight line is fitted to the points using linear regression. The value for S_o, the estimate of the standard deviation as concentration approaches zero, corresponds to the intercept term of the linear equation. The limit of detection (LOD) is defined as LOD = $3S_o$ and is the lowest concentration level that can be determined to be statistically different from a blank. If S_o is not available, the concentration of the lowest standard used for calibration is considered the method detection limit or the LOD of the method. The detection limit (DL) values, based on standards, are calculated to correspond to weight corrected samples. When the detection limits of analytes in unknown specimens are adjusted for the lipid content of the specimen, the lipid adjusted DL values (LP_DL.) are obtained. When there is a significant amount of analyte in the blank sample, the LOD becomes the lowest concentration level that is statistically different from the blank.

$$(5) \quad LOD = 3*SD_{BLK}$$

where SD_{BLK} is the standard deviation of the of the analyte from multiple

measurements in blank samples.

8.6.5 The precision of a duplicate sample analysis (P_D) is given by

$$(6) \quad P_D = \frac{C_{SAMPLE1} - C_{SAMPLE2}}{C_{AVERAGE}} \times 100$$

where $C_{SAMPLE\ 1}$ = the lipid adjusted concentration of the first analysis of the sample;

$C_{SAMPLE\ 2}$ = the lipid adjusted concentration of the duplicate analysis of the sample; and

$C_{average}$= the average lipid adjusted concentration.

9. REPORTABLE RANGE OF RESULTS

9.1 Criterion for Calibration Standards.

The ion current responses for each mass of a particular analyte or primary internal standard must maximize to within ± 1 second of each other. The isotope ratio of the primary internal standards must fall within the confidence intervals established for each analyte [see Tables 6]. These confidence intervals are based upon the theoretical ratio of the ion masses. The recovery of the internal quantitation standards should be between 90% and 120%.

9.2 Criterion for Quality Control Sample.

The Division of Laboratory Sciences has established QC criterion that must be met before results may be reported. The Division has provided Quality Control programs in SAS that calculate the QC limits for each analyte and evaluate the QC data against those limits.

The ion current responses for each mass of a particular analyte or primary internal standard must maximize to within ± 1 second of each other. The ion current intensities for a particular analyte must three time the noise level [S/N=3]. The isotope ratio of the analyte and the primary internal standard must fall within the confidence intervals established for each analyte [see Table 6]. The recovery of the internal quantitation standards should be between 10% and 120%. The calculated concentration of each analyte for at least two QC samples per run must be within the 99% confidence intervals established for each analyte. The confidence intervals are periodically updated. Ten (10) values in a row above or below the mean, but all values within the 95% confidence intervals shall initiate a search for an assignable cause. For a given analyst, if QC values from two consecutive runs are above or below the 95% confidence intervals, or two QC values from 2 consecutive runs all above or below the 99% confidence limits, analysis unknown specimen is halted and a search for an assignable cause is initiated. Analysis is resumed only after appropriate corrective action has been taken. For a given analyst, if the range among the replicate QC results falls outside their established range for 2 consecutive runs, a search for an assignable cause is initiated.

9.3 Criterion for Unknown Specimen

The three blank samples and the three QC samples associated with each set of 30 samples must first give valid results. If one or more of the requirements are not met for the blank or at least 2 of the QC samples, then

the 24 unknown sample results cannot be reported. The ion current responses for each mass of a particular analyte or primary internal standard must maximize to within ± 1 second of each other. The ion current intensities for a particular analyte must be 3 times the noise level (S/N=3). The isotope ratio of the analyte and the primary internal standard must fall within the confidence intervals established for each analyte [see Table 6]. The recovery of the internal quantitation standards should be between 10% and 120%. The instrument resolving power ratio for each sample must be within the upper 99th percentile established for this ratio. The capillary column isomer specificity ratio for each sample must be within the 99% confidence intervals established for this ratio. The relative retention time of each analyte peak must be within four-parts-per-thousand (ppt) of the relative retention time as determined for each analyte in the calibration standards which were analyzed at the beginning of the analytical run.

10. SUMMARY OF QUALITY CONTROL (QC) PROCEDURES

Quality assurance of analytical measurements has two essential elements. The first is quality control (QC), which involves developing and adhering, to standard operating procedures for all aspects of method performance. The second is quality assessment (QA), which involves the use of techniques (e.g., control charts) to assess the quality of the measurement process and the results.

10.1 Quality Control

We have developed standard operating procedures that provide detailed instructions for all aspects of data and sample handling, sample cleanup, and mass spectrometry. See section 9.

10.1.1 Multipoint calibration curves

A series of analytical standards (usually 6-10 analyses for each standard) are used to establish linear calibration curves for each analyte using the isotope-dilution technique. The standards are analyzed in ascending order at the beginning of each analytical run. The log transformed regression model ($y = a*b^X$) is used. The log transformed slope ranges from 0.97 to 1.03 and the log transformed intercepts range from -0.1 to +0.25.

10.1.2 Blanks (Bench Controls).

Three laboratory method blanks are prepared along with every 24 unknown samples and inserted into positions 1, 15, and 25 of each analytical run of 30 samples. The method blank is prepared by performing all the steps outlined in the procedure with the same reagents, spiking standards, equipment, apparatus, glassware, and solvents that are used for a sample analysis.

10.1.3 Control samples (Blind Controls)

Control samples are prepared by mixing large bulk pools of human or bovine serum and dispensing this bulk material into various sized aliquots for storage at -70ºC. These control materials are characterized over several weeks until there are at least 20 analyses of the pooled material that have processed by each analyst in cleanup and analyzed on each GC/MS. QC samples are inserted into positions 10, 20 and 30 of an analytical run of 24 unknown samples. QC charts are constructed for each analyte in the control pool, using the Division QC programs. The results from the analysis of individual samples from these pools are used to give a measure of precision from analytical run to analytical run over an entire study. For QA/QC purposes measurement of a target analyte in a set of samples was considered valid only after the QA/QC sample had fulfilled the following criteria: (i) the measurement of the target analyte in the QA/QC sample must not fall outside

the interval defined as plus/minus three standard deviations of the established mean of the QA/QC samples and (ii) ten or more consecutive measurements of the QA/QC sample may not fall above or below the established mean of the QA/QC samples after one QA/QC sample has failed criteria (i). Further, every measurement of a set of samples must fulfill the following criteria to be considered a valid measurement: (i) the ratio of the two ions monitored for every analyte and ^{13}C-labeled internal standard, must not deviate more than 20% from the theoretical value, (ii) the ratio of the retention time of the analyte over its corresponding ^{13}C-labeled internal standard must be within the range 0.99 – 1.01. For analytes that do not have an identical ^{13}C -labeled internal standard (IS), the ratio to the IS used may not deviate more than 1% from the average of the same ratio of the calibration standards analyzed in the same analytical run; and (iii) the measured recovery of the internal standard must be within the range 10-120%.

10.1.4 Duplicate sample analysis.

If the study protocol requires external blind duplicate samples on a subset of study samples, they are inserted "blind" into different analytical runs. The identity of this sample is "blind" to the laboratory and analyst. The precision is calculated as described in 8.6.5.

101.5 Proficiency Testing.

We participate in AMAP Ring Test for Persistent Organic Pollutants in Human Serum (Arctic Monitoring and Assessment Programme). There are 3 cycles/year consisting of 3 serum samples that have been spiked with the most common and most persistent PCBs, chlorinated pesticides and other organic pollutants in the Arctic environment. Results from each participating laboratory are compared to the theoretical concentrations in each sample based upon the weight of the compound added to a known volume of serum. For further information on AMAP see the Institut national de sante publique du Quebec Canada (INSPO) website (http://www.inspq.qc.ca/).

Twice a year, the AMAP samples are analyzed on all of the mass spectrometers that are used to analyze unknown specimen. The results are compared to insure that all the spectrometers are giving the same results. The correlation coefficient between mass spectrometers must be 0.95 or better.

10.1.6 Absolute recoveries of the internal quantitation standards.

The absolute recoveries of the ^{13}C-labeled internal quantitation standards are determined by comparing their responses with the recovery standard ($^{13}C_6$-1,2,3,4-TCDD), which is added just before mass spectral analysis. After analyzing more than 5,000 serum samples, we believe that absolute recoveries of the ^{13}C -labeled internal quantitation standards as low as 10% will still give valid quantitation. Recoveries above 120% (100% + coefficient of variability (CV)) may indicate potential interferences or an error in spiking the internal standards.

10.1.7 Mass spectrometer resolving power.

To separate the (P+6) ion of $^{13}C_6$-1,2,3,4-TCDD (m/z 331.9078) and the ion of $^{13}C_{12}$-2,3,7,8-TCDD (m/z 331.9368) requires > 11,400 resolving power (RP). Therefore, at 10,000 RP, the ratio of the peak on the $^{13}C_{12}$-2,3,7,8-TCDD (m/z 331.9368) channel which is due to $^{13}C_6$-1,2,3,4-TCDD, to the peak on the $^{13}C_6$-1,2,3,4-TCDD (m/z 331.9078) channel can be used as a QA parameter. A QC chart can be constructed with upper 99th and 95th percentiles to ensure that the mass spectrometer remains at 10,000 RP during the analysis of each sample. The RP ratio progressively increases as the number of analyses increases. We have found that this QC chart can be used to gauge the mass

spectrometers cleanliness. After an instrument bake out, the absolute magnitude of the RP ratio decreases.

10.1.8 Isotope ratio.

The analytical standards (Tables 5) can be used to determine the isotope ratios for the ^{13}C-labeled internal standards as well as for the unlabeled analytes over a range of concentrations. A QC chart can be constructed for each of these analytes with upper and lower 99% and 95% confidence intervals (See Table 6 for theoretical isotope ratios and confidence limits.)

10.3 Summary of Quality Assurance Functions.

All the QA functions outlined above have options that allow each PCB congener and chlorinated pesticide to be examined individually. Further, individual analysts, mass spectrometer operators, cleanup apparatus, time periods, and studies can also be monitored. Overall the quality assurance functions are used to document that the analytical measurement system is in statistical control. All quality assurance criteria have been incorporated into a Division wide computer program that is used by the Division statistician to review the final data. This program identifies those variables that do not meet specifications.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

For a given analyst, if QC values from two (2) consecutive runs are above or below the 95% confidence intervals, or two QC values from (2) consecutive runs all above or below the 99% confidence limits, analysis of new runs of unknown specimens is halted and a search for an assignable cause is initiated. Analysis is resumed only after appropriate corrective action has been taken. If additional serum is available, the specimen will be processed through cleanup and re-analyzed by HRGC/HRMS. Otherwise, the data from the unknown specimens cannot be reported.

12. LIMITATIONS OF METHOD

12.1 Potential Method Interferences. Some of the PCBs that are found in the environment but rarely found in human samples may co-elute with some of the PCBs reported in NHANES. The confirmed co-eluting PCBs are listed in Table 5.

Table 8 Potential PCB Interferences

NHANES Reported PCB	Verified Co-elutions on NHANES DB5-MS System
PCB138	PCB 158, 160,163,164
PCB153	PCB 132
PCB170	PCB190
PCB196	PCB-203

12.2 Potential Method Contamination.

The main sources of contamination seem to come from the environment. Sealants used in construction of new buildings sometimes out-gas lower chlorinated PCBs. Mud and dust from soil that has been contaminated with PCBs and pesticides in the past can enter the building on people's shoes or be blown in by the wind. Regular damp mopping and dusting minimizes the problems with dust and dirt.

13. REFERENCE RANGES (NORMAL VALUES)

Reference ranges for PCB and chlorinated pesticides have not been determined in a representative sample of the U.S. population, prior to NHANES 1999-2000 and NHANES 2001-2002. The "Third National Report on Human Exposure to Environmental Chemicals" gives the percentiles of serum concentrations for 22 PCB congeners and 11 chlorinated pesticides measured in NHANES 1999-2000 and 37 PCBs and 13 pesticides in NHANES 2001-2002(Web site: www.cdc.gov/exposurereport. The concentrations of some of the compounds were below their detection limits in most samples. NHANES 2003-2004 was done with larger sample sizes for PCBs, which resulted in lower the detection limits. Weighted 40g pools were prepared from the NHANES 2005-2006 specimen in order to estimate the concentrations of PCBs and pesticides in the U.S. population. The pooling was based on age, sex, and ethnicity.

14. CRITICAL CALL RESULTS

The human health effects resulting from exposure to PCBs and chlorinated pesticides are currently unclear. Therefore, no "panic values" have been established.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens will reach and maintain ambient temperature during analysis. If the sample preparation is to be delayed until the next day, samples should be refrigerated overnight. If the delay is longer than overnight, the sample should be refrozen at -20 ºC or below.

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

The congener specific analysis of PCBs in serum and at the parts-per-billion levels is a complex measurement. The alternative method for this analysis and the analysis of chlorinated pesticides is gas chromatography with an electrochemical detector (ECD). This method is very sensitive for chlorinated compounds but does not have the specificity of a mass spectrometer. If the analytical system fails, storage of the samples at -30ºC is recommended until the analytical system is again operational. Monitoring of serum samples which have been stored at -30 ºC for more than 5 years, indicates that the samples may be safely stored for this period of time.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE

Once the data has met the QC/QA criteria copy established by the division and has been approved by the statistician, a hardcopy (ASCII format) and an

electronic copy (EXCEL) of the data will be generated. 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). Once approved at the division level, they will be sent to the contact person who requested the analyses.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

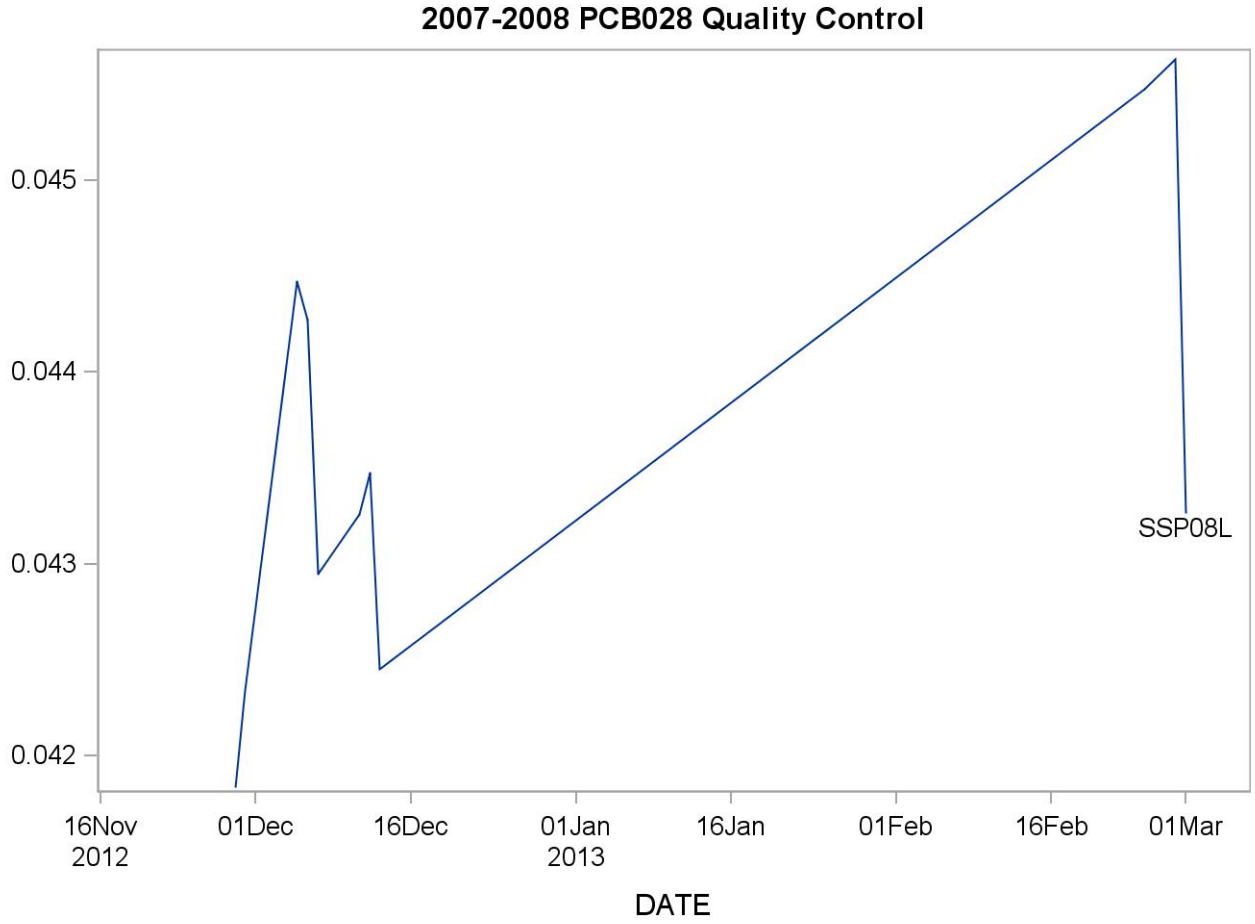
The sample remaining after the analysis, should be returned to storage a -30°C. Standard record keeping means (database, sample logs, optical disc files) are used to track specimens. Records are maintained for three (3) years, including related QA/QC data; duplicate records are kept in electronic format. All personal identifiers should be available only to the medical supervisor to maintain confidentiality. The various forms and specimen accountability and tracking are outlined in Section 3.

19 SUMMARY STATISTICS AND QC GRAPHS

See following pages.

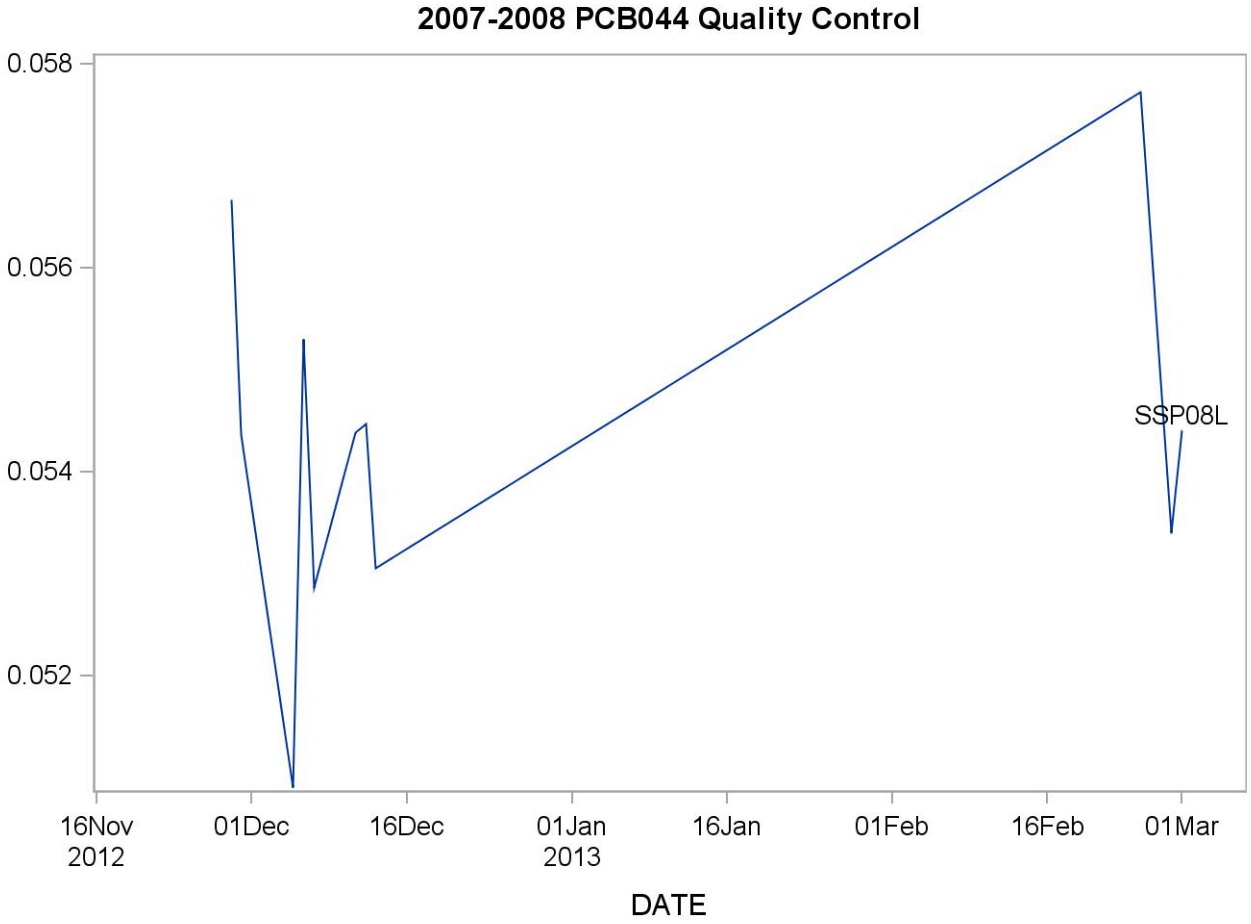
Summary Statistics for PCB028

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08L	18	29NOV12	01MAR13	0.04	0.00	3.4



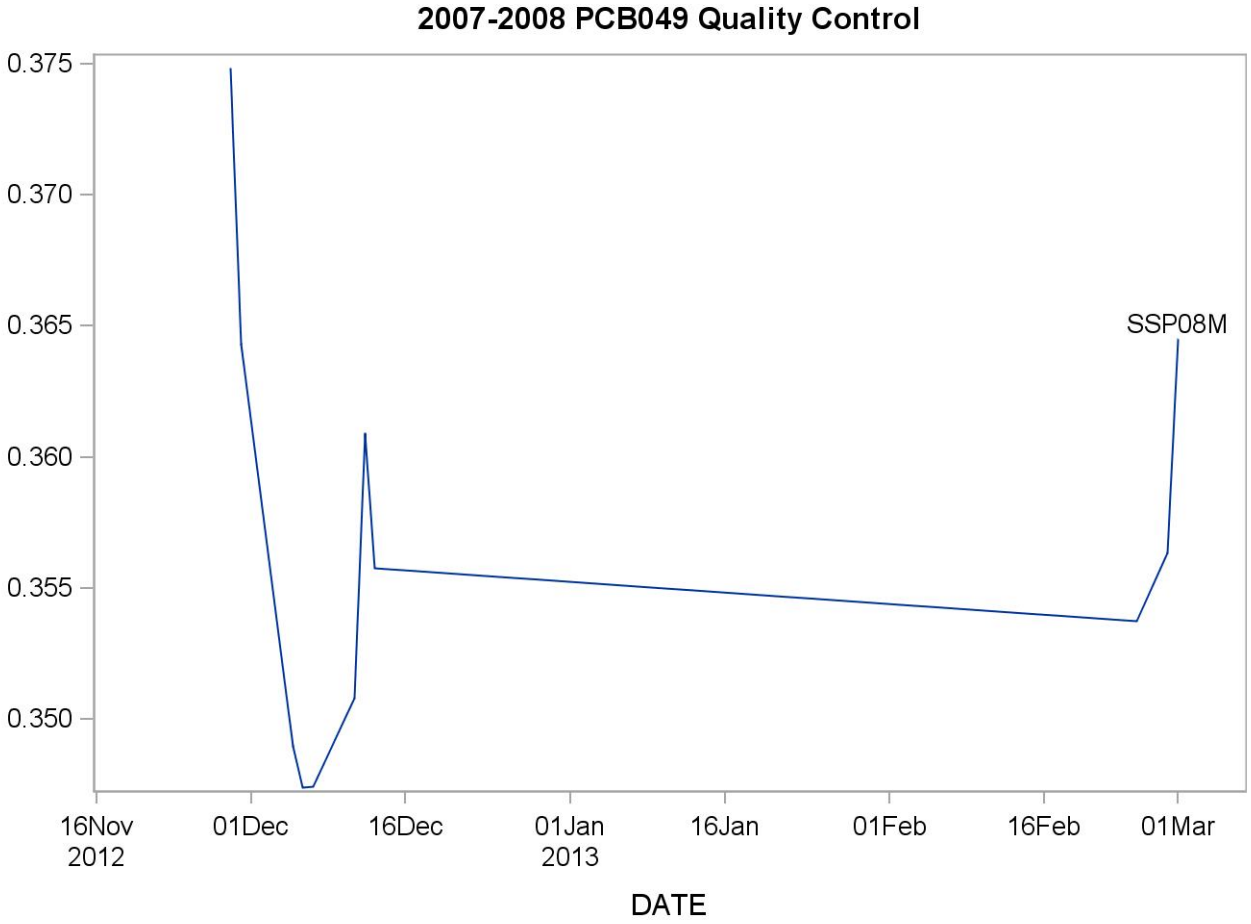
Summary Statistics for PCB044

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08L	14	29NOV12	01MAR13	0.05	0.00	4.7



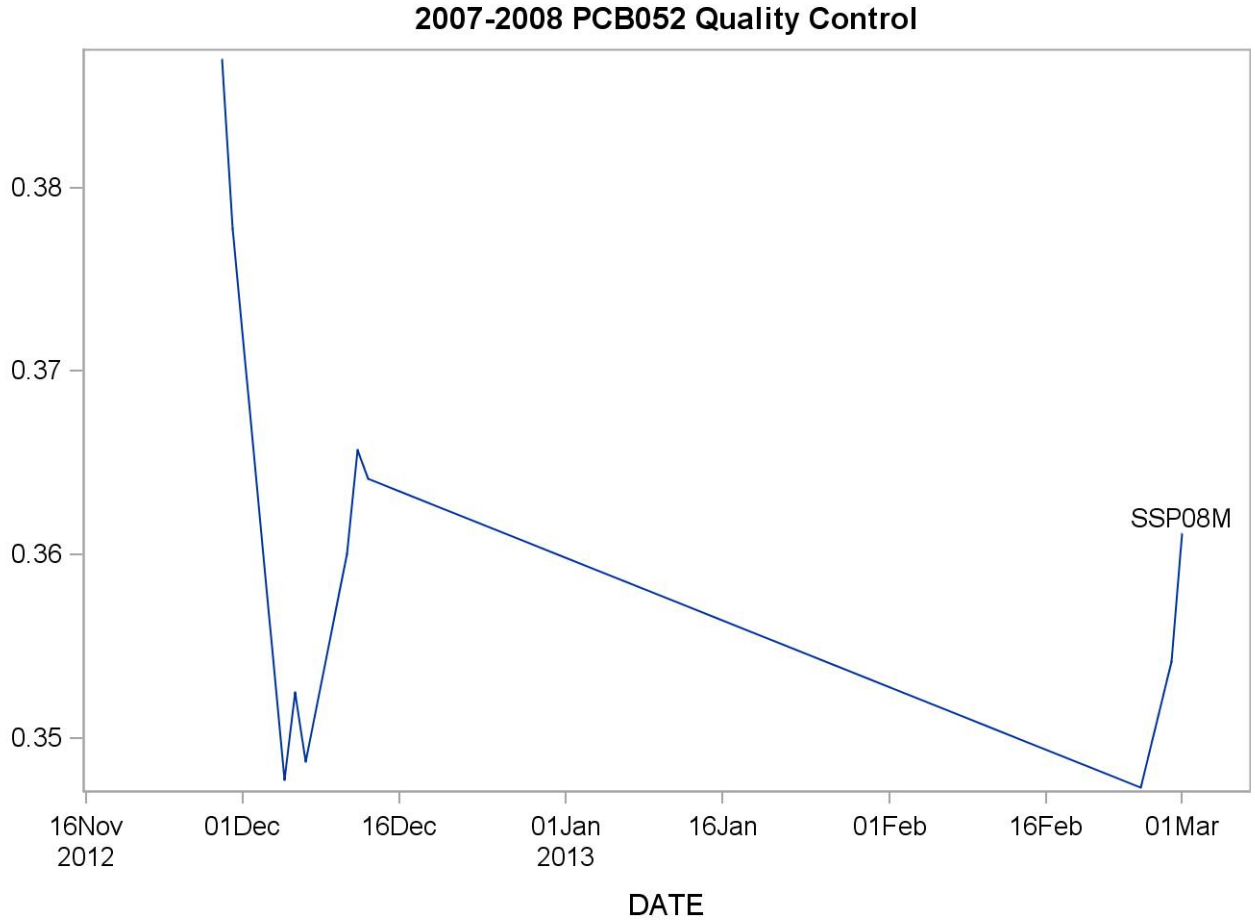
Summary Statistics for PCB049

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	21	29NOV12	01MAR13	0.36	0.01	3.4



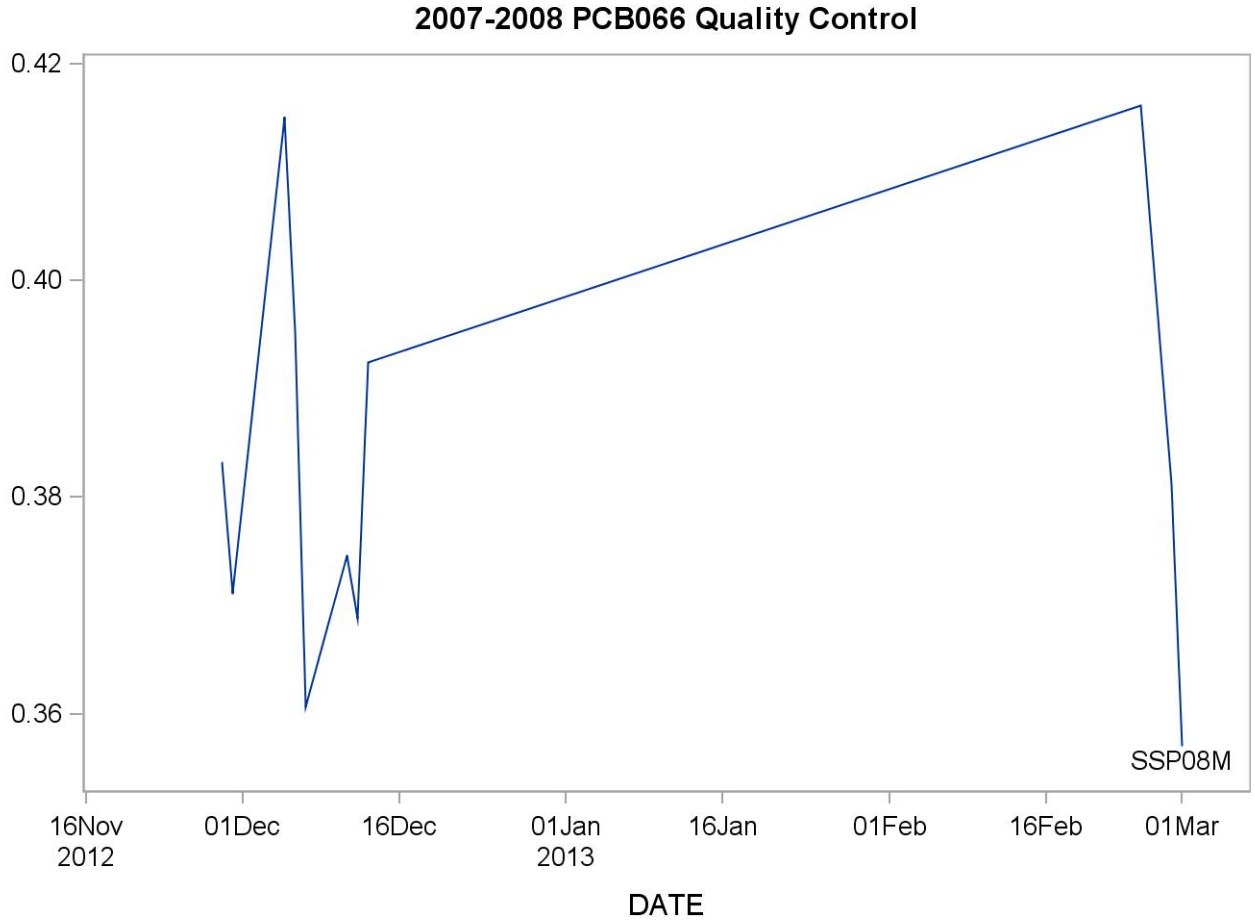
Summary Statistics for PCB052

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.36	0.02	5.3



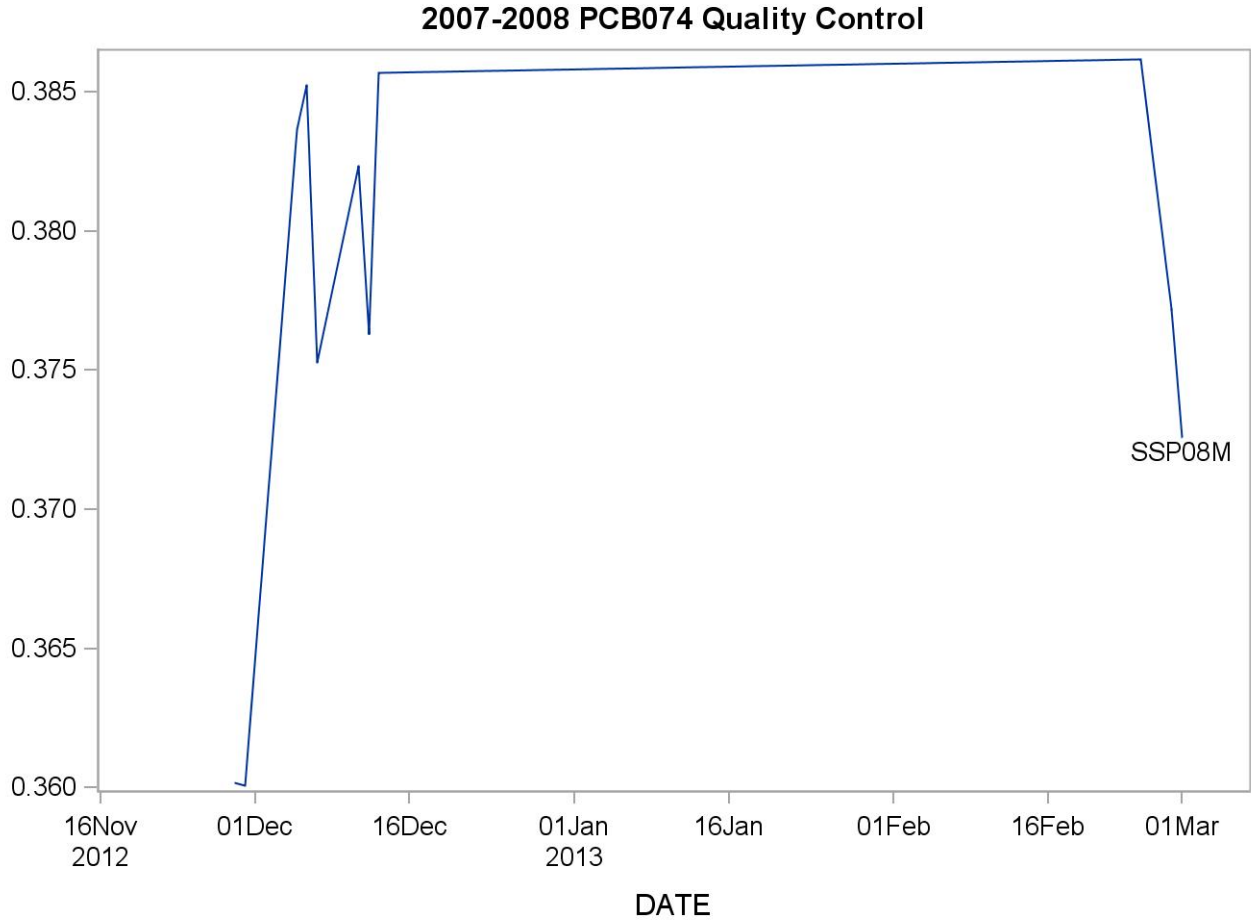
Summary Statistics for PCB066

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	14	29NOV12	01MAR13	0.38	0.02	5.5



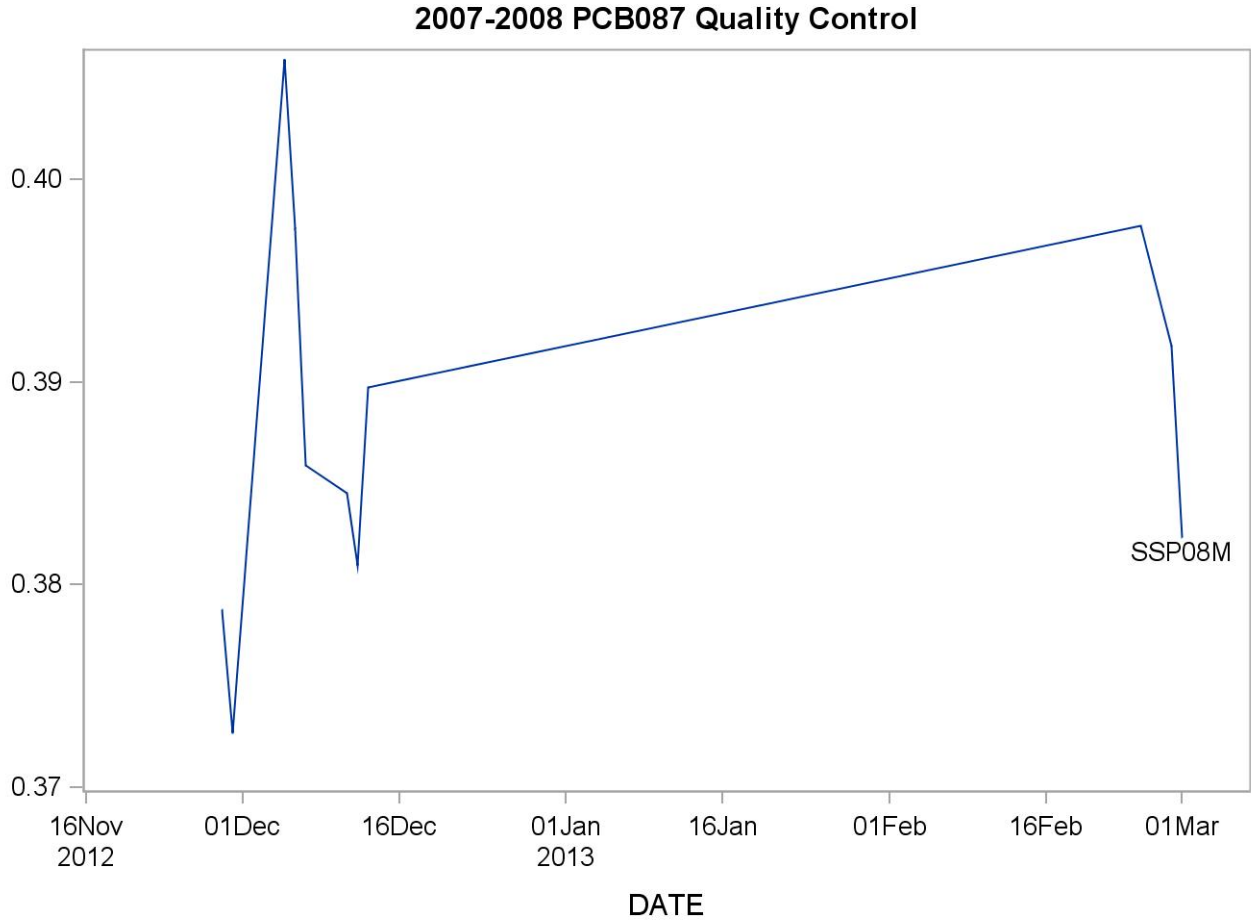
Summary Statistics for PCB074

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	20	29NOV12	01MAR13	0.38	0.01	3.3



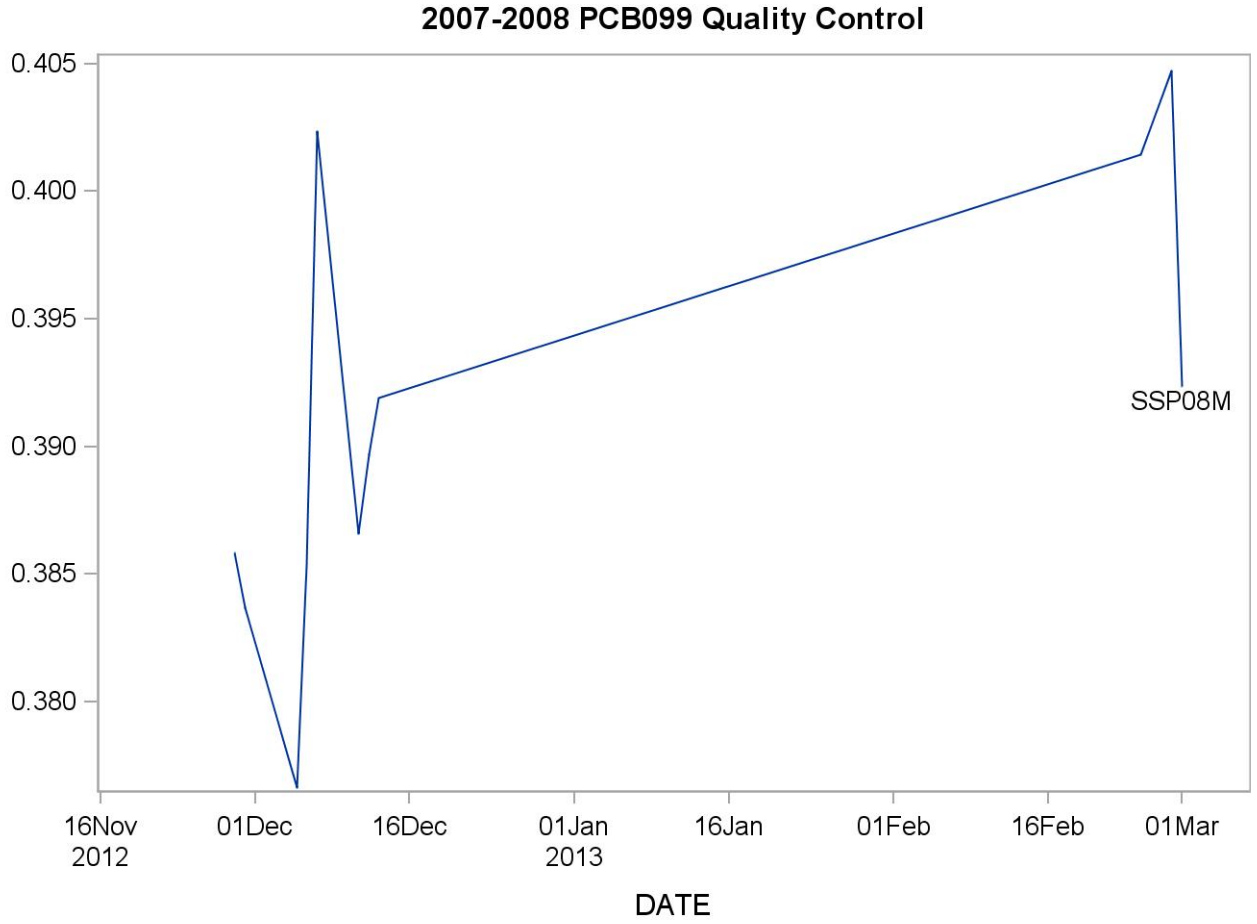
Summary Statistics for PCB087

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.39	0.01	3.1



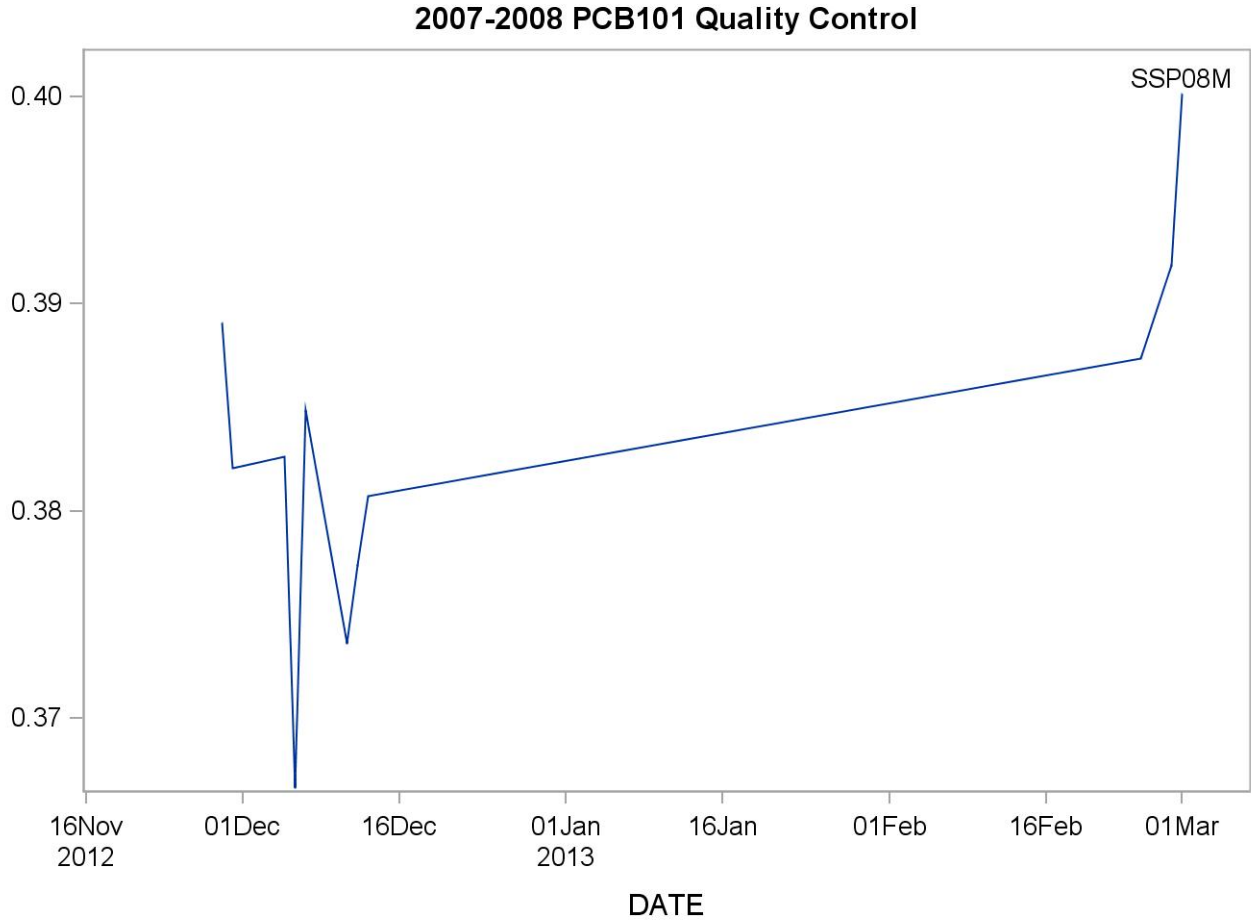
Summary Statistics for PCB099

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	21	29NOV12	01MAR13	0.39	0.01	2.8



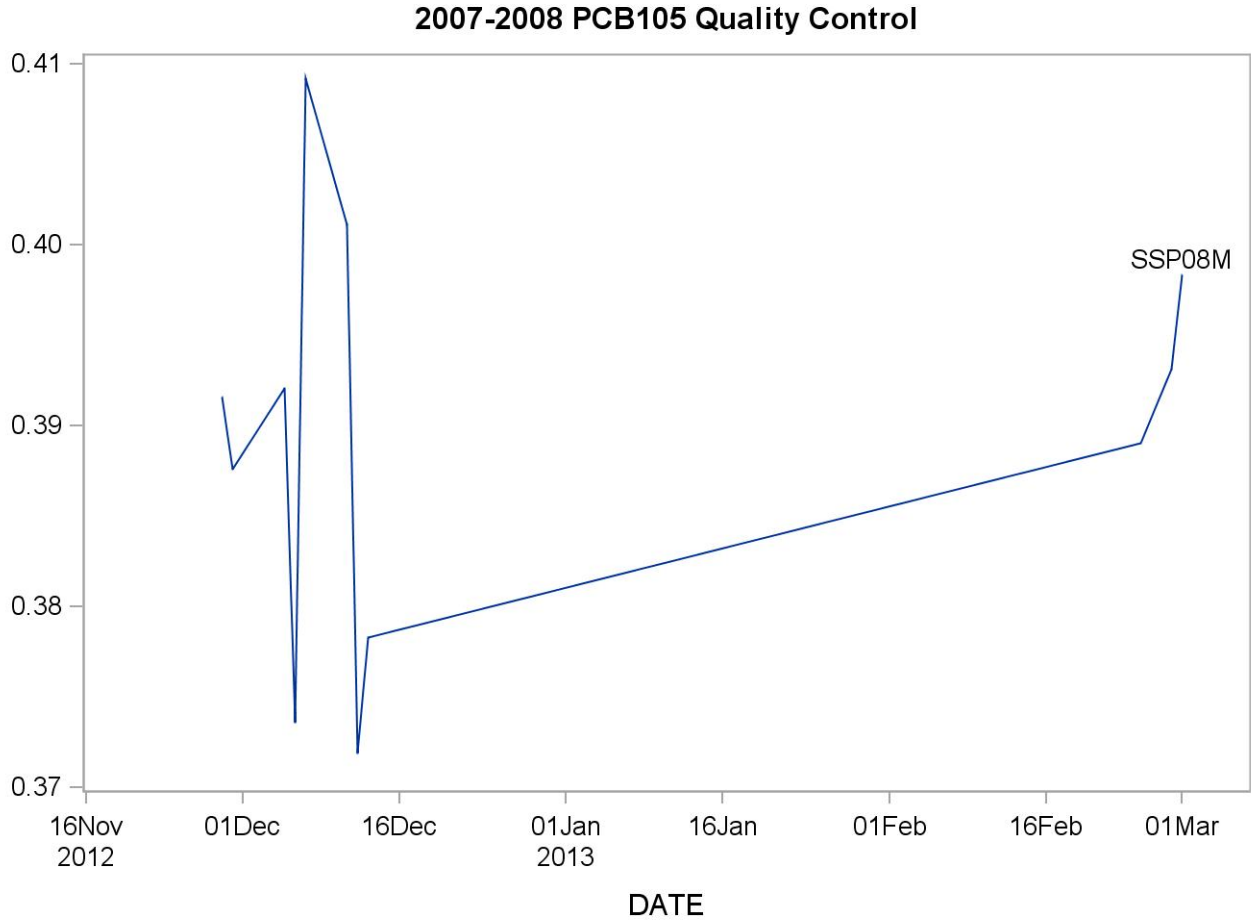
Summary Statistics for PCB101

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	20	29NOV12	01MAR13	0.38	0.01	3.5



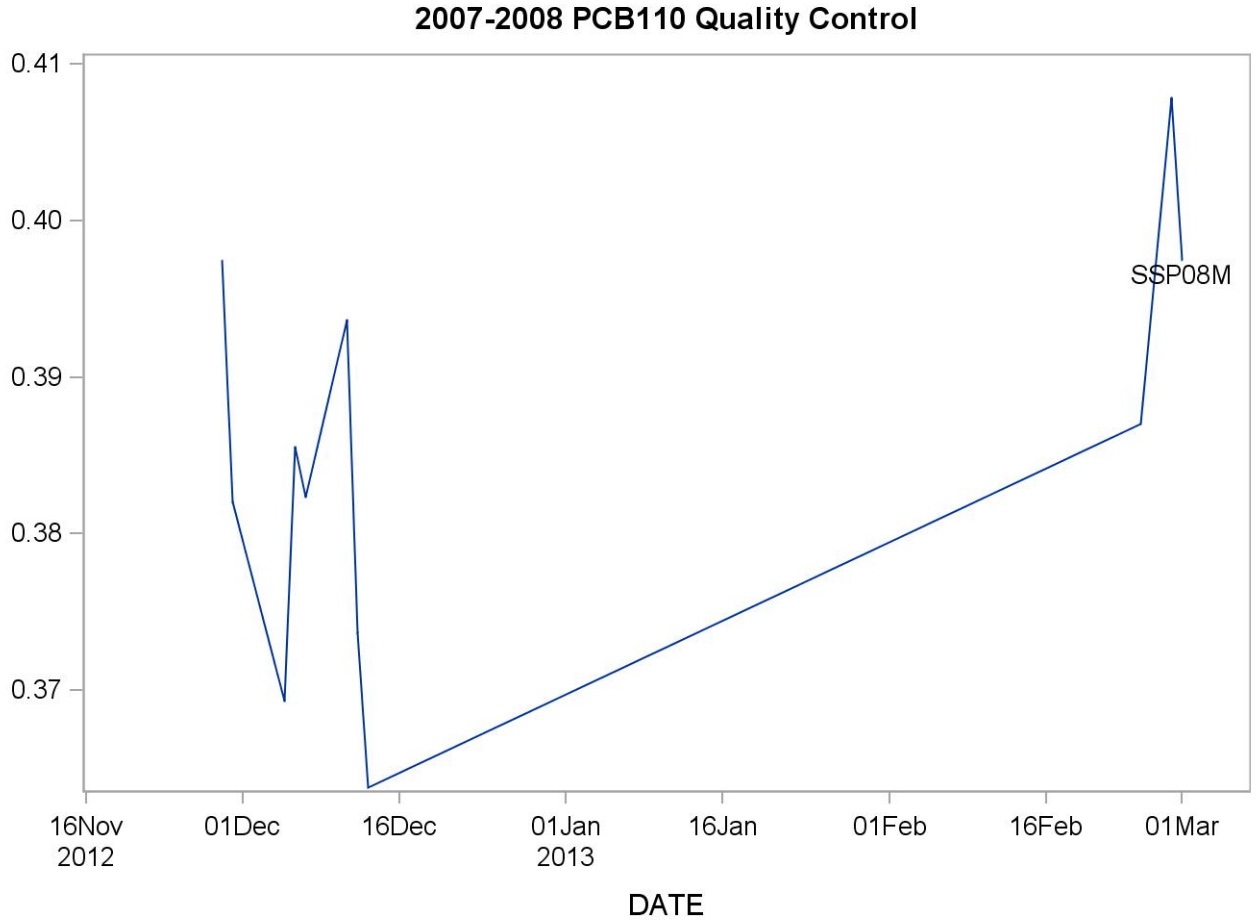
Summary Statistics for PCB105

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.39	0.02	5.3



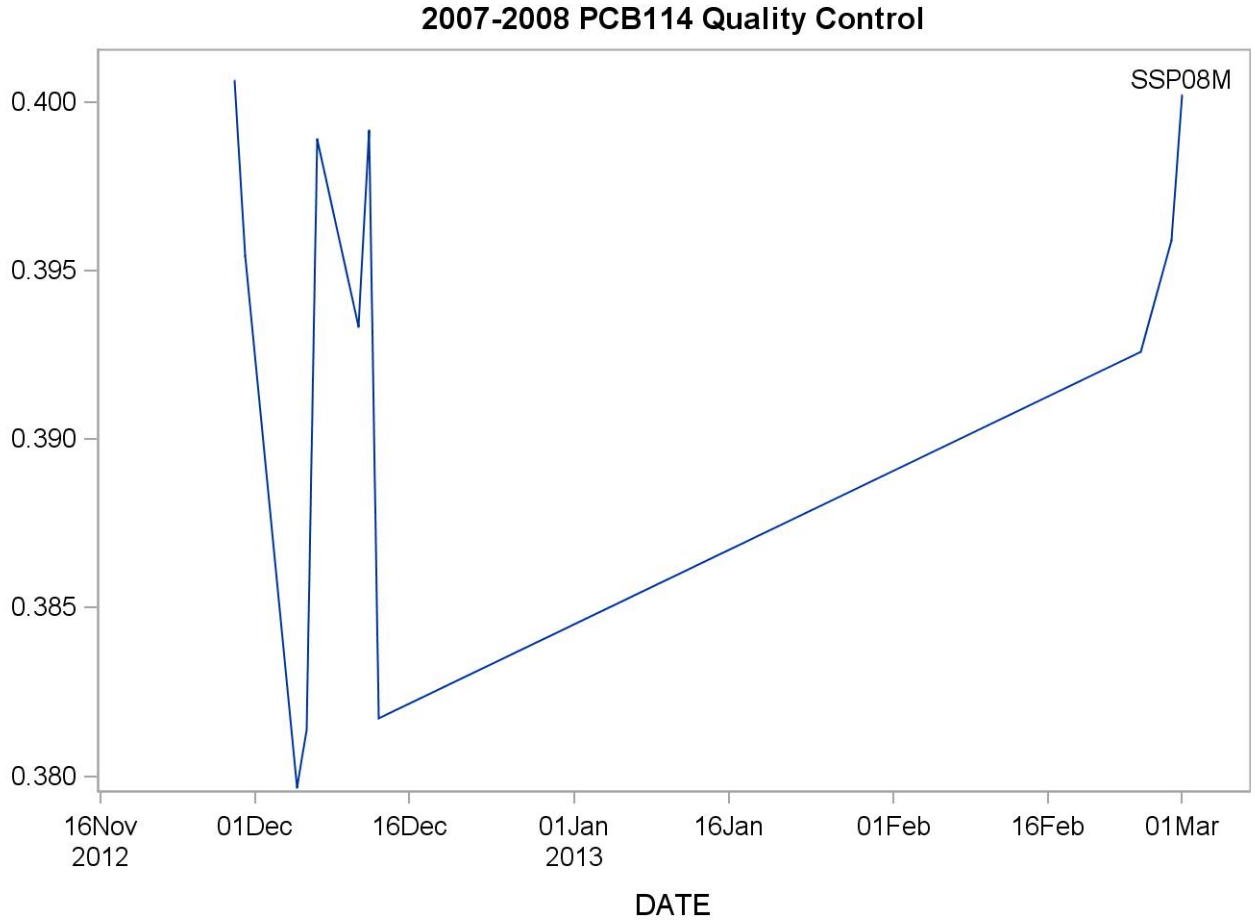
Summary Statistics for PCB110

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	23	29NOV12	01MAR13	0.39	0.02	4.1



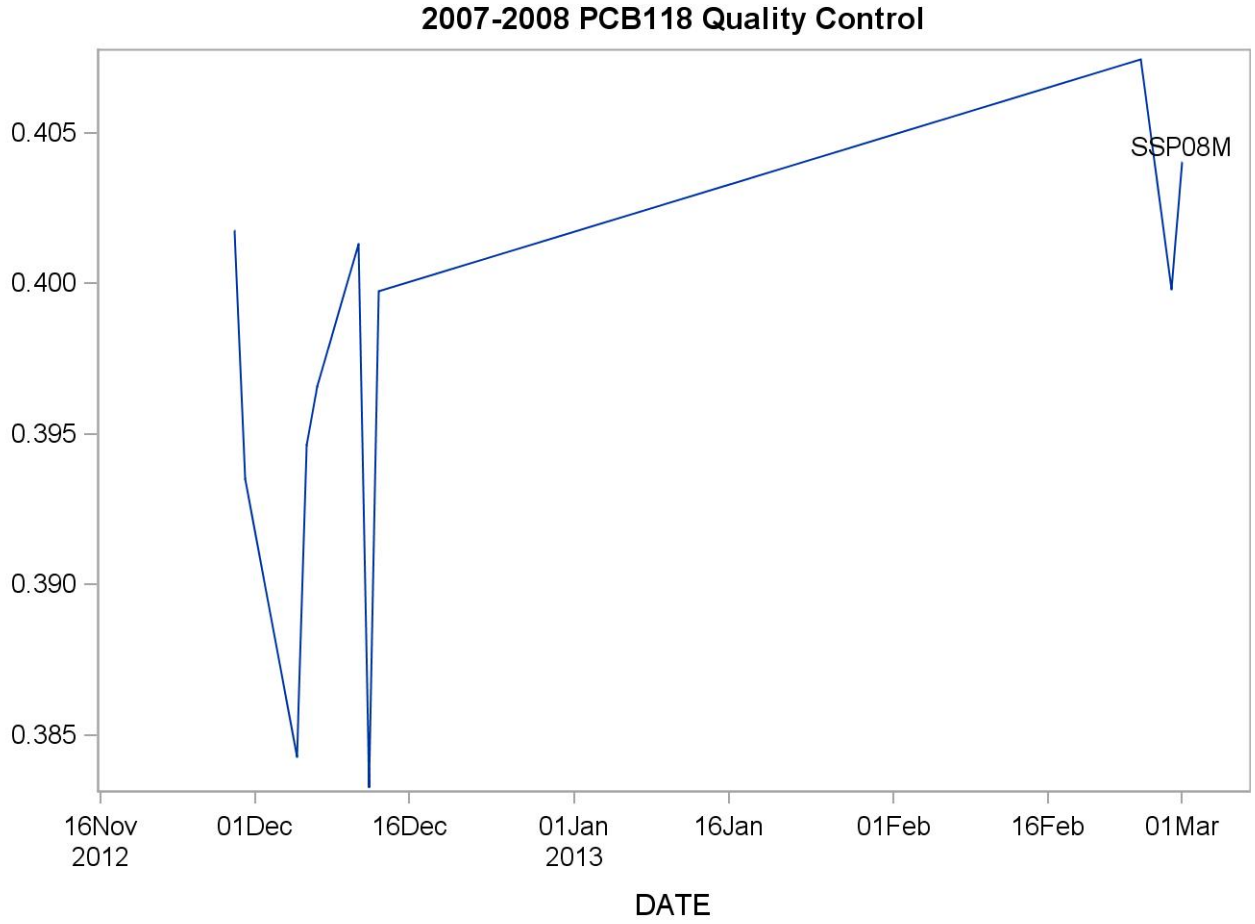
Summary Statistics for PCB114

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.39	0.01	2.8



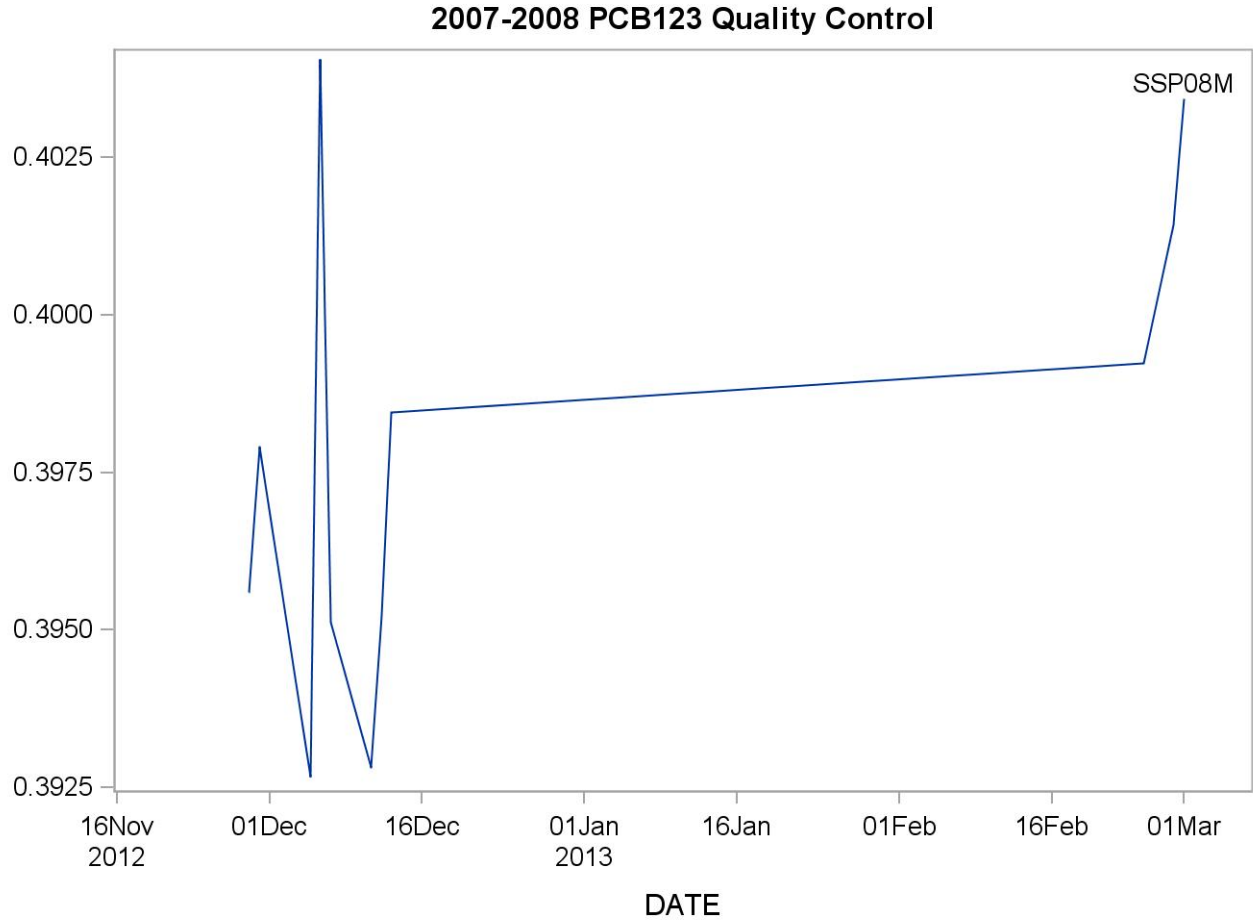
Summary Statistics for PCB118

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	23	29NOV12	01MAR13	0.40	0.01	2.1



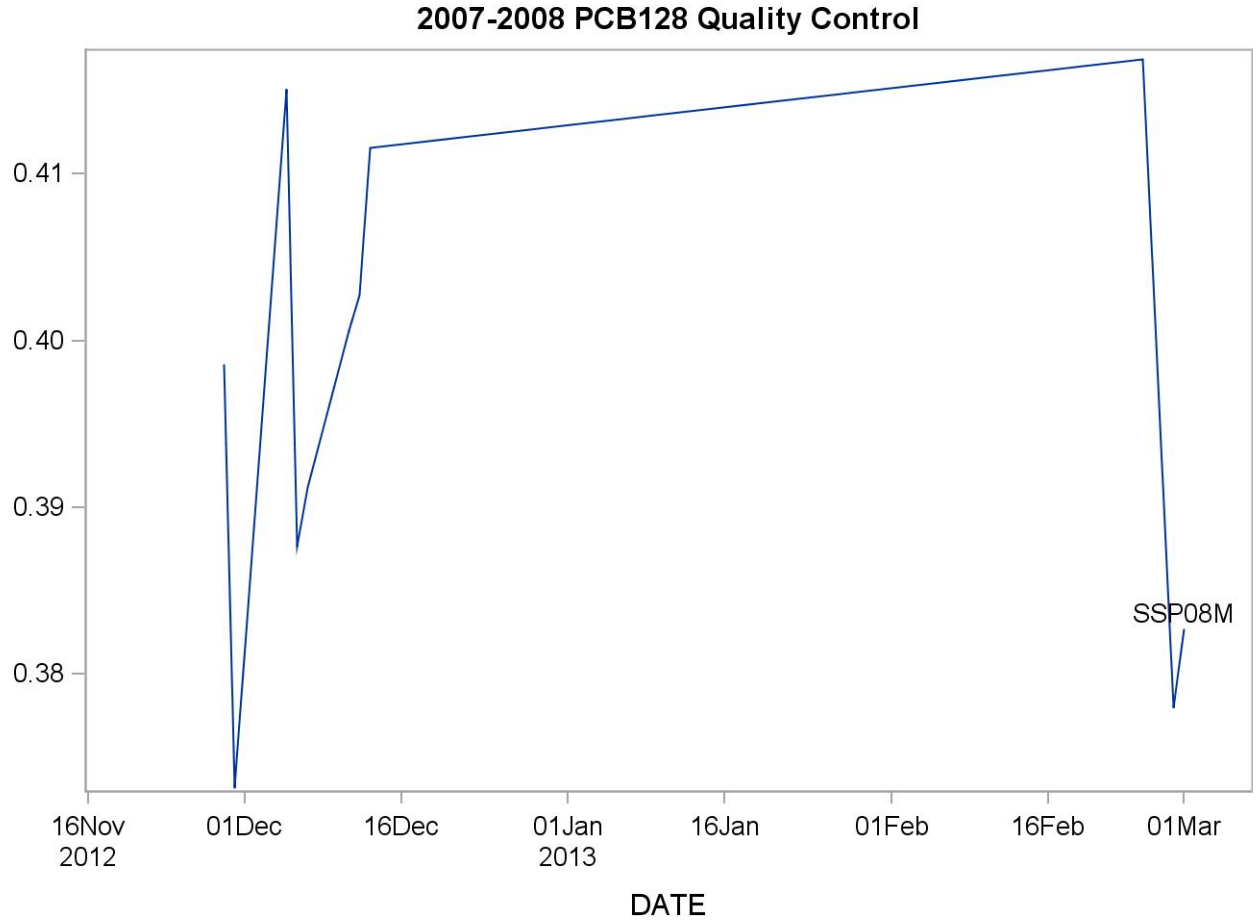
Summary Statistics for PCB123

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	15	29NOV12	01MAR13	0.40	0.01	2.1



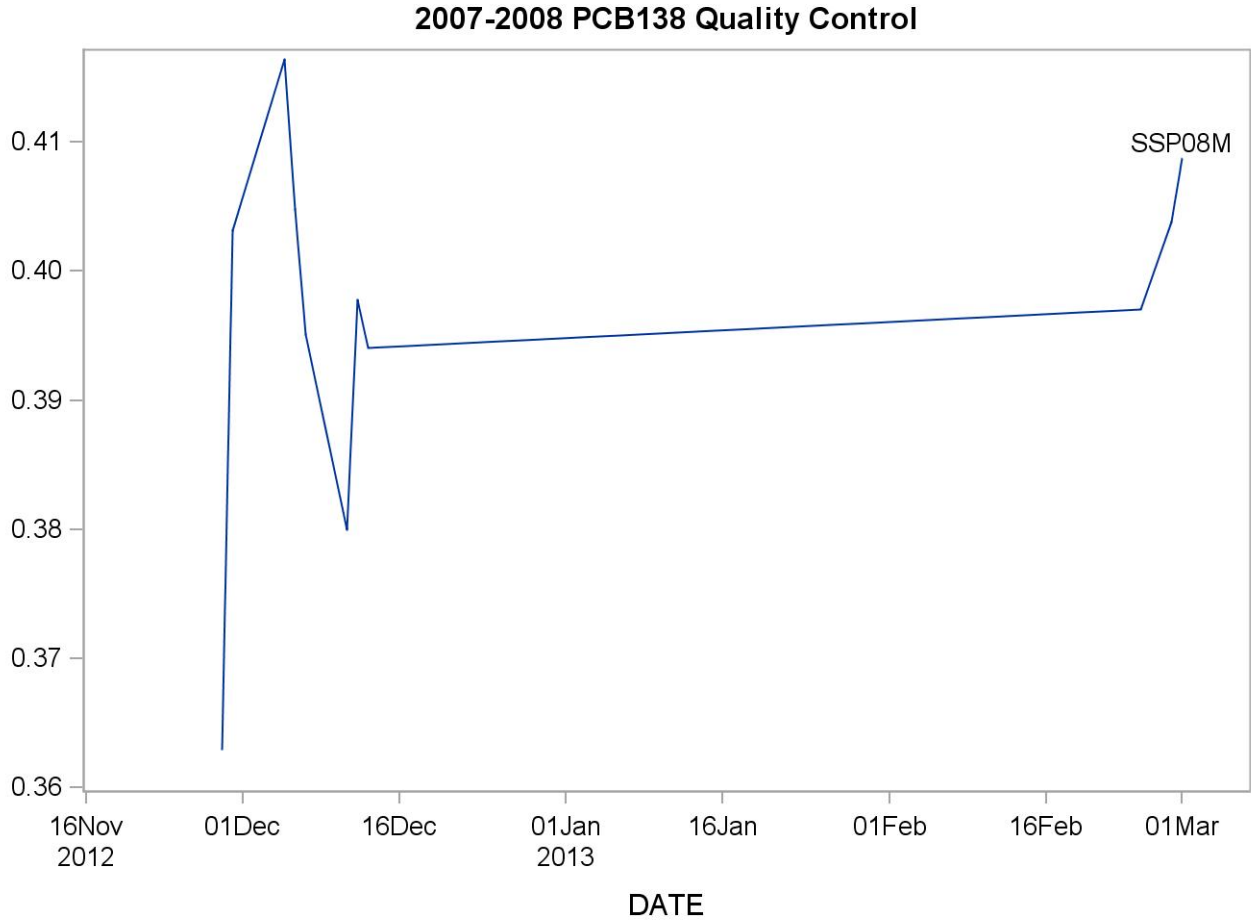
Summary Statistics for PCB128

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	13	29NOV12	01MAR13	0.39	0.02	5.2



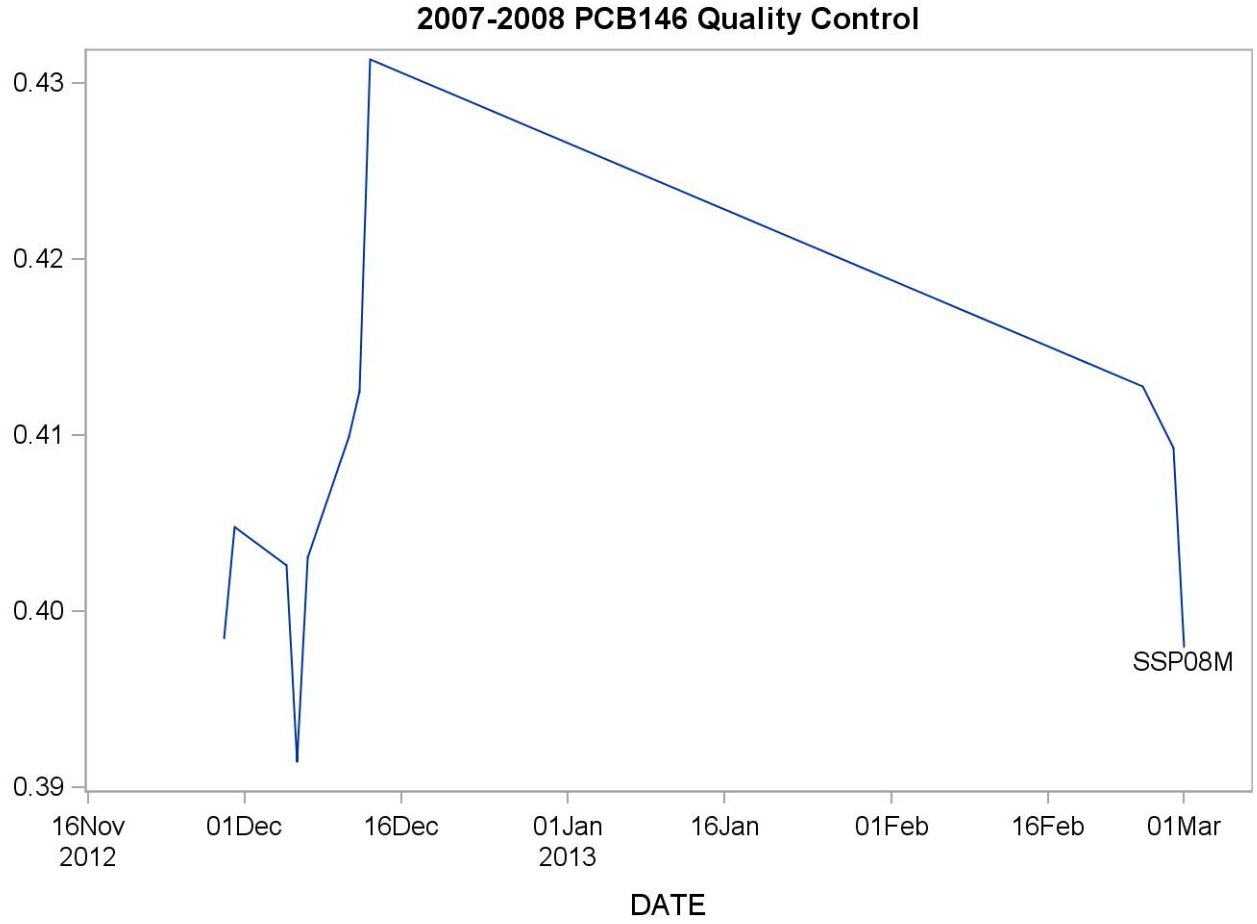
Summary Statistics for PCB138

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	21	29NOV12	01MAR13	0.40	0.01	3.6



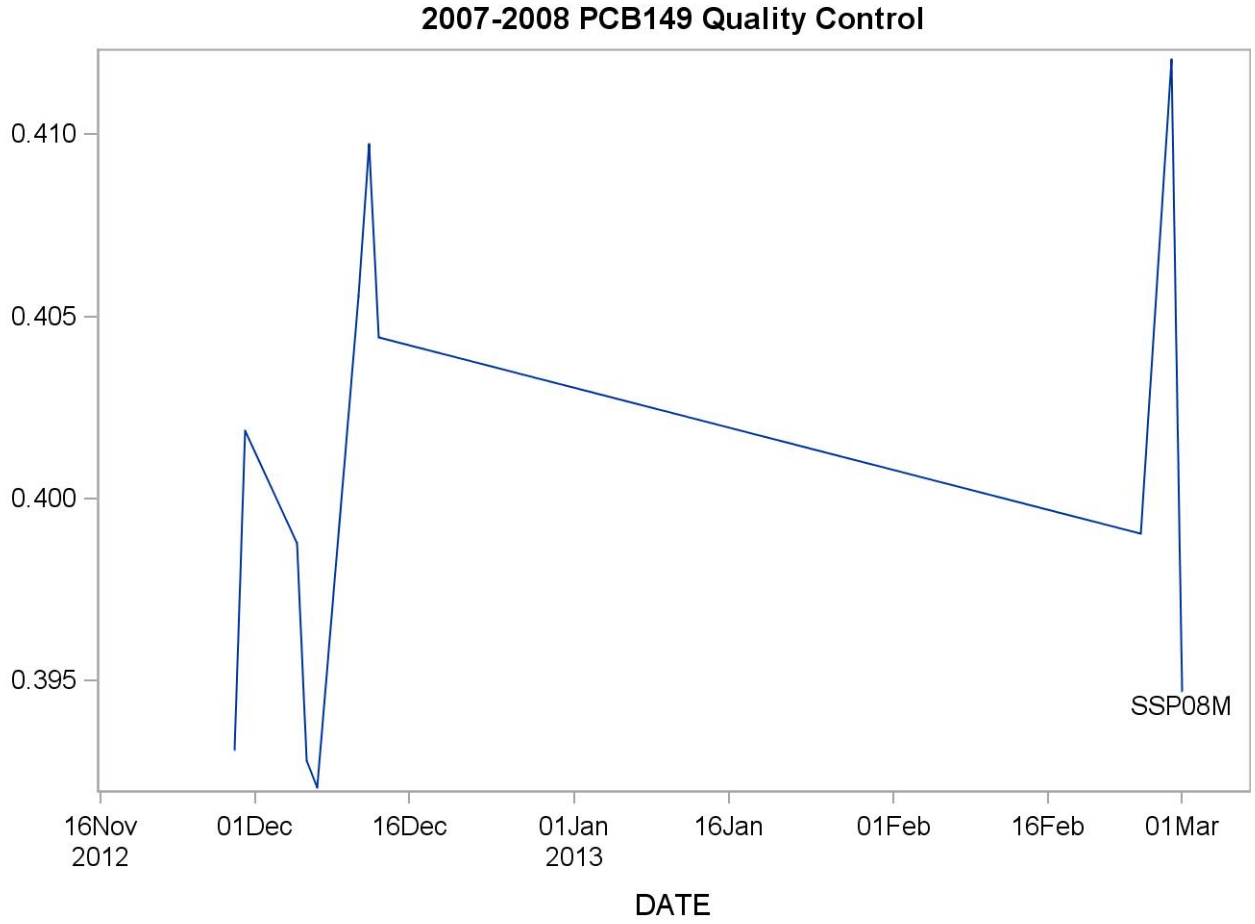
Summary Statistics for PCB146

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.41	0.01	3.6



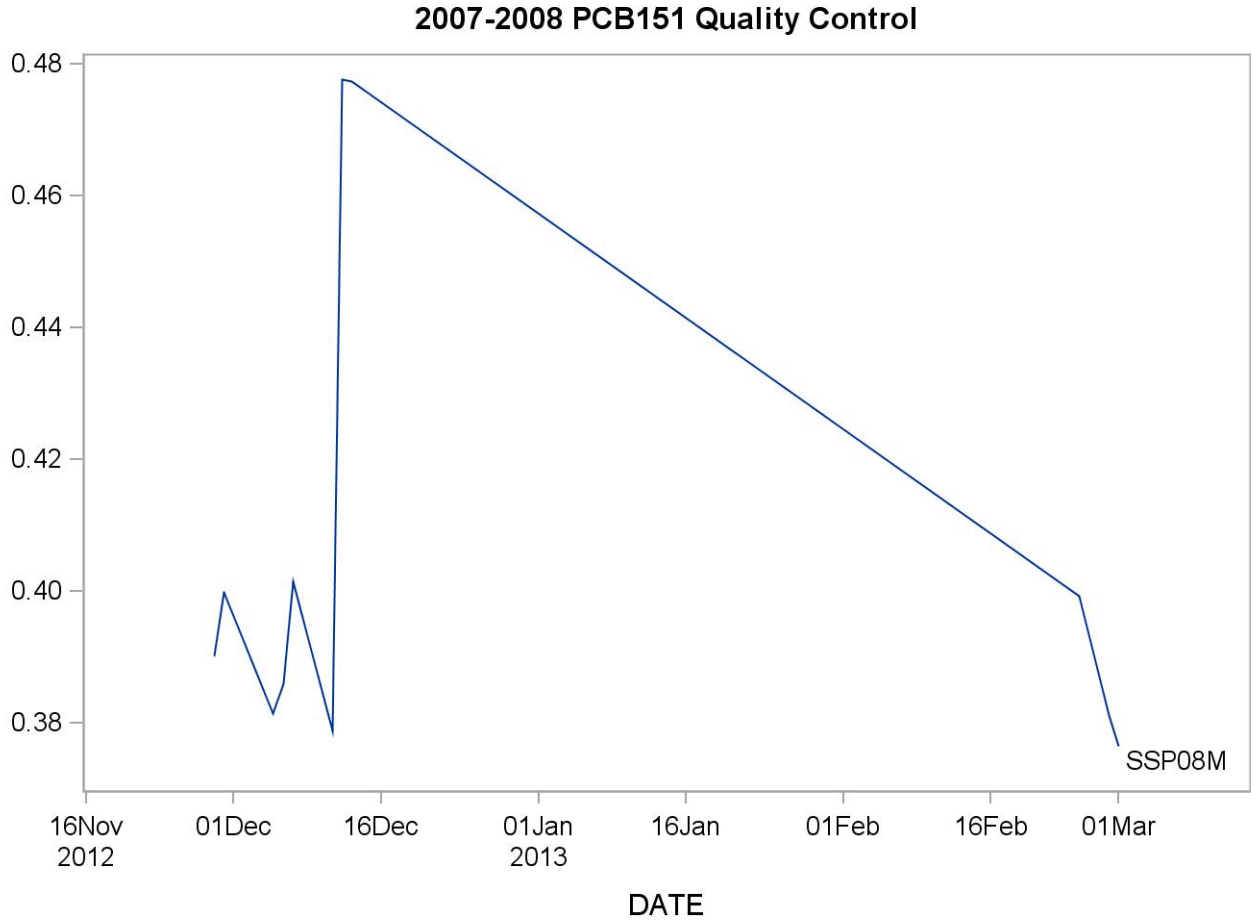
Summary Statistics for PCB149

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.40	0.01	2.7



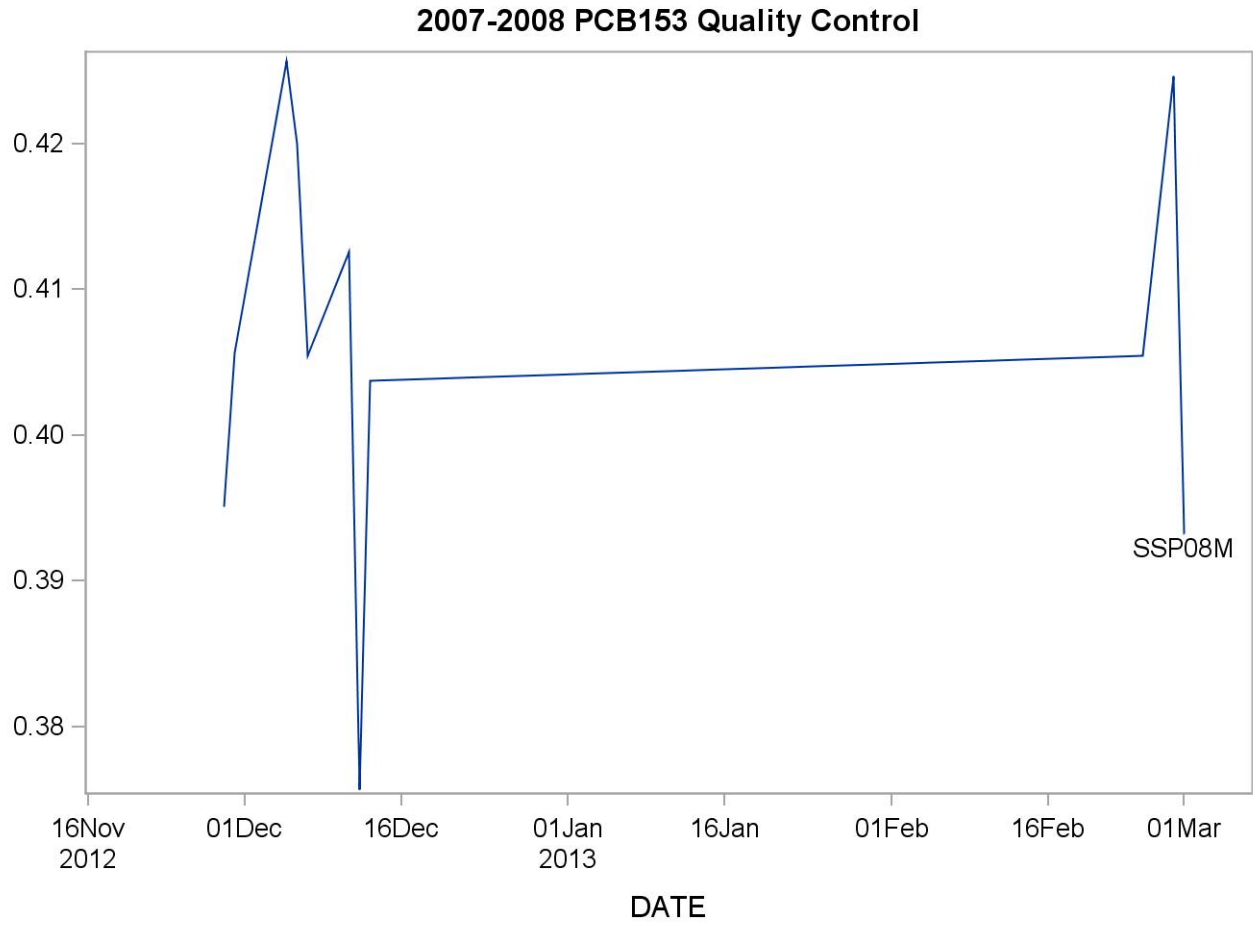
Summary Statistics for PCB151

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	13	29NOV12	01MAR13	0.40	0.04	9.2



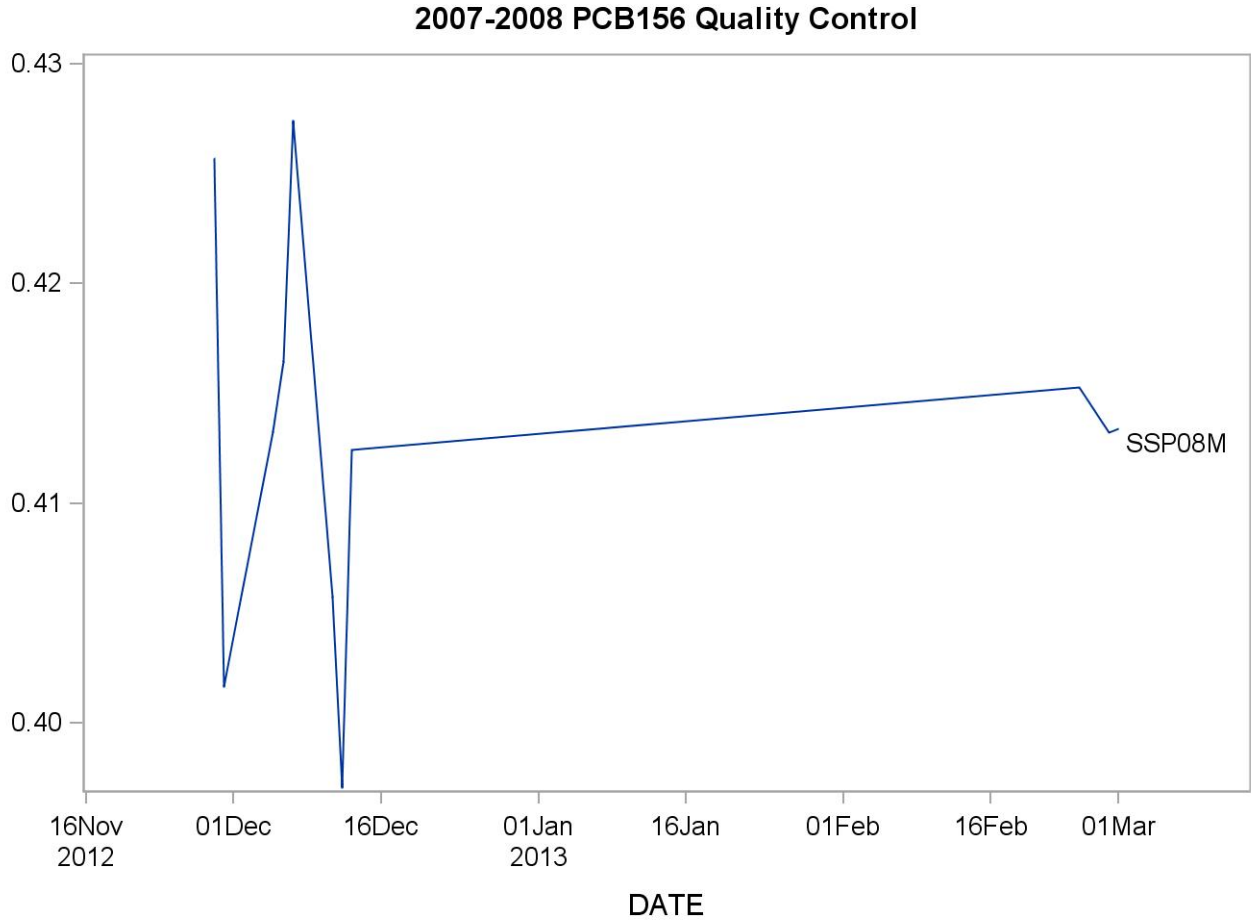
Summary Statistics for PCB153

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	13	29NOV12	01MAR13	0.41	0.02	4.6



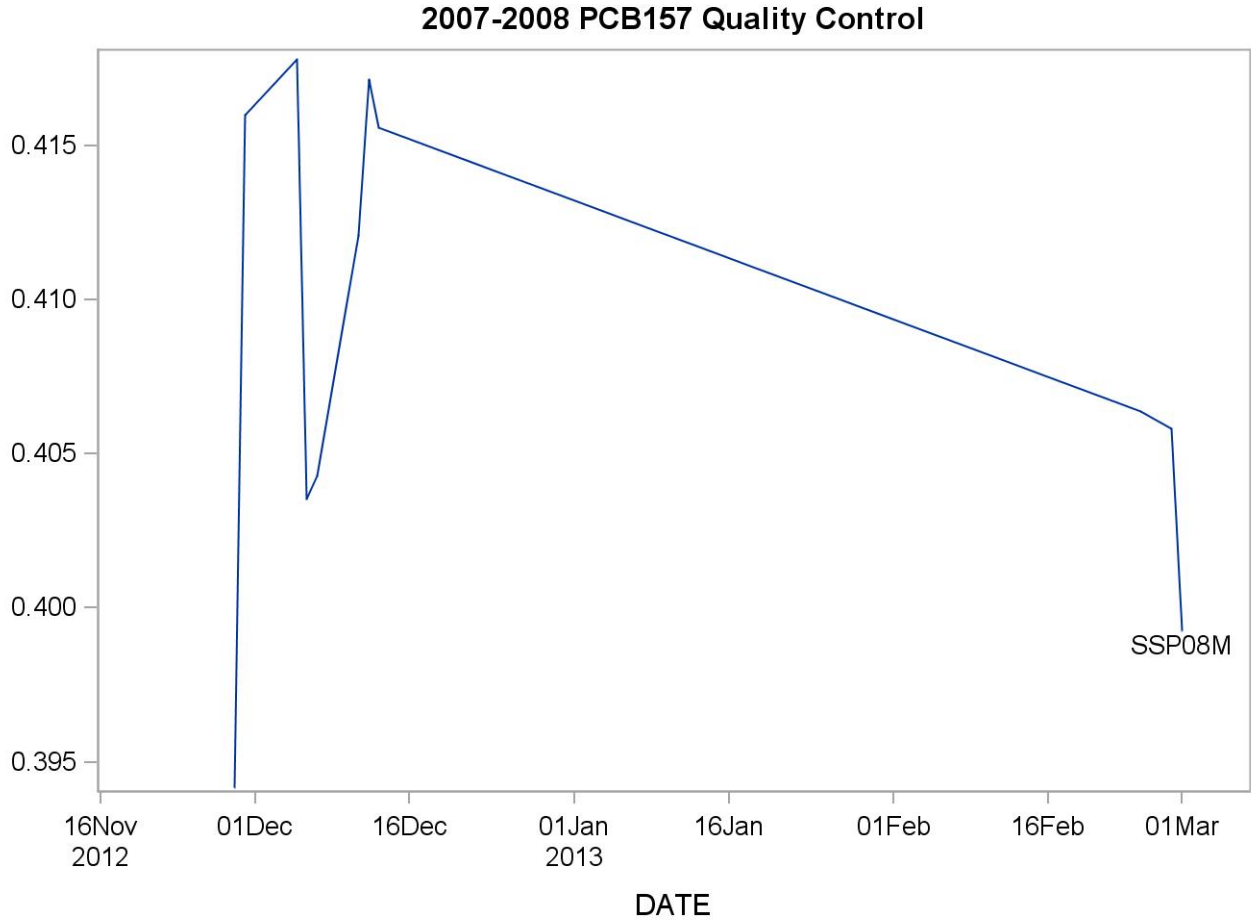
Summary Statistics for PCB156

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.41	0.01	3.3



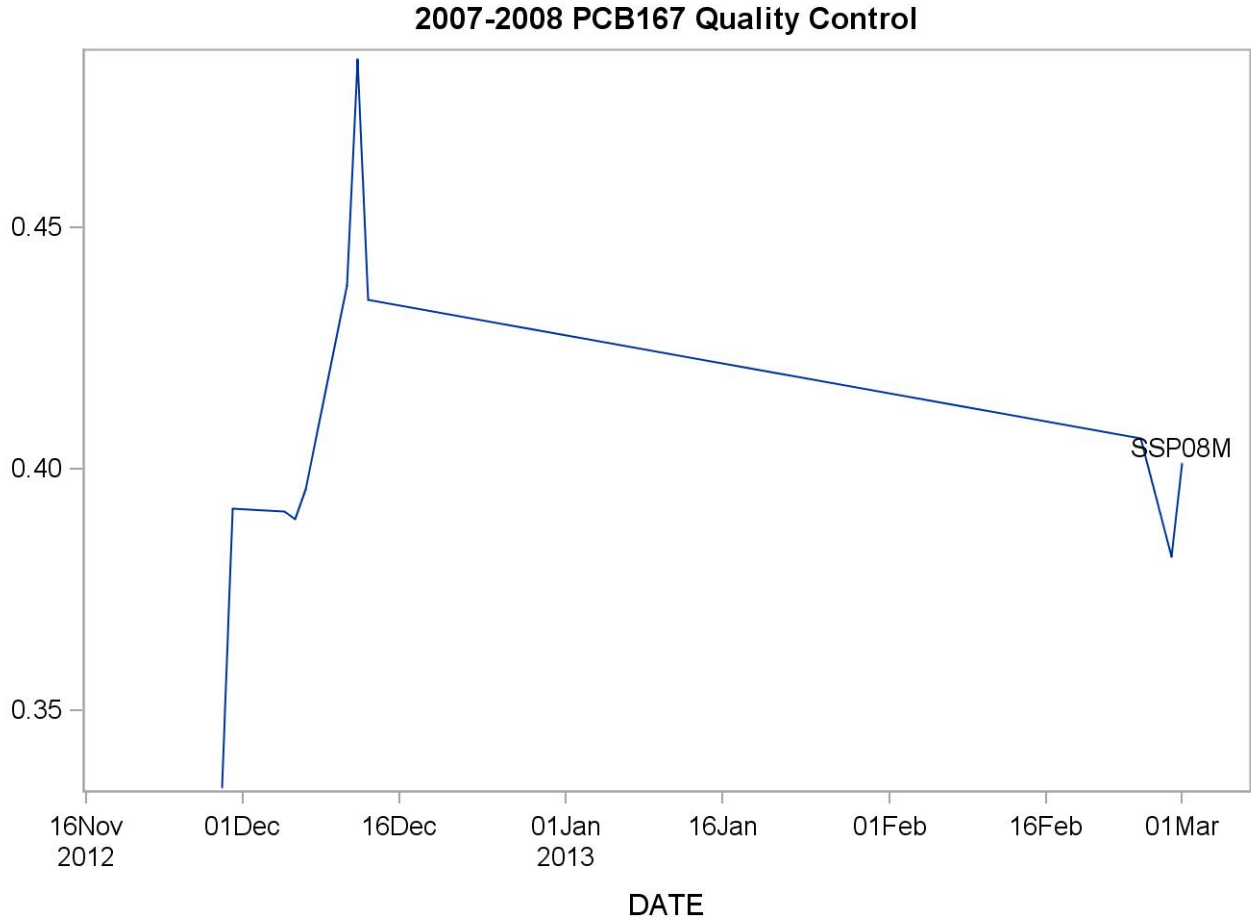
Summary Statistics for PCB157

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.41	0.01	2.6



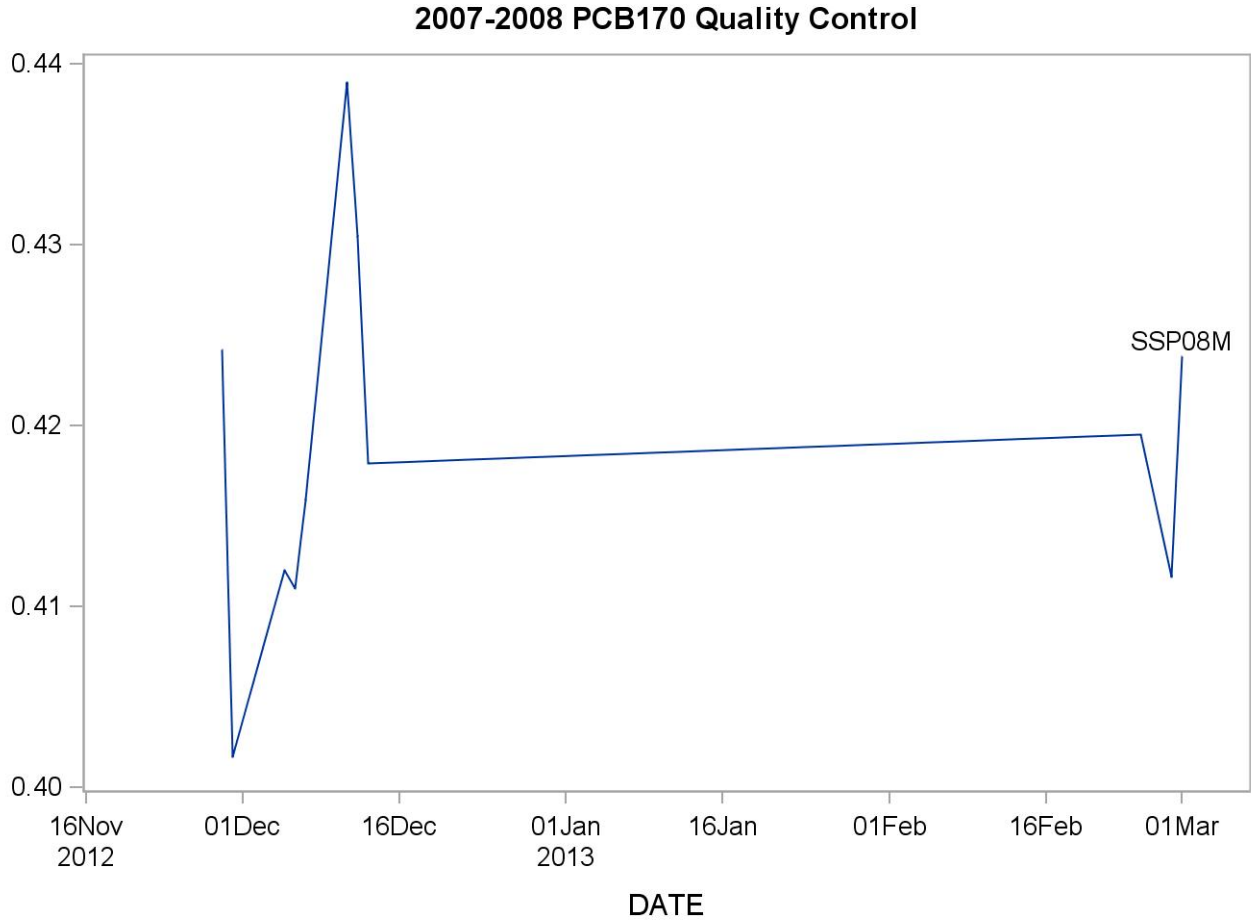
Summary Statistics for PCB167

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.40	0.05	12.7



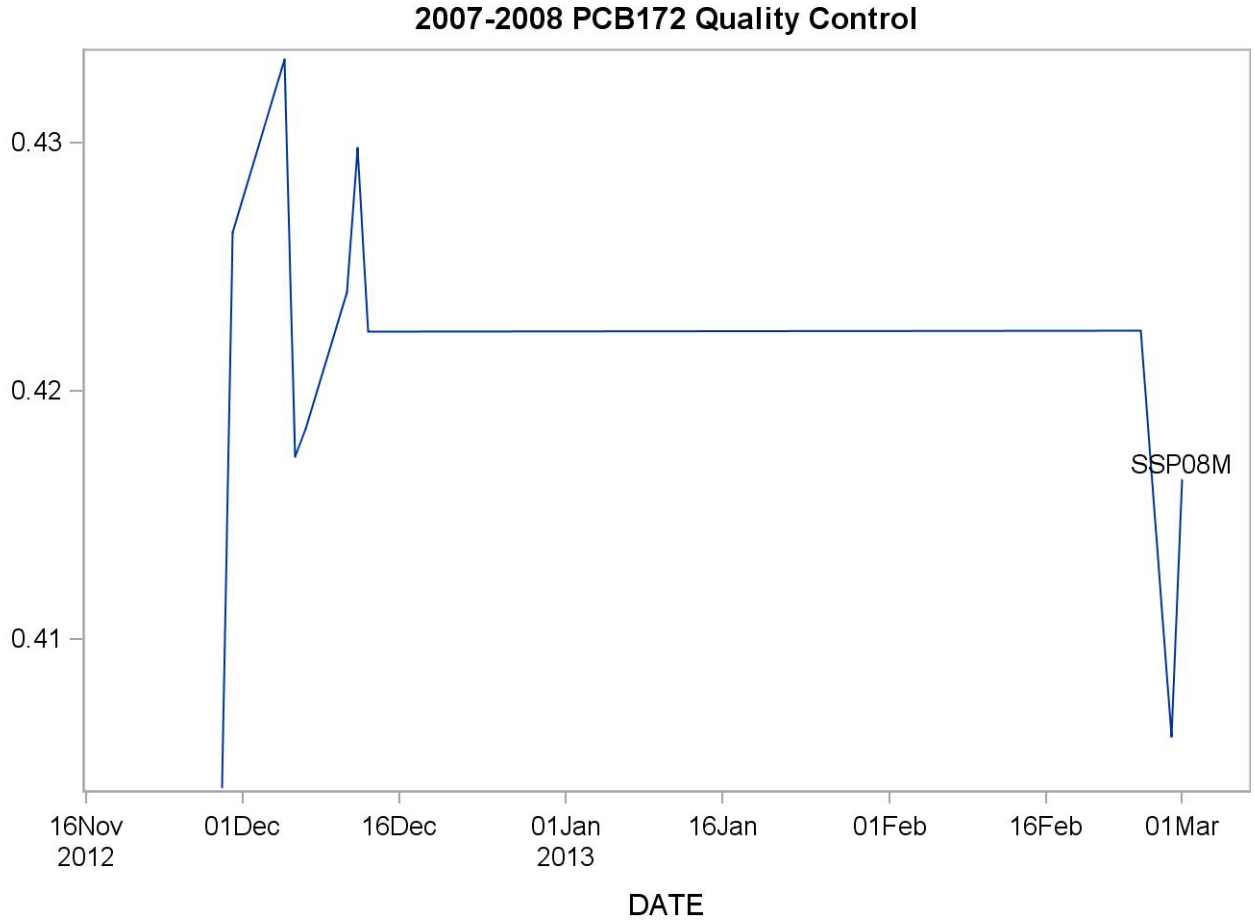
Summary Statistics for PCB170

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.42	0.01	3.2



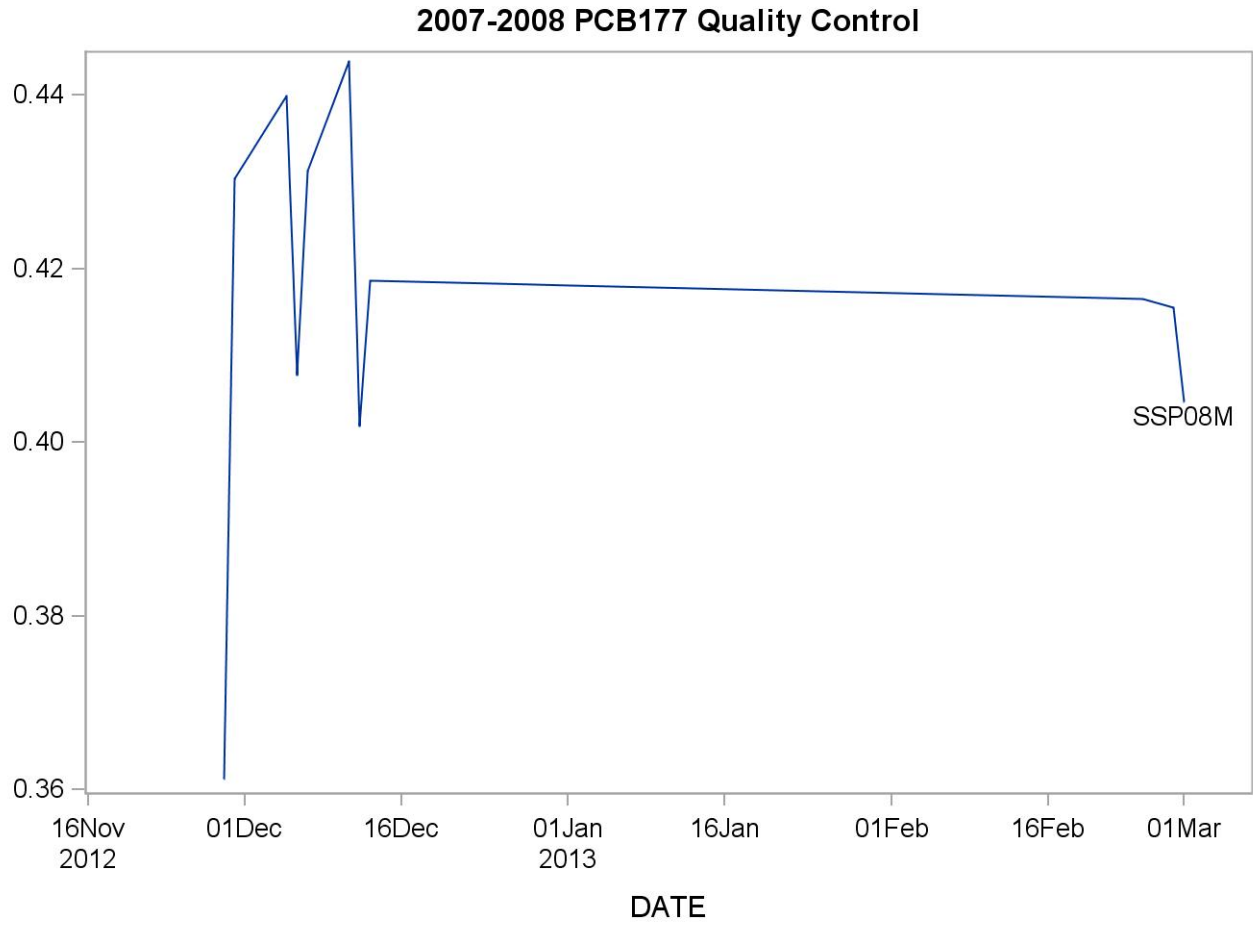
Summary Statistics for PCB172

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.42	0.01	2.9



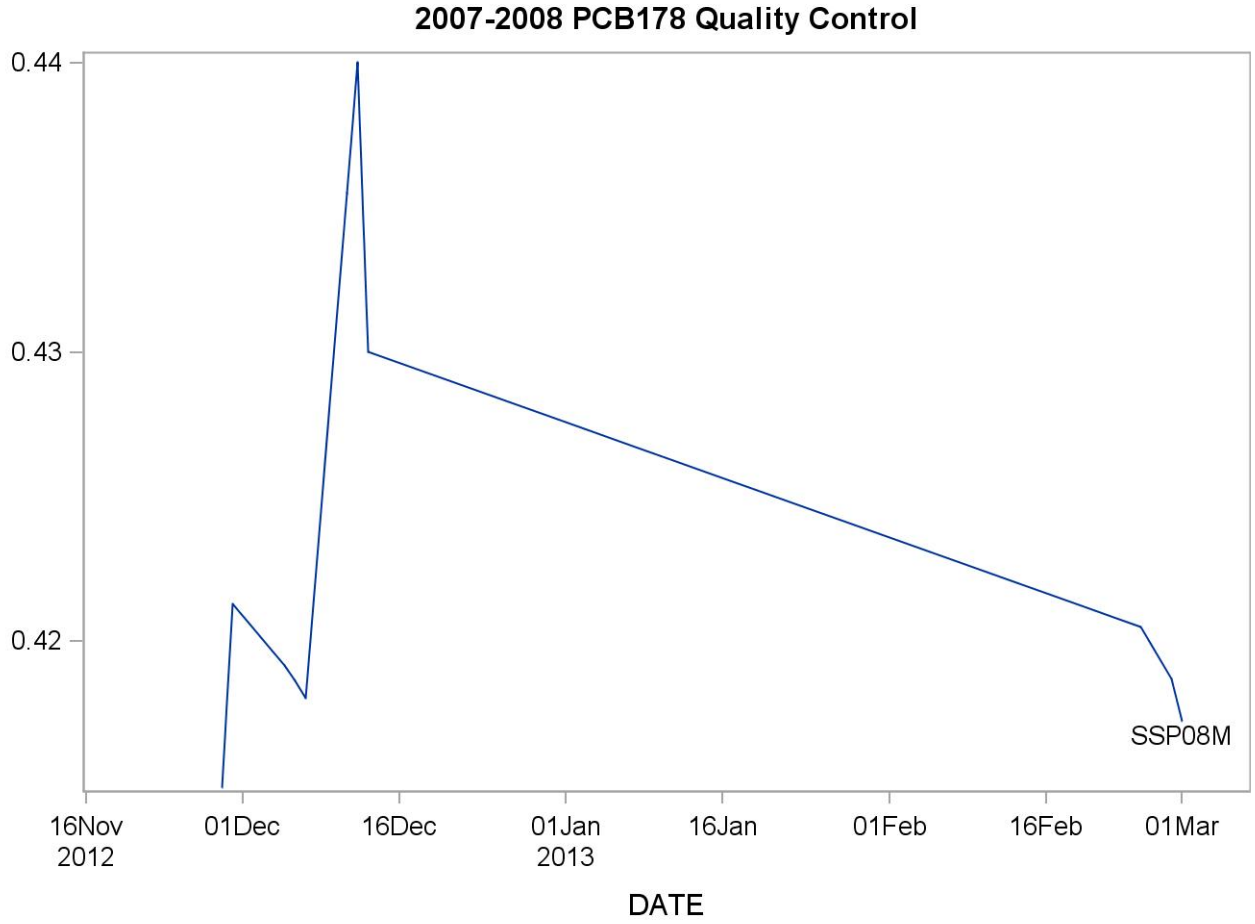
Summary Statistics for PCB177

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.42	0.03	6.8



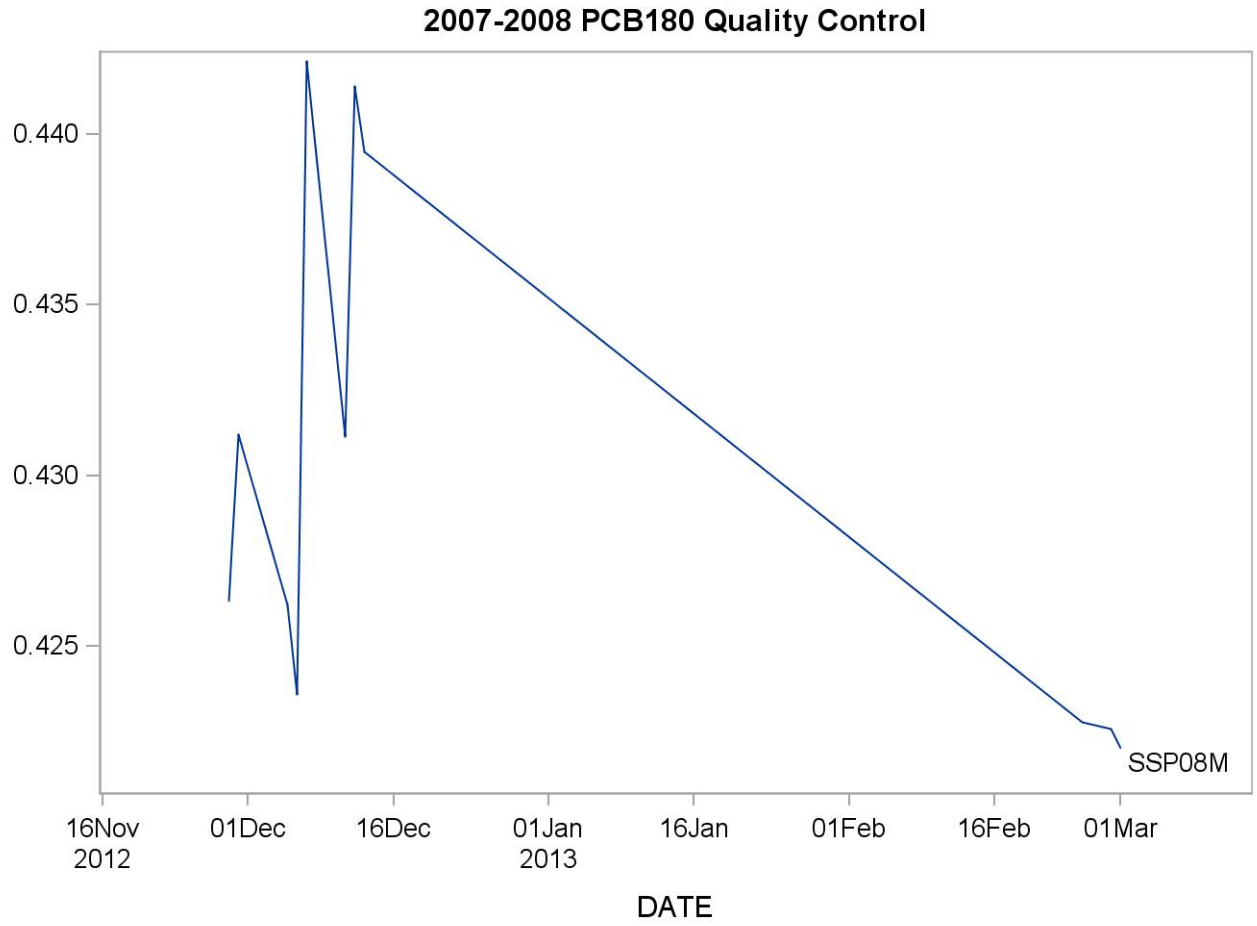
Summary Statistics for PCB178

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.42	0.01	2.3



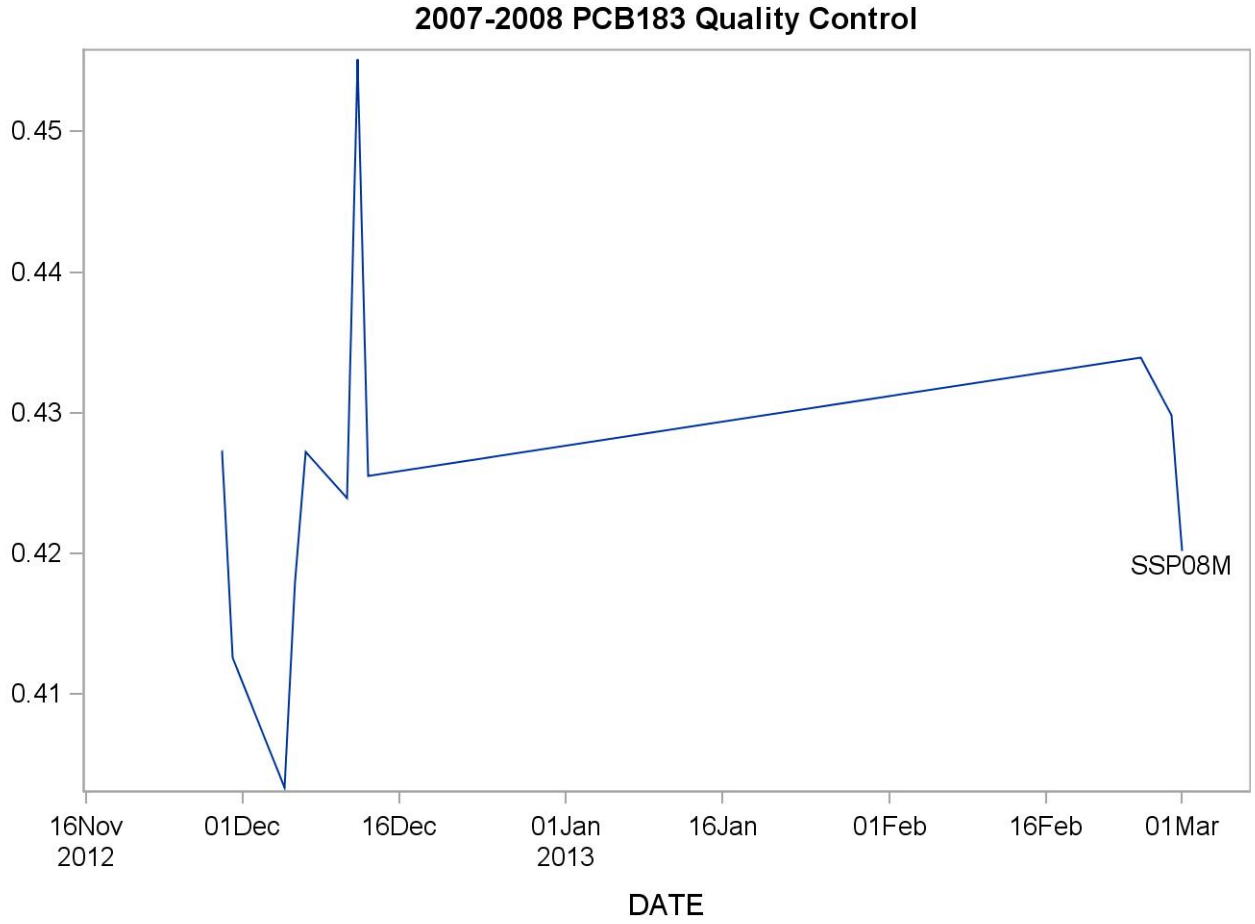
Summary Statistics for PCB180

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.43	0.01	2.7



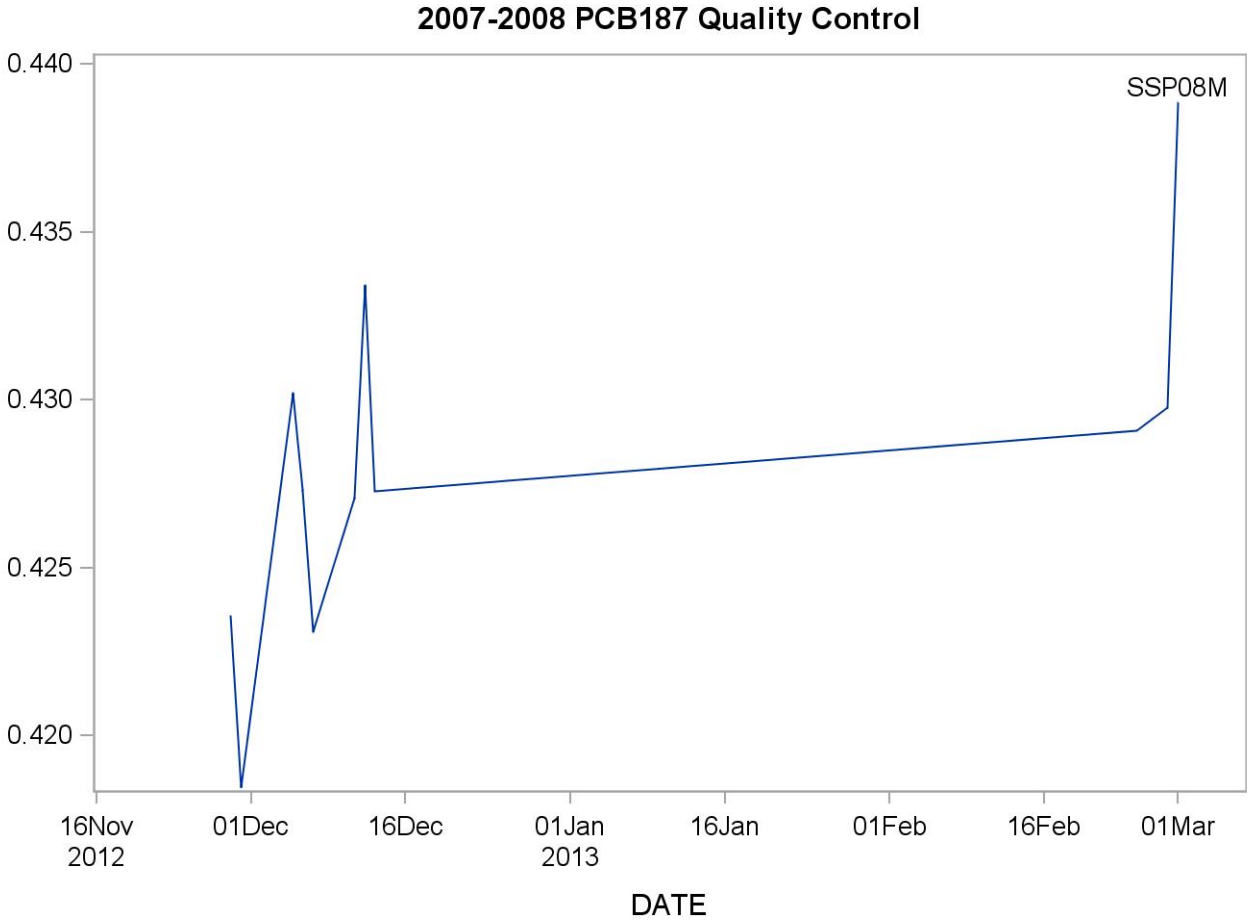
Summary Statistics for PCB183

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	12	29NOV12	01MAR13	0.43	0.01	3.5



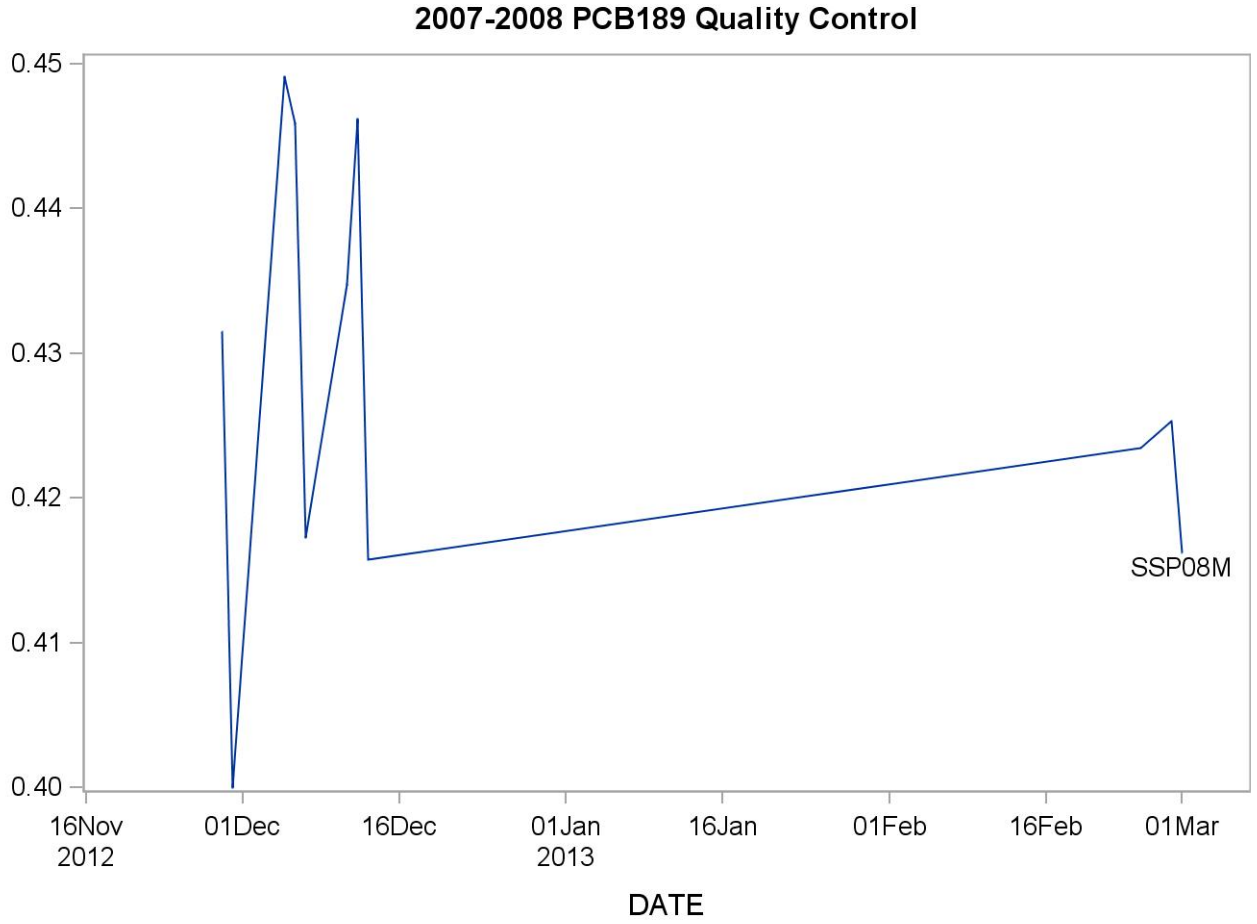
Summary Statistics for PCB187

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	20	29NOV12	01MAR13	0.43	0.01	2.1



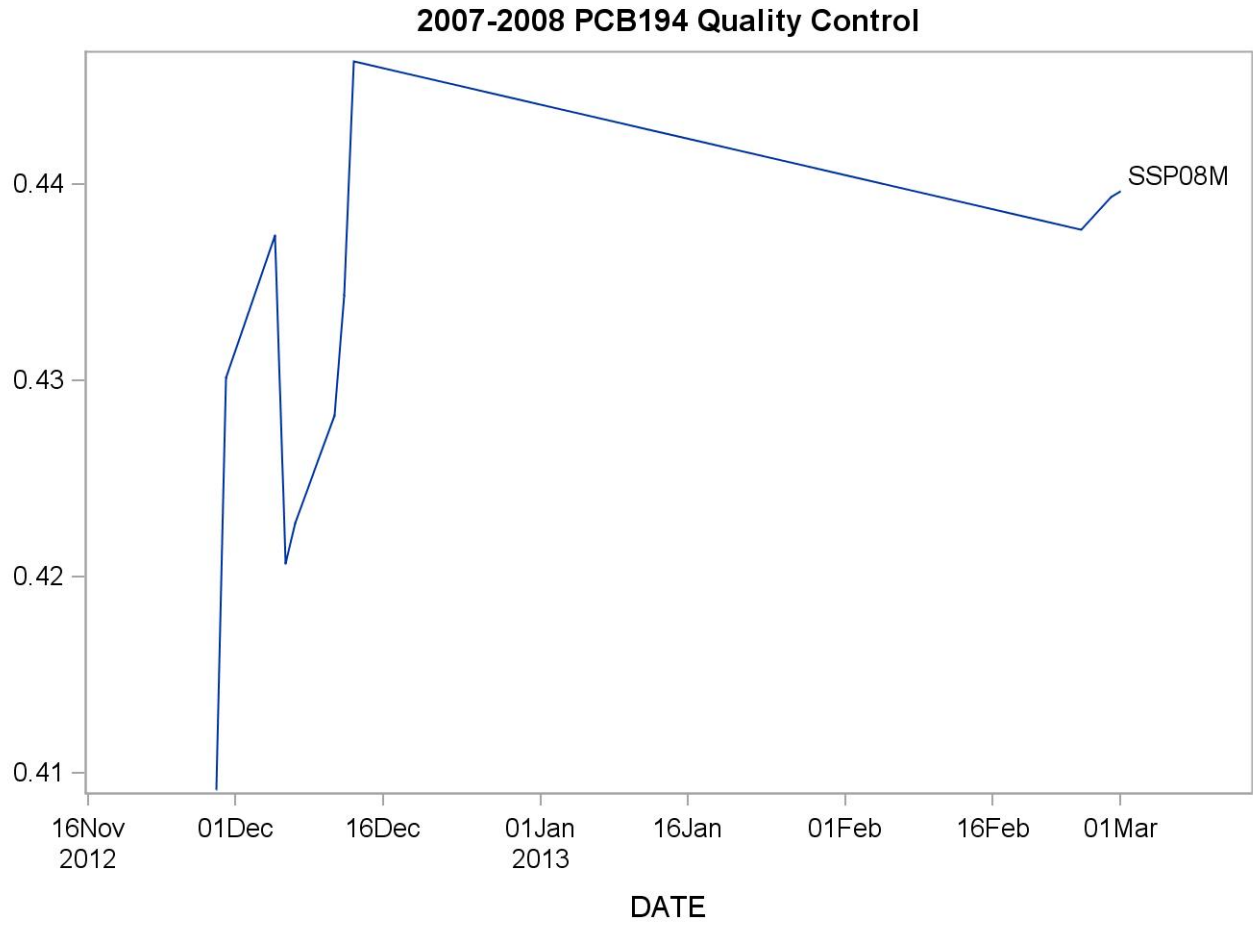
Summary Statistics for PCB189

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.43	0.02	4.5



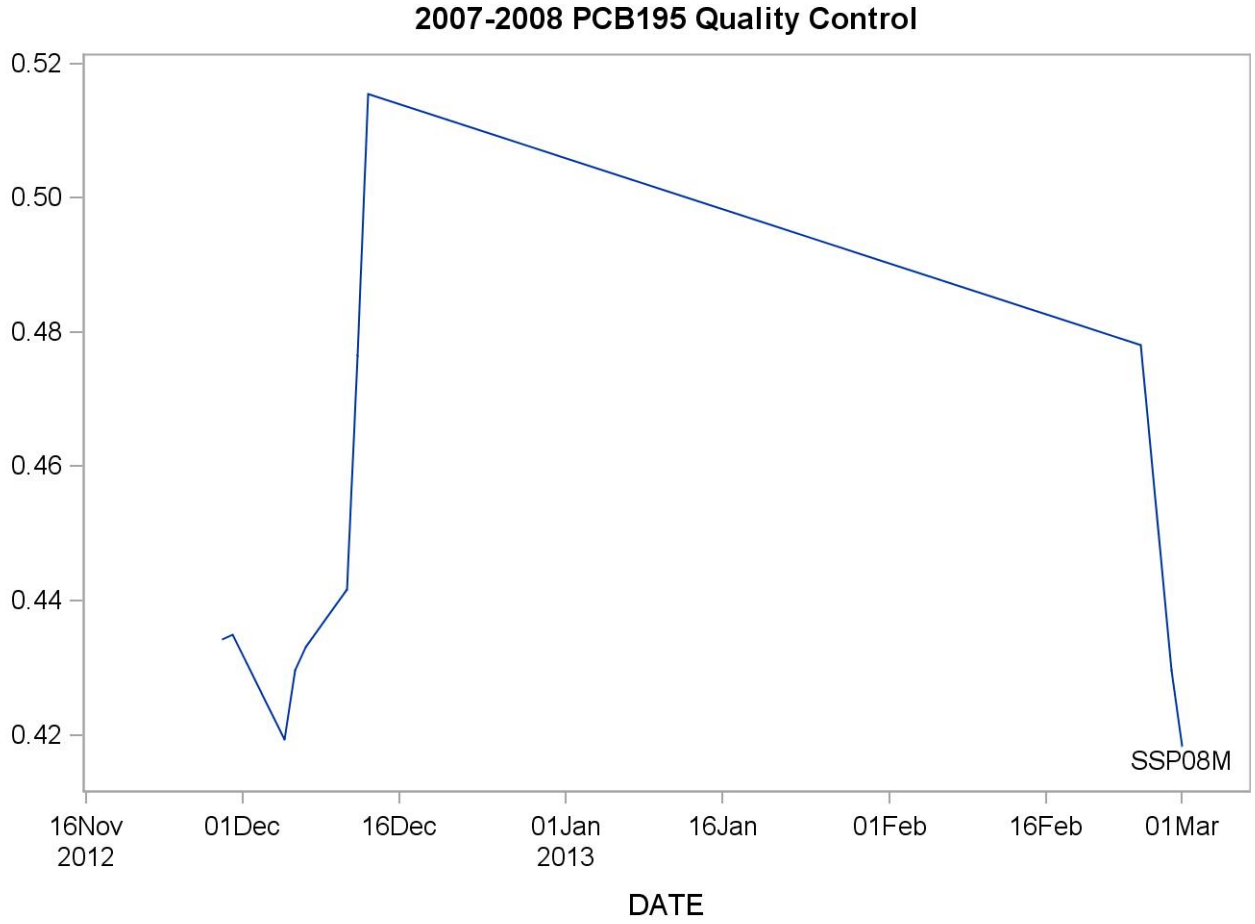
Summary Statistics for PCB194

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.43	0.01	3.4



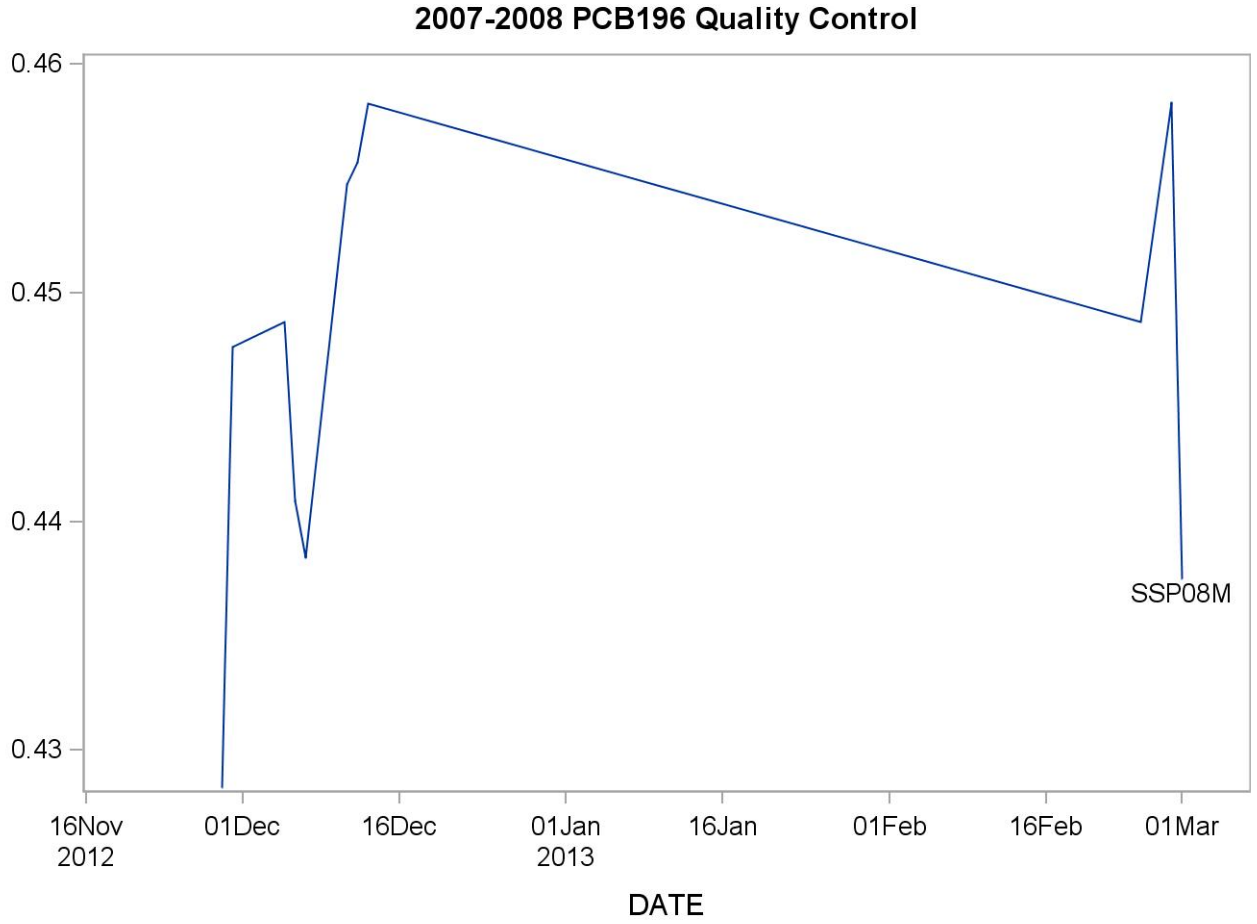
Summary Statistics for PCB195

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.45	0.04	9.1



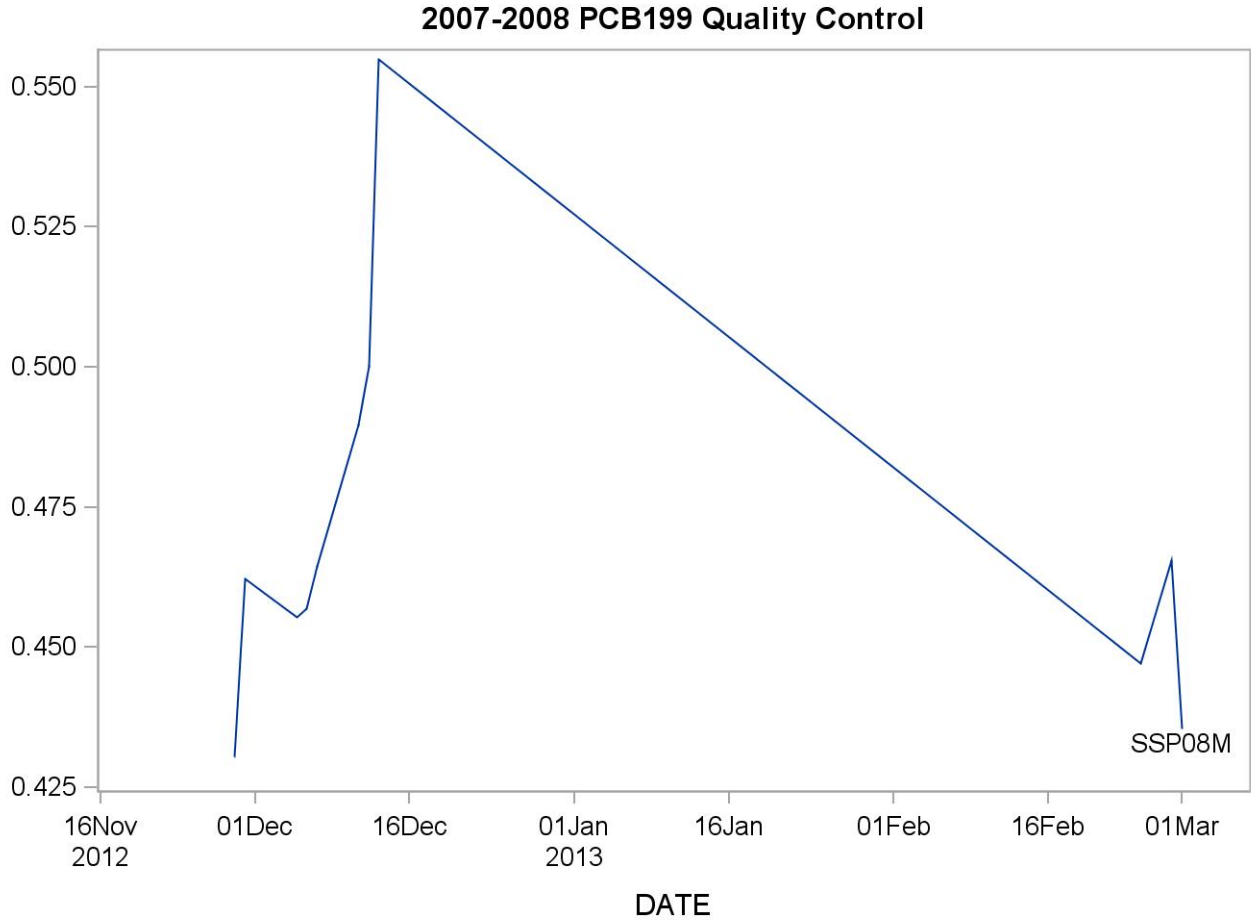
Summary Statistics for PCB196

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	22	29NOV12	01MAR13	0.45	0.01	3.2



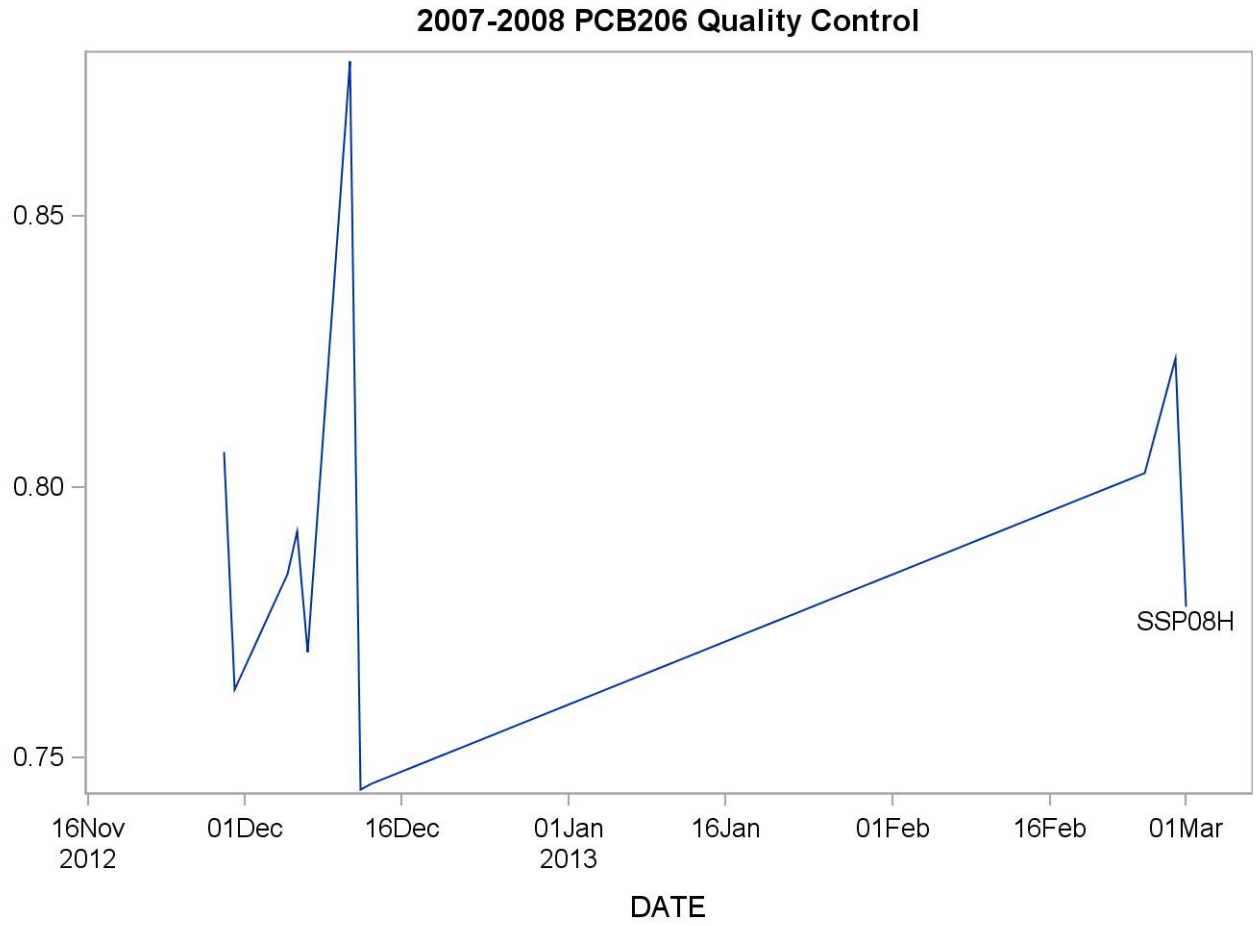
Summary Statistics for PCB199

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08M	19	29NOV12	01MAR13	0.47	0.04	9.1



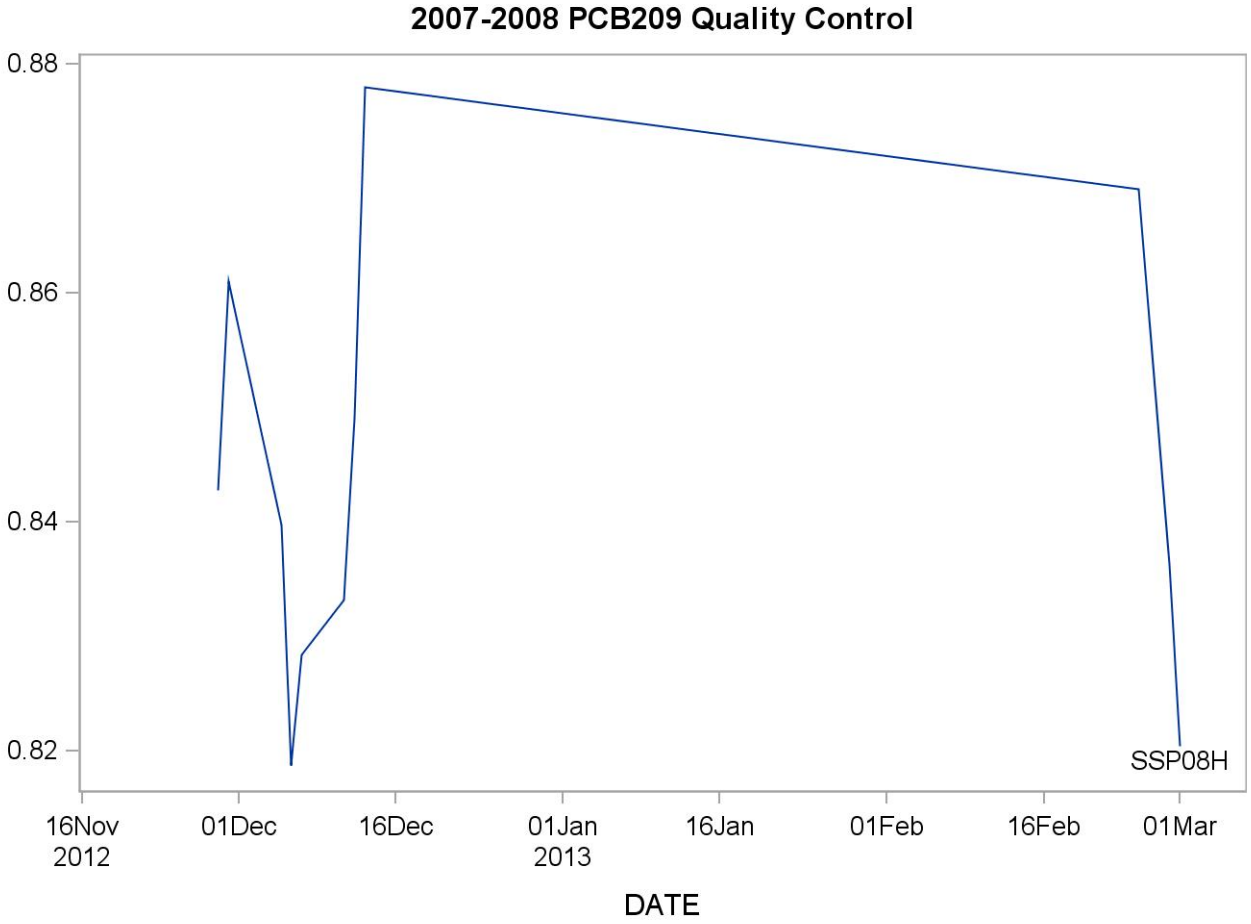
Summary Statistics for PCB206

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08H	11	29NOV12	01MAR13	0.79	0.04	4.9



Summary Statistics for PCB209

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
SSP08H	11	29NOV12	01MAR13	0.84	0.02	2.3



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