Laboratory Procedure Manual

Analyte: **PCBs and Persistent Pesticides**

Matrix: **Serum**

Method: **HRGC/ID-HRMS**

Method No.: **28**

Revised:

as performed by: *Organic Toxicology Branch*
Division of Laboratory Sciences
National Center for Environmental Health, CDC

Contact: *Donald G. Patterson Jr. or Wayman Turner*
(770)-488-4207 *(770)-488-7974*

Important Information for Users

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

Public Release Data Set Information

This document details the Lab Protocol for NHANES 1999-2000 data.

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
lab28	LBX028	PCB28 (ng/g)
	LBX052	PCB52 (ng/g)
	LBX066	PCB66 (ng/g)
	LBX074	PCB74 (ng/g)
	LBX087	PCB87 (ng/g)
	LBX099	PCB99 (ng/g)
	LBX101	PCB101 (ng/g)
	LBX105	PCB105 (ng/g)
	LBX110	PCB110 (ng/g)
	LBX118	PCB118 (ng/g)
	LBX128	PCB128 (ng/g)
	LBX138	PCB138 (ng/g)
	LBX146	PCB146 (ng/g)
	LBX149	PCB149 (ng/g)
	LBX151	PCB151(ng/g)
	LBX153	PCB153(ng/g)
	LBX156	PCB156(ng/g)
	LBX157	PCB157 (ng/g)
	LBX167	PCB167 (ng/g)
	LBX170	PCB170 (ng/g)

	LBX172	PCB172 (ng/g)
	LBX177	PCB177 (ng/g)
	LBX178	PCB178 (ng/g)
	LBX180	PCB180(ng/g)
	LBX183	PCB183(ng/g)
	LBX187	PCB187(ng/g)
	LBX189	PCB189(ng/g)
	LBX194	PCB194 (ng/g)
	LBX195	PCB195 (ng/g)
	LBX196	PCB196 (ng/g)
	LBX201	PCB201 (ng/g)
	LBX206	PCB206 (ng/g)
	LBXBHC	Beta-hexachloro-cyclohexane (ng/g)
	LBXGHC	Gamma-hexachloro-cyclohexane (ng/g)
	LBXHCB	Hexachlorobenzene (ng/g)
	LBXHPE	Heptachlor Epoxide (ng/g)
	LBXMIR	Mirex (ng/g)
	LBXODT	opDDT (ng/g)
	LBXOXY	Oxychlordane (ng/g)
	LBXPDE	ppDDE (ng/g)
	LBXPDT	ppDDT (ng/g)
	LBXTNA	Trans-nonachlor (ng/g)
	LBXDIE	Dieldrin (ng/g)
	LBXALD	Aldrin (ng/g)
	LBXEND	Endrin (ng/g)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

1.1 Summary of Test Principle

Thirty-eight ortho-substituted polychlorinated biphenyls (PCBs), 13 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 (1–1.5 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 hexane using a C_{18} solid phase extraction (SPE) procedure followed by extraction through neutral silica and Florosil SPE columns. PCBs and pesticides are eluted from the Florosil column with hexane and 1:1 dichloromethane /hexane. For PCBs and pesticides, each analytical run consists of nine unknown specimens, one method blank, and two 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 6890 gas chromatograph operated in the splitless injection mode with a flow of 1 mL/min helium through a DB-5ms capillary column (30 m x 0.25 mm x 0.25 µm film thickness) where analytes are separated prior to entering a Thermo Finnigan MAT95 XP (5 kV) 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 (^{12}C) compound and it corresponding ^{13}C-internal standard. The instrumental response factor for each analyte is calculated as the sum of the two ^{12}C 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 ^{12}C compound and its corresponding ^{13}C 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 3 parts-per-thousand compared to a standard, response ratios of the two ^{12}C and ^{13}C 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 and recovery. 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 “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 dioxin/furan 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

Databases (i.e. NHPCB02) have been set up on the PC using R:BASE version 6.5. The databases will be used for storage, retrieval, and analysis of data from projects of the dioxin and related compounds 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 or to the mainframe. 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 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 10 mL. The minimum amount is about 1 mL of serum.

Specimen Collection Materials for Each Participant, up to 10-g Serum Sample.

- Gauze sponges, sterile, individually wrapped 2"x 2" (2 ea).
- Alcohol wipe
- Band-aid

- Red-top Vacutainers (size depends on volume to be collected)
- 21 gauge multi sample needle, sterile
- Pre-printed labels
- Tourniquet
- Vacutainer holder
- Freezer
- Pasteur pipette (1 each*)
- Qorpak bottle (1 each*)
- Teflon-lined stoppers for above Qorpak bottle (2 ea.*)
- Aluminum seals (2 each)
- Pre-printed labels
- Pipette bulb
- 16 Racks
- Centrifuge
- Freezer (–20°C)

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

Collection of one 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

- 1 Styrofoam shipper
- 3-4 lbs. dry ice
- 4 bubble-pack bags 4"x 7"
- Safety glasses or eye shield
- Strapping tape
- Gloves for handling dry ice and frozen specimens
- Sheets of bubble-pack packing material
- CDC "Specimen Shipping List" filled out
- 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"x 7" 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°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 Reagent Preparation

6.1.1 50% dichloromethane/hexane solution (v/v).

With a 2-L graduated cylinder, measure 1.5-L dichloromethane, and pour into a clean labeled 4-L bottle. Measure 1.5 L of hexane with the same graduated cylinder and pour into the same 4-L bottle. Gently swirl to mix.

6.1.2 10% Dimethyldichlorosilane (DMCS) Silanizing Solution.

A 10% DMCS in toluene solution (v/v) is prepared for silanizing glass vessels and TurboVap tubes. The silane solution is stored in a glass reagent bottle at 4°C and may be reused until it begins to turn yellow.

Before silanizing the glass vessels or tubes, they are rinsed with acetone and dried in an oven at 130°C for 10 min. The vessels or tubes are filled with 10% DMCS solution and allowed to stand for 10 min. The silanizing solution is then decanted and saved for reuse. The vessels or tubes are rinsed with toluene and filled with methanol and allowed to stand for 5 min. The methanol is discarded. The vessel or tubes are rinsed again with methanol, followed by toluene and acetone.

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 38 PCBs found in humans and 13 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 1A and 1B list the components of the isotope dilution standards. ^{13}C-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 1A and 1B list the internal standards used for each of the 38 PCBs measured. The concentrations of each of the PCB congeners in each of the IDMS standards are shown in Table 2A and the concentrations of each pesticide are shown in Table 2B.

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 1) 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 1A. 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	PCB32
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	PCB70
2,3',4,4'-Tetrachloro biphenyl	$C_{12}H_6Cl_4$	PCB66	Yes	PBC70
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	PCB111
2,3,3',4',6-Pentachloro biphenyl	$C_{12}H_5Cl_5$	PCB110	Yes	PCB111
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	PCB111
2,2',3,4',5',6-Hexachloro biphenyl	$C_{12}H_4Cl_6$	PCB149	Yes	PCB118
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
2,4',6–Trichloro biphenyl	$C_{12}Cl_3$	PCB32	No	Yes
2,3',4',5-Tetrachloro biphenyl	$C_{12}Cl_4$	PCB70	No	Yes
2,3,3',5,5'-Pentachloro biphenyl	$C_{12}Cl_5$	PCB111	No	Yes
$^{13}C_6$ 1,2,3,4-TCDD	Recovery standard			

Table 1B. 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
Aldrin	$C_{12}H_8Cl_6$	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
Endrin	$C_{12}H_8OCl_6$	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 2A. 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 2B. 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.

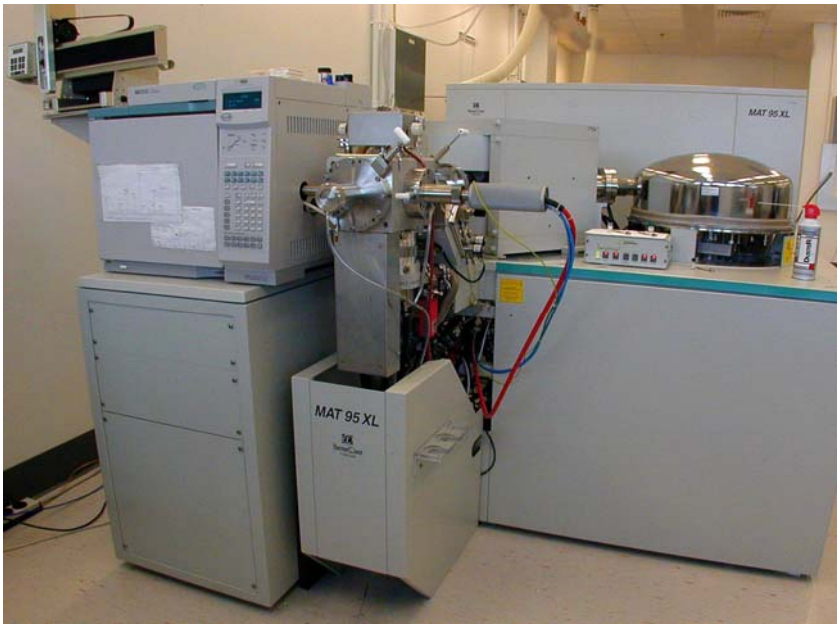
6.3 Other Materials

- Nitrogen gas, ultra-pure grade (Airgas South, Hapeville, GA).
- Detergent, Micro liquid laboratory cleaner (Cole-Parmer, Chicago, IL). [A 5% solution of Micro in deionized water (v/v), is used for washing glassware].
- Solvent rinsed 20x125 and 20x150 mm disposable glass tubes with Teflon lined caps size 18.
- 1.1 mL screw top vials [1.1CTVG] with Teflon faced silica septa [11-AC-TST1] (Sun SRI, Wilmington, NC).
- DB-5MS 30 m, 0.25 mm I.D., 0.25 µm film thickness gas chromatography column (J&W Scientific, Folsom, CA).
- GC syringe, 10 µL [019390] (SGE, San Antonia, TX).
- TurboVap II Concentration Workstation [ZW8001] for 200 mL tubes with 0.5 mL (Caliper Life Sciences, Hopkinton, MA).
- Microman M25, M50, and M250 positive-displacement pipets with capillaries and pistons. Ranin EDP 10 µL and 100 µL; EDP PLUS 10 µL and 100 µL Motorized Pipettes (Rainin Instrument Co., Woburn, MA).
- Eppendorf 1000µL adjustable pipette (Brinkman Instrument Company, Westbury, NJ).
- Wrist action shaker, model 75 (Burrell, Pittsburg, PA).
- SPE vacuum manifold (J.T. Baker, Phillipsburg, NJ).
- Balance model BP310S (Sartorius, Goettinger, Germany).
- Solvents: glass-distilled dichloromethane, acetone, HPLC grade water, and hexane, methanol, ethanol (anhydrous reagent), and ACS grade formic acid (Tedia, Fairfield, OH). Dodecane, and decane (Aldrich Chemical Co., Milwaukee, WI).
- Dimethyldichlorosilane (Aldrich, Chemical Co., Milwaukee, WI).
- Water, deionized (Culligan Water Systems, Inc., Marietta, GA).
- SPE cartridges: 500 mg BakerBond Octadecyl (C_{18}) disposable extraction columns, 1000 mg BakerBond Silica Gel disposable extraction columns (J.T. Baker, Phillipsburg, NJ) and 900 mg MAXI-CLEAN Florisil cartridges and 2 g MAXI-CLEAN sodium sulfate cartridges (Alltech, Deerfield, IL).

6.4 Instrumentation

High-resolution gas chromatography/high-resolution mass spectrometry systems: Thermo Finnigan MAT95 XP Mass Spectrometer (5kv), with X-caliber data systems (Thermo Finnigan, San Jose, California) and Agilent Technologies 6890 Gas Chromatograph (Agilent Technologies, Palo Alto, California) and a GC-Pal autosampler (Leap Technologies, Carrboro, North Carolina). Sample extracts are analyzed for PCBs and pesticides by HRGC/ID-HRMS. Two microliters of extract are 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 DB-5ms 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 40 eV, using selected ion monitoring (SIM) at 10,000 resolving power (10% valley).

Figure 1 Thermo Finnigan MAT95 XP



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 (carbon-12) compound and its corresponding ^{13}C-internal standard. The quantitative analytical standards are listed in Table 2A for PCBs and Table 2B for pesticides. The standards are analyzed in ascending and descending order for several days. At least 6 analyses for each standard are made before performing a preliminary linear regression analysis of the data to estimate a slope (b) and an intercept (a) for each congener. The slopes and intercepts are periodically updated as additional standard data become available. 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 sample in every clean-up run is an analytical blank. 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 3A and 3B) 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 the average ion ratios observed in the calibration standards 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 average 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 the limits are based upon +15% of their average ratios.

Table 3A. Ion Ratios for Analysis of PCBs

Compound	Ions Monitored	Average 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 3B. Ion Ratios for Analysis of Chlorinated Pesticides

Compound	Ions Monitored	Average Ratio	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	224.9317/226.9287	2.09	(1.68-2.50)
γ-HCCH	218.9115/220.9085	2.09	(1.54-2.64)
$^{13}C_6$-γ-HCCH	224.9317/226.9287	2.09	(1.68-2.50)
Aldrin	260.8599/262.8570	0.61	(0.45-0.77)
$^{13}C_{12-}$ Aldrin	267.8834/269.8805	0.61	(0.49-0.73)
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	260.8599/262.8570	0.61	(0.65-0.77)
$^{13}C_{10}$ trans-Nonachlor	267.8834/269.8805	0.61	(0.49-0.73)
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	0.61	(0.45-0.77)
$^{13}C_{12}$-Dieldrin	267.8834/269.8805	0.61	(0.49-0.73)
Endrin	260.8599/262.8570	0.61	(0.45-0.77)
$^{13}C_{12}$ Endrin	267.8834/269.8805	0.61	(0.49-0.73)
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 4 contains a list of all the mass ions used for the determination of the 38 PCBs and the 13 chlorinated pesticides and their relative order of elution. Figure 2 and 3 show reconstructed ion chromatograms of PCBs and pesticides showing peak identities and retention times.

Table 4. Ions Monitored for High-Resolution Mass Spectrometric Analysis of PCBs and Pesticides on a MAT95 XP Mass Spectrometer.

WINDOW 1	**Start Time**	5.92	**Label IDMS Std**	**Lock Mass**	242.9856	
	End Time	8.98		**Cali Mass**	292.9824	
	Low Mass	216.9145				
	High Mass	291.8273		**Cycletime**	0.60sec	
	Ratio	1.35				
	Analytes			**Mass**		**Fragment**
	Tri-PCB	-18	32	255.9613		M
		-28	28	257.9584		M+2
		-32		268.0016		
				269.9986		
	HCB			283.8102		M+2
				285.8072		M+4
				289.8303		
				291.8273		
	HCH	beta	C13	216.9145		M
		gamma	C13	218.9113		M+2
				222.9347		
				224.9317		
WINDOW 2	**Start Time**	9.00		**Lock Mass**	292.9824	
	End Time	12.48				
				Cali Mass	366.9792	
	Low Mass	268.0016				
	High Mass	398.8358		**Cycletime**	0.60sec	

	Ratio	1.49				
	Analytes			**Mass**		**Fragment**
	Tetra-PCB	-52	52	289.9224		M
		-49	52	291.9194		M+2
		-44	52	301.9626		
		-74	70	303.9597		
		-66	70			
		-70	70			
	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**	12.50		**Lock Mass**	242.9856	
	End Time	13.50				
				Cali Mass	292.9824	
	Low Mass	323.8834				
	High Mass	418.8176		**Cycletime**	0.50sec	
	Ratio	1.293				
	Analytes			**Mass**		**Fragment**
	DDE		C13	246.0003		M-Cl2
				247.9973		M+2-Cl2
				258.0406		
				260.0376		
	t-Nonachlor		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		
WINDOW 4	**Start Time**	13.52		**Lock Mass**	242.9856	

End Time	16.80			
			Cali Mass	292.9824
Low Mass	235.0081			
High Mass	303.9411		**Cycletime**	0.80sec
Ratio	1.438			

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
t-Nonachlor			262.857	M+2C5H6ClO
			267.8834	
			269.8805	
Penta-PCB	-111			
	-87	111	323.8834	M
	-110	111	325.8804	M+2
	-118	118	335.9237	
	-105	105	337.9207	
Hexa-PCB	-151	111	289.9037	M+2-Cl2
	-149	149	291.9008	M+4-Cl2
	-146	153	335.9237	
	-153	153	337.9207	

			Lock Mass	242.9856
WINDOW 5	**Start Time**	16.80		
	End Time	18.83		
			Cali Mass	292.9824
	Low Mass	235.0081		
	High Mass	337.9021	**Cycletime**	0.80sec
	Ratio	1.438		

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	335.9237	
	-167	167	337.9207	
Hepta-PCB	-178	178	393.8025	M+2-Cl2
	-187	178	395.7995	M+4-Cl2

	-183	178	405.8428	
			407.9021	
1234D			327.9137	
			327.9465	
2378D	Label		331.9368	
			333.9338	

WINDOW 6	**Start Time**	18.85	**Lock Mass**	292.9824
	End Time	20.50		
			Cali Mass	316.9824
	Low Mass	289.9037		
	High Mass	337.9021	**Cycletime**	0.50sec
	Ratio	1.166		

Analytes			**Mass**	**Fragment**
Hexa-PCB	-156	156	289.9037	M+2-Cl2
	-157	157	291.9008	M+4-Cl2
			335.9237	
			337.9207	
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**	20.52	**Lock Mass**	292.9824
	End Time	22.67		
			Cali Mass	366.9792
	Low Mass	271.8102		
	High Mass	405.8241	**Cycletime**	0.60sec
	Ratio	1.493		

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	

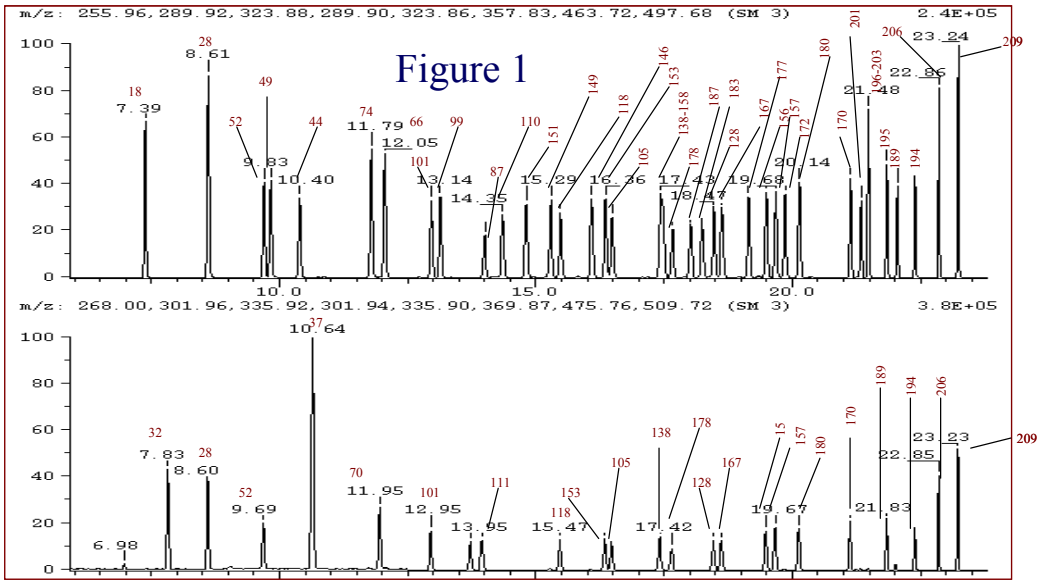
			337.9021	
Octa-PCB	-199	170	357.8258	M+2-Cl2
	-196	170	359.8229	M+4-Cl2
	-203	170	369.8661	
	-195	194	371.8631	
	-194	194		

WINDOW 8

Start Time	22.68	**Lock Mass**	454.9728
End Time	23.80		
		Cali Mass	504.9697
Low Mass	454.9728		
High Mass	511.7199	**Cycletime**	0.50sec
Ratio	1.125		

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	

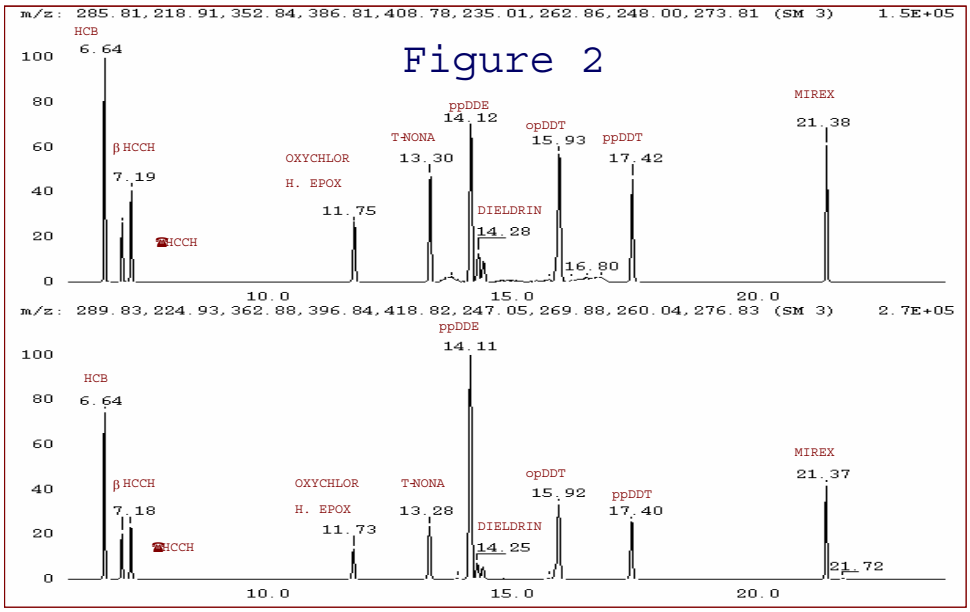
Figure 2. Ion Chromatogram for PCBs.



Top : Native PCBs

Bottom: ^{13}C-labeled PCBs

Figure 3. Ion Chromatogram of Pesticides



Top: Native Pesticides
Bottom: ^{13}C-labeled Pesticides

7.2 Calibration Verification

7.2.1 Daily Function Checks.

Before analyzing a run, the analyst is required to verify the existing calibration by analyzing a randomly selected analytical standard from Table 1A and Table 1B and compute the slope function check(s).

7.2.2 Calibration verification -- within instrument.

At least once every six months or prior to starting a new study, a within instrument calibration verification will be performed over the reportable range of the method to ensure that the accuracy of the measurement process over the reportable range is maintained over time. For PCB and pesticides analyze the 0.01, 0.10, 1.0, and 10.0 pg/µL calibration standards (Table 1) within a 3-day time period. The calculated concentration of each of the above standards must be within the confidence intervals established for each standard. Records of the calibration verification will be maintained in the QC manual for each instrument and checked by the Supervisor.

The within instrument calibration verification procedure described above will be performed after any change in the analytical procedure which is likely to make a non-trivial difference in sample results such as changing a GC capillary column, changing a photomultiplier, a major factory maintenance involving the removal and changing an outer source or changing an ion volume.

8. OPERATING INSTRUCTIONS FOR SAMPLE CLEANUP

8.1 An analytical run consists of 12 samples: the first is a reagent blank and the sixth and twelfth are quality control samples that have been well characterized in our laboratory. Quality assurance criteria for the blank and quality control sample are described in Section 10. These blanks and quality control samples are treated in the same manner as other specimen.

8.2 Serum – (C_{18}) Solid-Phase Extraction (SPE) Method for 500 mg C18 Cartridges and Sample Weights 1-2 g.

Rinse all Teflon and glassware before use. The solvent rinse order is 1) acetone; 2) toluene; 3) 1:1 dichloromethane/hexane. Wear gloves, lab coat, and safety glasses whenever handling chemicals or serum samples. Each day, the first person to use balance must check the accuracy of the balance and enter the result in the log book. All balances are to be checked for accuracy on the date of use. To check the balance, zero balance. Using forceps, place a NIST class I test weight on the pan. (Choose a test weight close to the range that you intend to use.) Determine the measured weight and record it in the log book with date and your initials. Compare measured weight with labeled weight of test weight. If weights do not agree within acceptable limits notify supervisor and DO NOT USE BALANCE. If weights agree within limits - proceed with weighing. CLEAN balance after use and re-set weights to ZERO.

The acceptable weight limits are:

	Balance Weight Limits
Sartorius	100 g ± 0.1 g
BP310s	50 g ± 0.1 g
	10 g ± 0.05 g
	1,2, or 5 g ± 0.01 g

Thaw the serum samples overnight in the refrigerator and bring to room temperature. Vortex the serum sample to homogenize the sample. Weigh serum into a solvent rinsed container with a tight fitting lid on the analytical balance. Record specimen number, run number, notebook number, and sample weight in

lab notebook. Enter data into R:BASE. Add 10 µL each of PCB and pesticide internal standards. Check the pipette for correct dispensing amount. (Sonicate the standard for 2 minutes before spiking). Vortex the spiked sample for 15–20 seconds and equilibrate for 15 minutes. Add 1 mL high purity water to the serum and mix vigorously. Then measure a volume of formic acid equal to the weight of the serum and add it to the serum. Vortex the serum/formic acid mixture and allow it to degas for 30 min. (Contact between the serum and formic acid for longer than 30 minutes can result in the formation of a gelatinous material, which is unsuitable for C_{18} extraction.) Measure a volume of high purity water equal to the volume of formic acid and add it to the serum formic acid mixture. Vortex and then allow gas to escape from the solution.

Activation of the C_{18} cartridges. Attach one 500 mg C_{18} SPE cartridge per sample to the SPE vacuum manifold. Activate the C_{18}. (Do not let C_{18} dry out during activation – when the solvent reaches the frit above the C_{18}, add more solvent.) Pour 2 volumes methanol through the C_{18} cartridge followed by 2 volumes of high purity water. Discard methanol in appropriate waste container.

C_{18} Extraction of PCBs and pesticides. As the water level from the second volume approaches the frit above the C_{18}, the vacuum should be turned off to allow the analyst adequate time to apply sample to the cartridges. Pour sample mixture into the SPE cartridge. Rinse the sample container with 1 mL high purity water and add water to SPE cartridge. Allow the liquid to completely drain through the C_{18} column. DO NOT USE THE VACUUM. (Use gravity flow.) Rinse the SPE cartridge twice with about 1 mL high purity water. Dry the SPE cartridge under vacuum (10-15 psi) for about 60 minutes on the vacuum manifold.

On the SPE vacuum manifold, stack a sodium sulfate SPE cartridge below each C_{18} cartridge. Elute PCBs and pesticides into labeled solvent rinsed 20x125 mm glass tube. Measure hexane elution solvent with a hexane dispenser, apply to cartridge and collect eluate as follows:

4 mL hexane – pump dry using gravity flow or no more than 5 psi;
4 mL hexane – pump dry using gravity flow or no more than 5 psi;
4 mL hexane – pump dry using gravity flow or no more than 5 psi;
4 mL hexane – pump dry using gravity flow or no more than 5 psi.

8.3 Extraction of PCBs and Pesticides through silica and Florisil.

Place each silica and Florisil SPE cartridge on the vacuum manifold and rinse the adsorbents with 1:1 dichloromethane/hexane solvent followed by hexane. On the vacuum manifold, stack the silica and the Florisil SPE cartridges with the Florisil on the bottom and place a labeled 20 x 125 mm collection tube under each pair of cartridges. Pour the 16 mL of hexane eluate from the C_{18} extraction of the serum into the silica cartridge with the matching label. Rinse the C_{18} collection tube with 1 mL hexane and add this to the silica cartridge. Using minimum vacuum, allow all of the hexane to drain through both SPE cartridges. Elute the PCBs and pesticides with 1:1 dichloromethane/hexane solvent as follows:

4 mL dichloromethane/hexane – pump dry using minimal vacuum;
4 mL dichloromethane/hexane – pump dry using minimal vacuum;
4 mL dichloromethane/hexane – pump dry using minimal vacuum.

Transfer the eluate to a 200 mL solvent rinsed TurboVap tube. Rinse the 20 x 125 mm tube 3 times with 1:1 dichloromethane/hexane solvent. Evaporate the solvent to 0.35 mL at 35ºC and 8–10 psi using TurboVap II with automatic sensor shutoff. Add 2µL dodecane and 8 µl of decane ‘keeper’ to 1 mL silanized conical glass vial and transfer 0.35 mL 1:1 dichloromethane/hexane extract into the vial. Rinse the TurboVap tube with 0.5 mL dichloromethane. Transfer rinse to vial. Allow the remaining solvent to evaporate at room temperature (overnight) in plastic box – the 2 µL dodecane ‘keeper’ will be retained in the vial. Seal vial using Teflon-faced silicone septa and plastic screw cap. Place the 12 samples associated with the same notebook number (one run) and its transfer sheet in rack for transfer to the Mass Spec Lab.

8.4 HIGH RESOLUTION MASS SPECTROMETRY ANALYSIS OF PCBs

8.4.1 GC Conditions

30 m X 0.25 mm i.d.× 0.25 µm thickness DB-5ms

Splitless injection

Injection port temperature	275°C
Oven temperature program	100°C, hold 0.6 min; 4°C/min to 200°C, hold 5 min; 4.5°C/min to 250°C; 50°C/min to 320°C, hold 3 min
Carrier gas	Helium flow rate 1 cc/min

Constant flow mode; vacuum correct off; pressure correct off

8.4.2 MASS SPECTROMETRY CONDITIONS:

Ion Source	High Sensitivity
Ionizing electron energy	40 eV
Accelerating Voltage	7638 V
Trap Current	500 µA
Source temperature	270°C
Transfer line temperature	270°C
Mass Resolution	10,000

8.4.3 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 38 PCBs and 13 pesticides on the Finnegan MAT95 XP mass spectrometers consist of eight groups. Table 4 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 DB-5ms 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. During a run, the mass spectrometer is recalibrated and the instrument resolution is rechecked as needed (i.e. loss of sensitivity, bad peak shape) by injecting 0.5 µL of 250 high boiling PCR in the septum reservoir.

8.4.4 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 Checks8.3.5 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 30

m DB-5 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 ºC 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 30:1 before analyzing specimen. If the S/N ratio is less than 30:1, check the tuning (retune if necessary), cut 1-2 inches from the GC end of the DB-5 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.

8.4.6 Daily Slope Function Check.

Inject 2 µL of a randomly selected calibration standard for the PCBs and pesticides (Table 2) and compute the Slope Checks for each compound. This standard serves as a check on the calibration and as recovery standard for the day. These calculations are performed in R:BASE and appear in the daily report of the data

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.

8.5 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 for each analytical standard. In addition, a glass syringe used in analyzing an unknown or QC sample is not reused.

The 12 samples in the cleanup run are analyzed as an analytical run. Samples with notebook numbers containing F and L are usually the QC samples in the analytical run. The area counts and retention times for each ion in Table 3 are measured and sent to the mass spec table in R:BASE. The sum of the area responses for each ion (Tables 3) 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. Analyst may continue with a second analytical run from cleanup as time permits. Calibration is checked as needed. Another calibration standard may be run whenever the analyst deems it necessary (i.e. retention time shift) or if the run proceeds past midnight.

For each congener, the following will be calculated in R:BASE: the mass fraction, the absolute recovery

of the primary internal standard ,the isotope ratio (Table 3) 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 .

8.6 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.

8.7 Replacement and periodic maintenance of key components

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 30:1. If the S/N ratio is unsatisfactory, check the tuning (retune if necessary), cut 1–2 inches from the GC end of the DB-5ms 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 monthly. The multiplier is changed every 6–12 months, once the setting is greater than 2.3. The outer source is replaced annually. GC column is replaced as needed usually every 2 months. Reference inlet septum and autosampler syringe are replaced weekly. Magnetic calibration (MCAL) is performed monthly. Electric calibration (ECALIB) is performed weekly. Instrument preventive maintenance (changing vacuum pump oil, etc) is performed by service technician annually.

8.8 Calculations

All computations and statistical analyses were carried out using the SAS v.9 statistical software package (SAS Institute 2005).

8.8.1 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).

8.8.2 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.

8.8.3 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× TCHOL + TRIG + 62.3);
TCHOL = total cholesterol mg/dL and
TRIG = triglycerides

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

TL = the total lipids in mg/dL; and 102.6 = the average density of serum in g/dL.

The estimated minimum detectable lipid adjusted concentration (C_E) is calculated using equation (4) where C_E is substituted for CONC. C_E is calculated using equations (1) and (2).

8.8.4 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. The detection limit (DL) values, based on standards, are calculated to correspond to weight corrected samples (See Figure 2). 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.

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

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

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 Table 3]. These confidence intervals are periodically updated. The recovery of the internal quantitation standards should be between 90% and 120%.

9.2 Criterion for Quality Control Sample.

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 3]. The confidence intervals are periodically updated. The recovery of the internal quantitation standards should be between 10% and 120%. The calculated concentration of each analyte for at least one QC sample 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 (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 specimen is halted and a search for an assignable cause is initiated. Analysis is resumed only after appropriate corrective action has been taken.

9.3 Criterion for Unknown Specimen.

The blank sample and the two QC samples associated with each set of nine unknown samples must first give valid results. If one or more of the requirements are not met for the blank or at least 1 of the QC samples, then the nine 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 3] The confidence intervals are periodically updated. 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 analytical standard which was 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.

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. These data are used to establish confidence intervals for standards. The calibration curves are updated periodically as data become available.

10.1.2 Blanks (Bench Controls).

A laboratory method blank is prepared along with every nine unknown samples and inserted into position A of each analytical run of 12 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 –70°C. 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 F and L of an analytical run of nine unknown samples. QC charts are constructed for each analyte in the control pool. 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 then 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 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 Section 8(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 three 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 their Website www.amap.no.

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. This lower limit (10%) for the absolute recovery has been validated in QC samples at a concentration as low as 22 ppq. 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 (Table 2) 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 3 for theoretical isotope ratios and confidence limits.)

10.2 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 5 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 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 "Second 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(Web site: www.cdc.gov/exposurereport. The concentrations of many of the congeners were below their detection limits in most samples. The next national exposure report, containing the 2001–2002 NHANES data, will be released in 2005.

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°C 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 –30°C is recommended until the analytical system is again operational.

Monitoring of serum samples which have been stored at –30°C 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

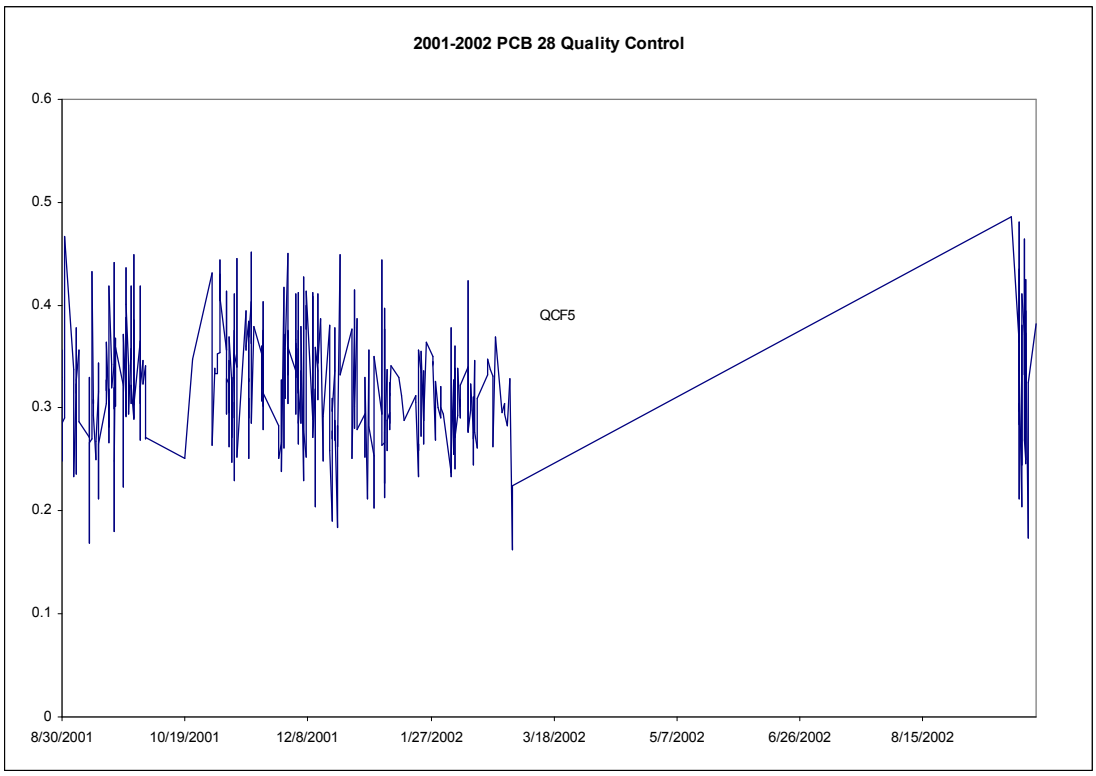
The sample remaining after the analysis, should be returned to storage at –30°C. Standard record keeping means (database, sample logs, optical disc files) are used to track specimens. Records are maintained for 3 years, including related QA/QC data; duplicate records are kept in electronic format. All 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

A. PCB28

Summary Statistics for PCB 28 by Lot

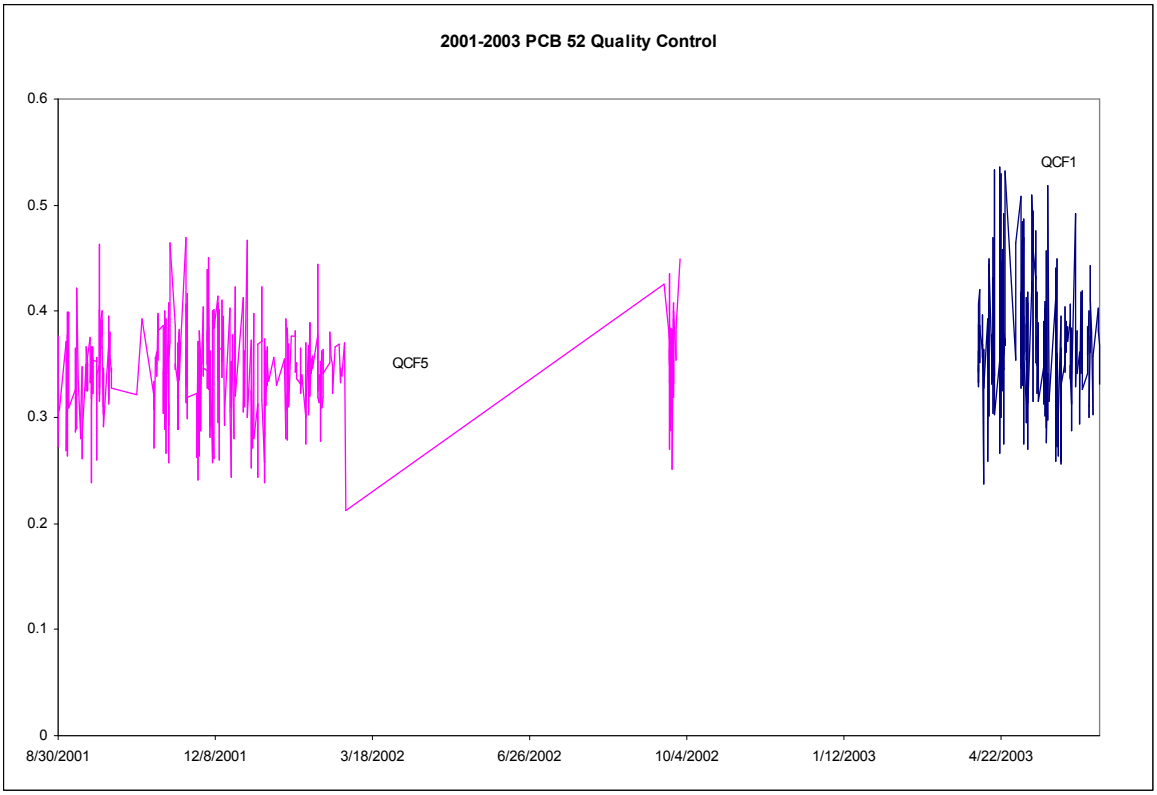
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	424	8/30/2001	9/30/2002	0.3207	0.0564	17.6



B. PCB52

Summary Statistics for PCB 52 by Lot

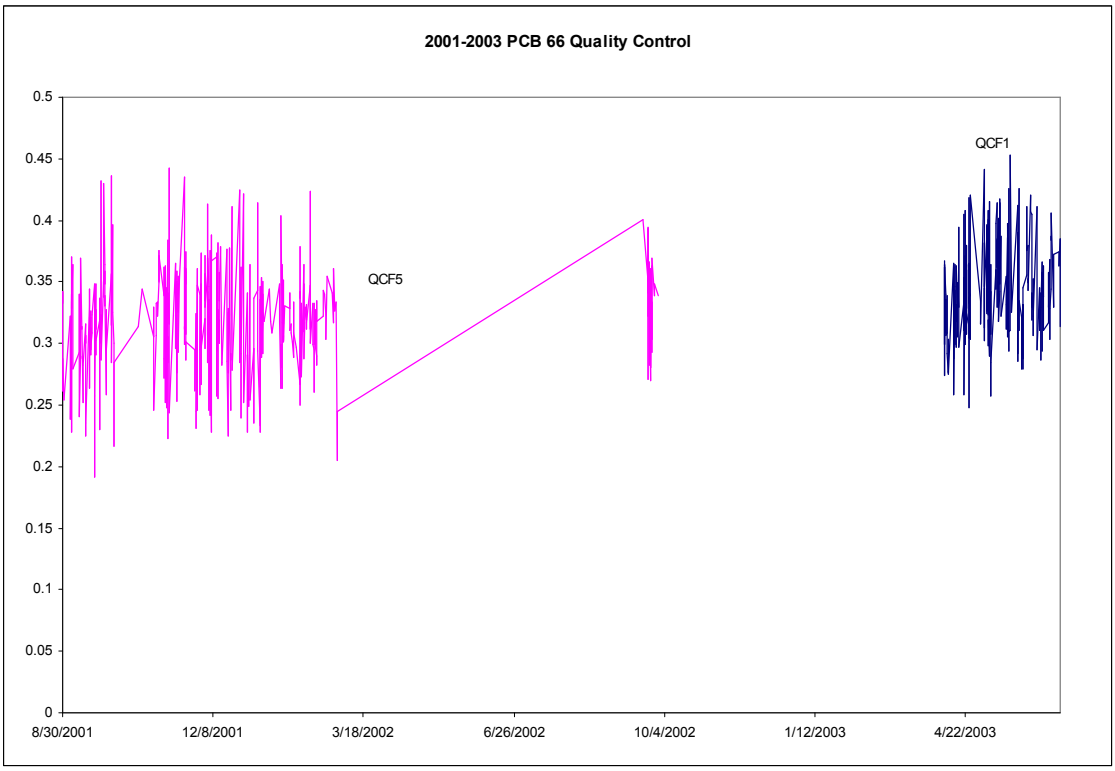
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	423	8/30/2001	9/30/2002	0.3452	0.042	12.2
QCF1	270	4/8/2003	6/24/2003	0.3722	0.0573	15.4



C. PCB 66

Summary Statistics for PCB 66 by Lot

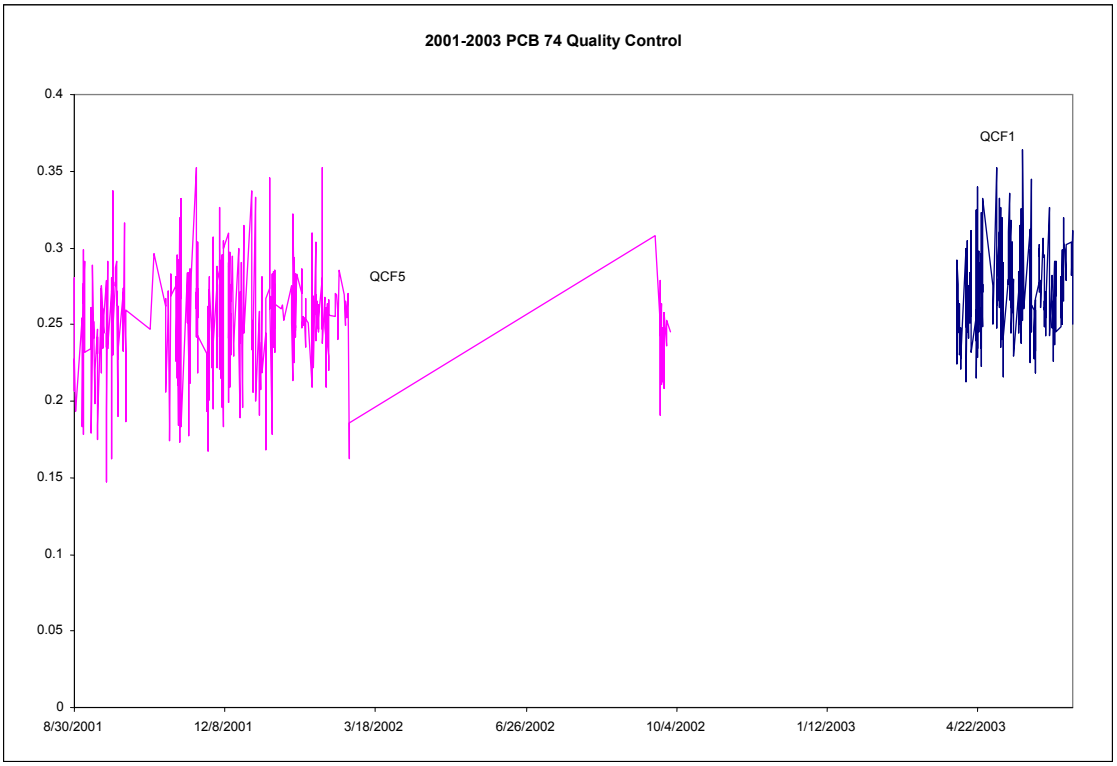
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	426	8/30/2001	9/30/2002	0.3181	0.0418	13.2
QCF1	270	4/8/2003	6/24/2003	0.3424	0.0371	10.8



D. PCB 74

Summary Statistics for PCB 74 by Lot

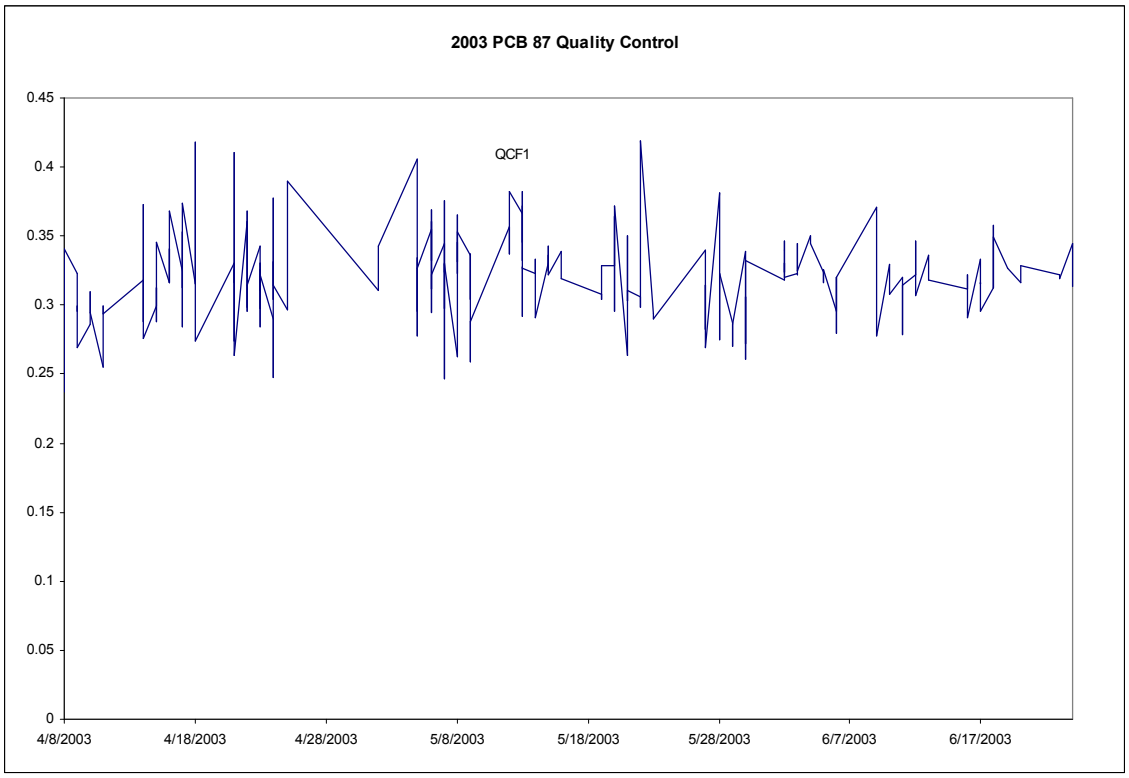
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	425	8/30/2001	9/30/2002	0.251	0.0333	13.3
QCF1	270	4/8/2003	6/24/2003	0.2712	0.0268	9.9



E. PCB 87

Summary Statistics for PCB 87 by Lot

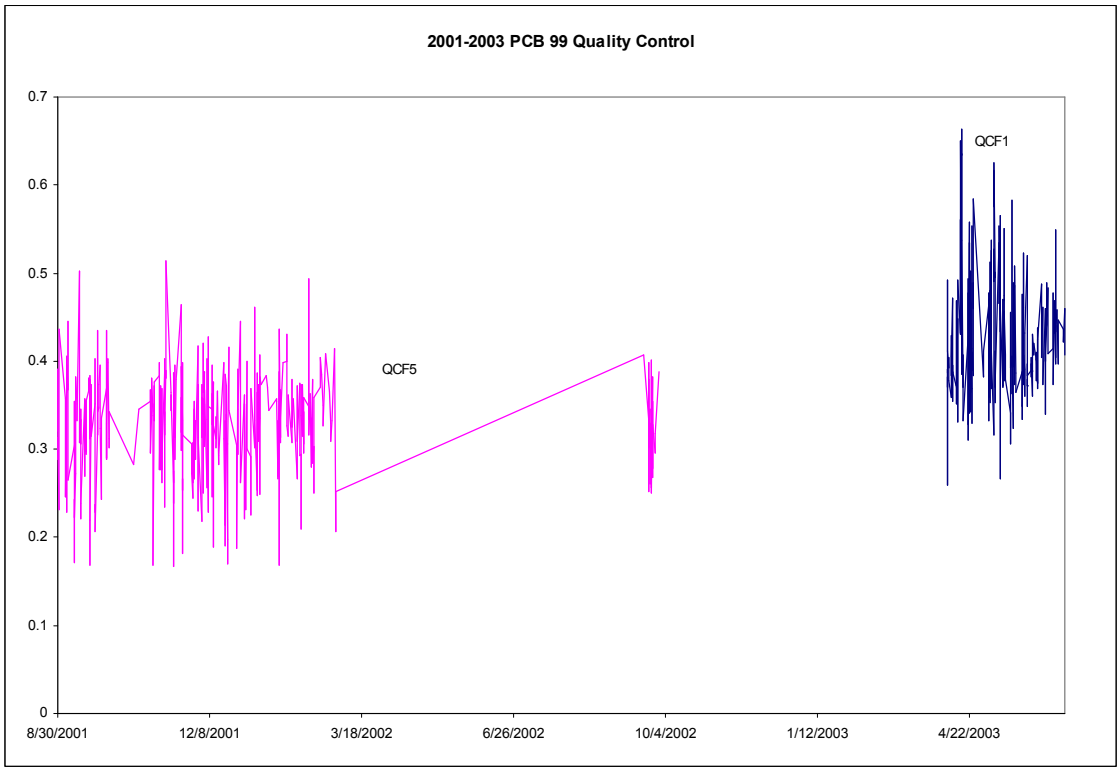
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF1	269	4/8/2003	6/24/2003	0.3199	0.0302	9.4



F. PCB 99

Summary Statistics for PCB 99 by Lot

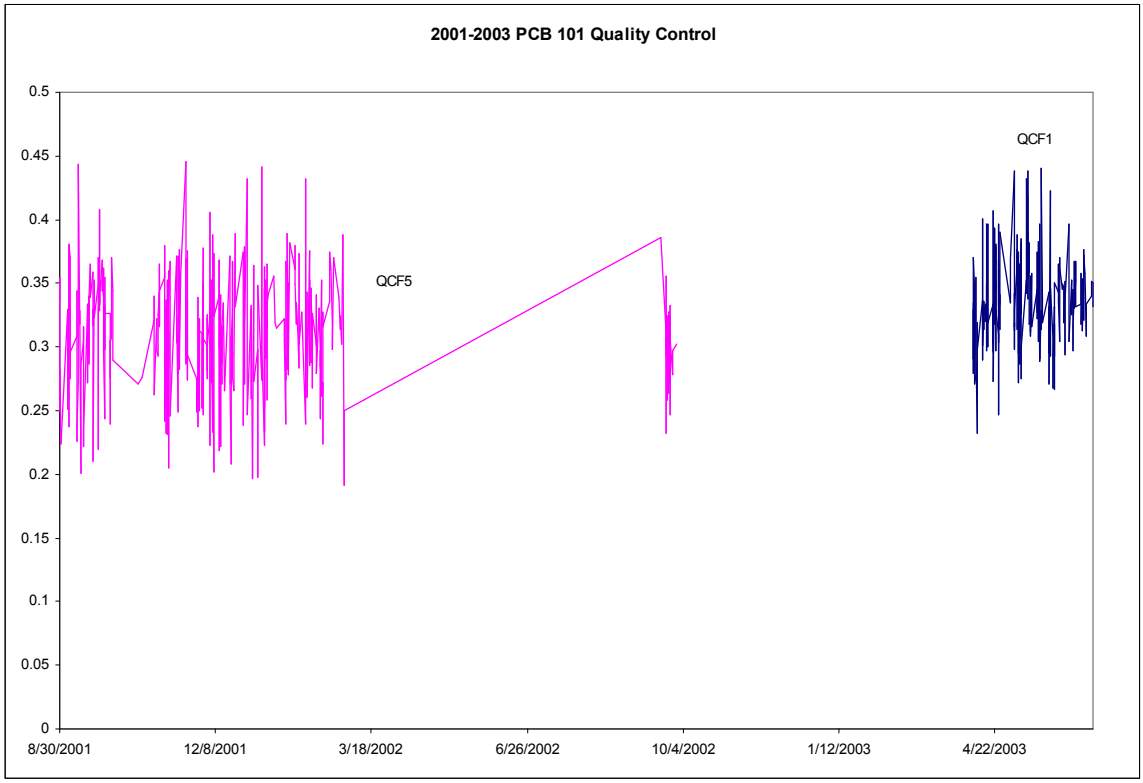
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	416	8/30/2001	9/30/2002	0.3287	0.0571	17.4
QCF1	267	4/8/2003	6/24/2003	0.4281	0.0674	15.7



G. PCB 101

Summary Statistics for PCB 101 by Lot

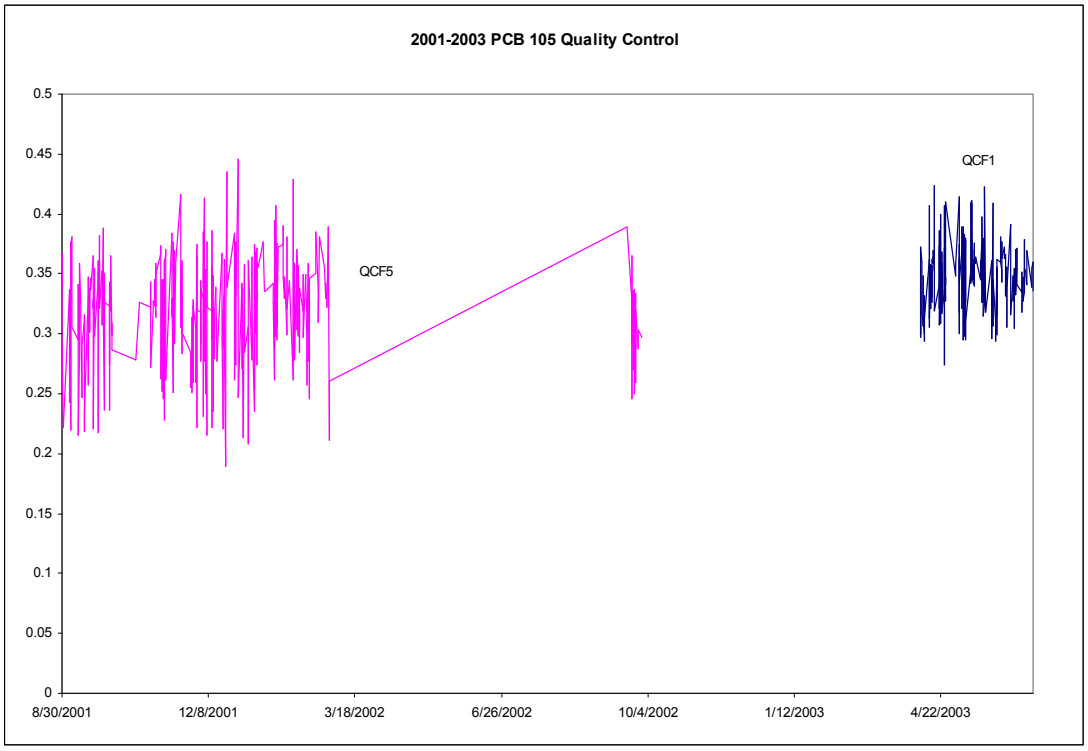
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	421	8/30/2001	9/30/2002	0.3103	0.0445	14.4
QCF1	270	4/8/2003	6/24/2003	0.3369	0.0339	10



H. PCB 105

Summary Statistics for PCB 105 by Lot

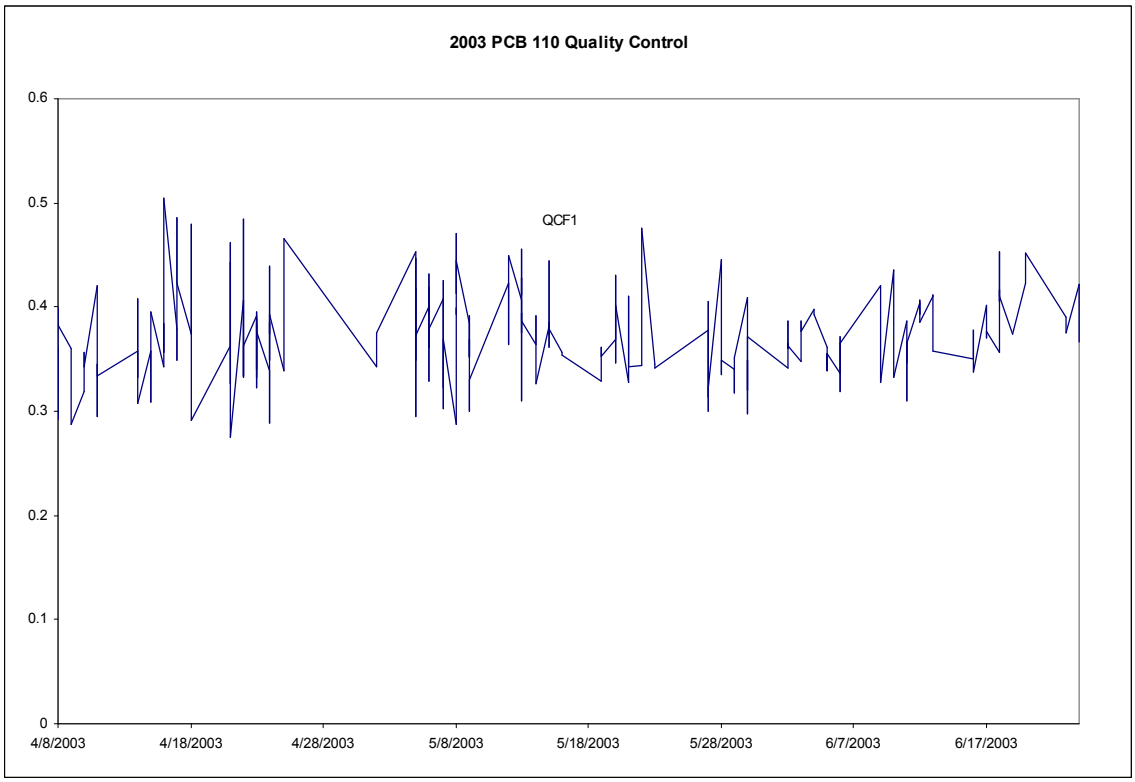
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	420	8/30/2001	9/30/2002	0.3178	0.0423	13.3
QCF1	270	4/8/2003	6/24/2003	0.3477	0.0267	7.7



I. PCB 110

Summary Statistics for PCB 110 by Lot

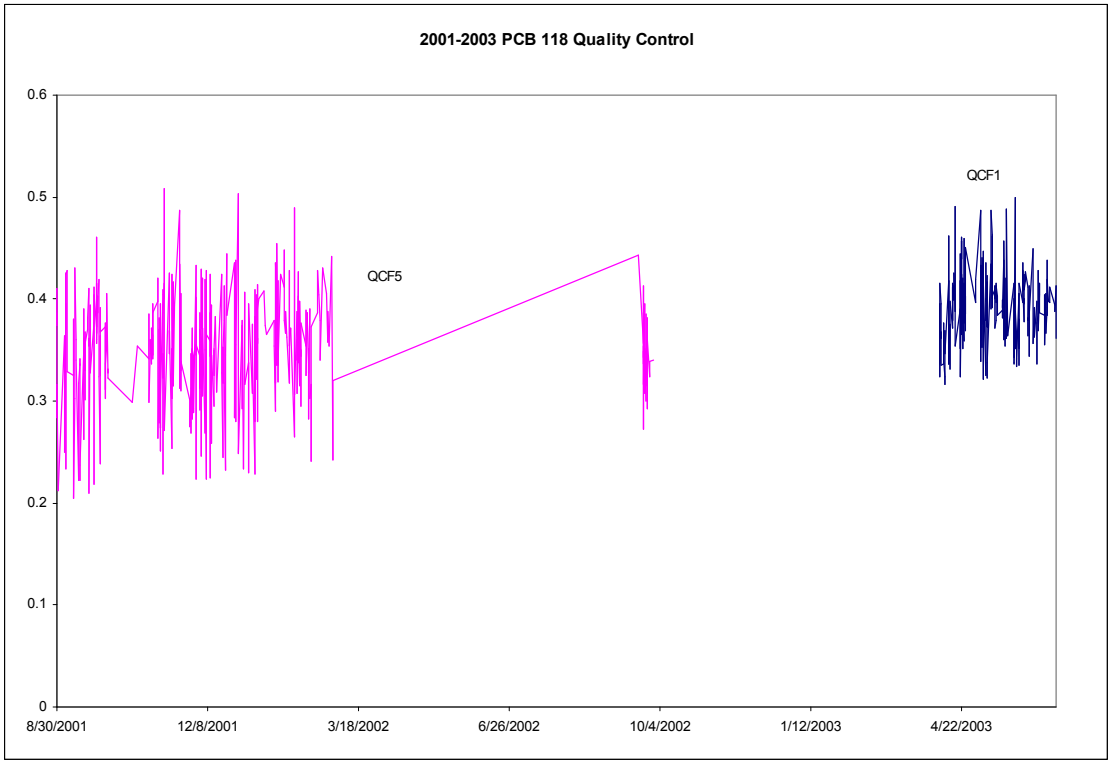
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF1	269	4/8/2003	6/24/2003	0.3726	0.0417	11.2



J. PCB 118

Summary Statistics for PCB 118 by Lot

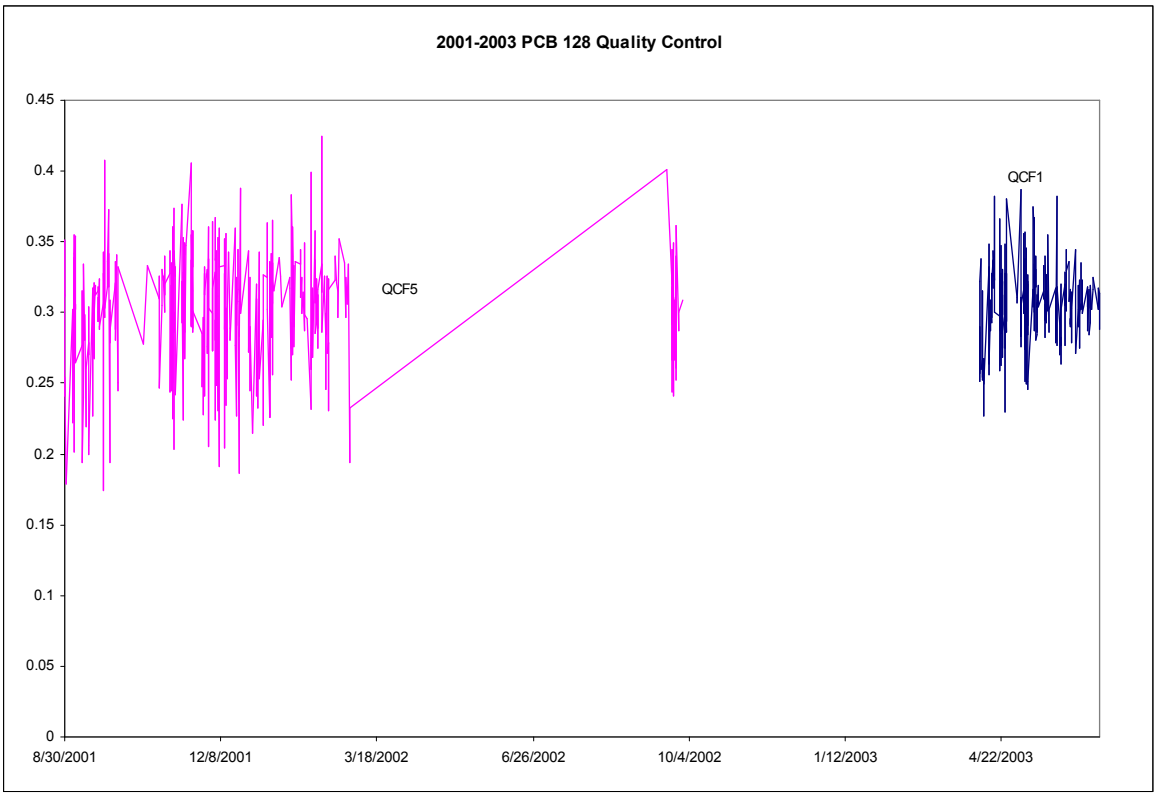
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	422	8/30/2001	9/30/2002	0.3491	0.053	15.2
QCF1	270	4/8/2003	6/24/2003	0.3923	0.0339	8.6



K. PCB 128

Summary Statistics for PCB 128 by Lot

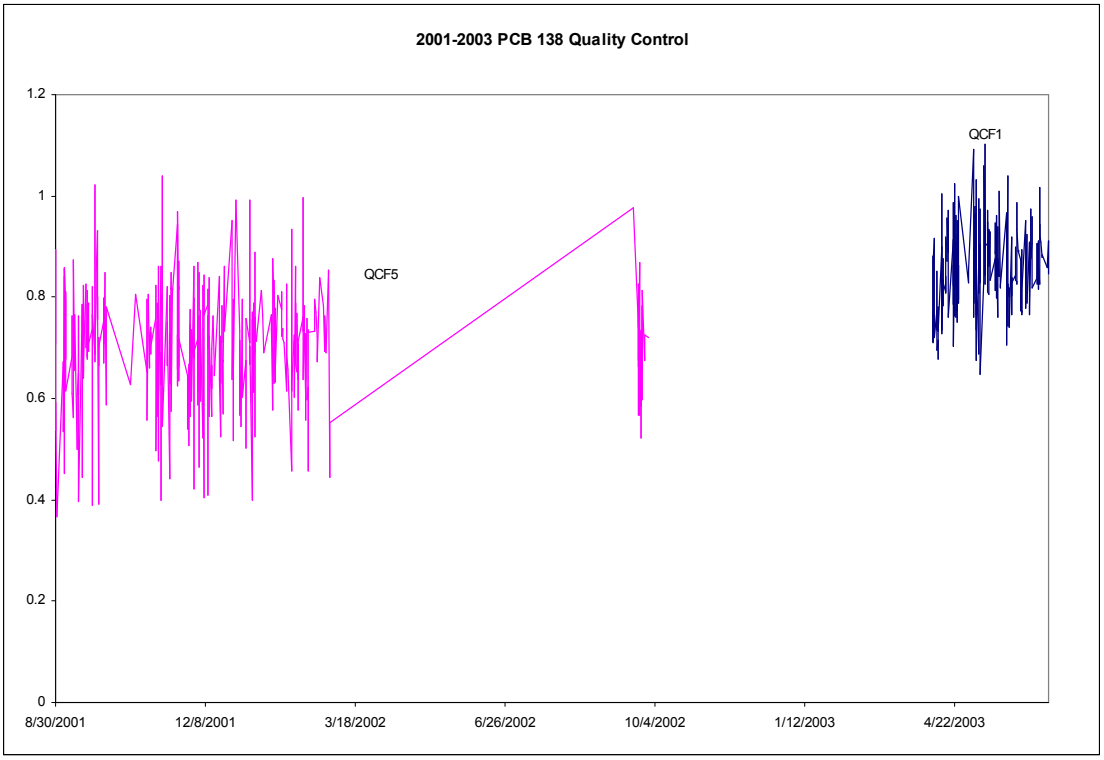
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	420	8/30/2001	9/30/2002	0.3011	0.0413	13.7
QCF1	269	4/8/2003	6/24/2003	0.3052	0.0266	8.7



L. PCB 138

Summary Statistics for PCB 138 by Lot

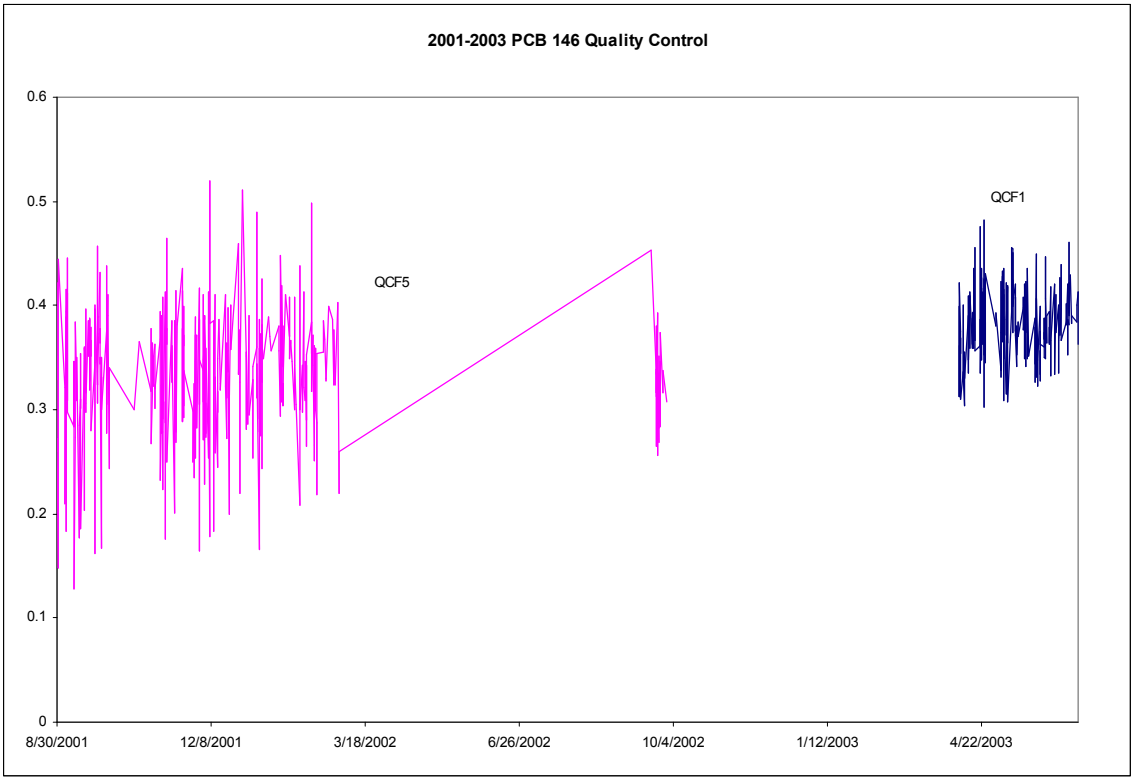
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	420	8/30/2001	9/30/2002	0.7006	0.1129	16.1
QCF1	268	4/8/2003	6/24/2003	0.8581	0.0784	9.1



M. PCB 146

Summary Statistics for PCB 146 by Lot

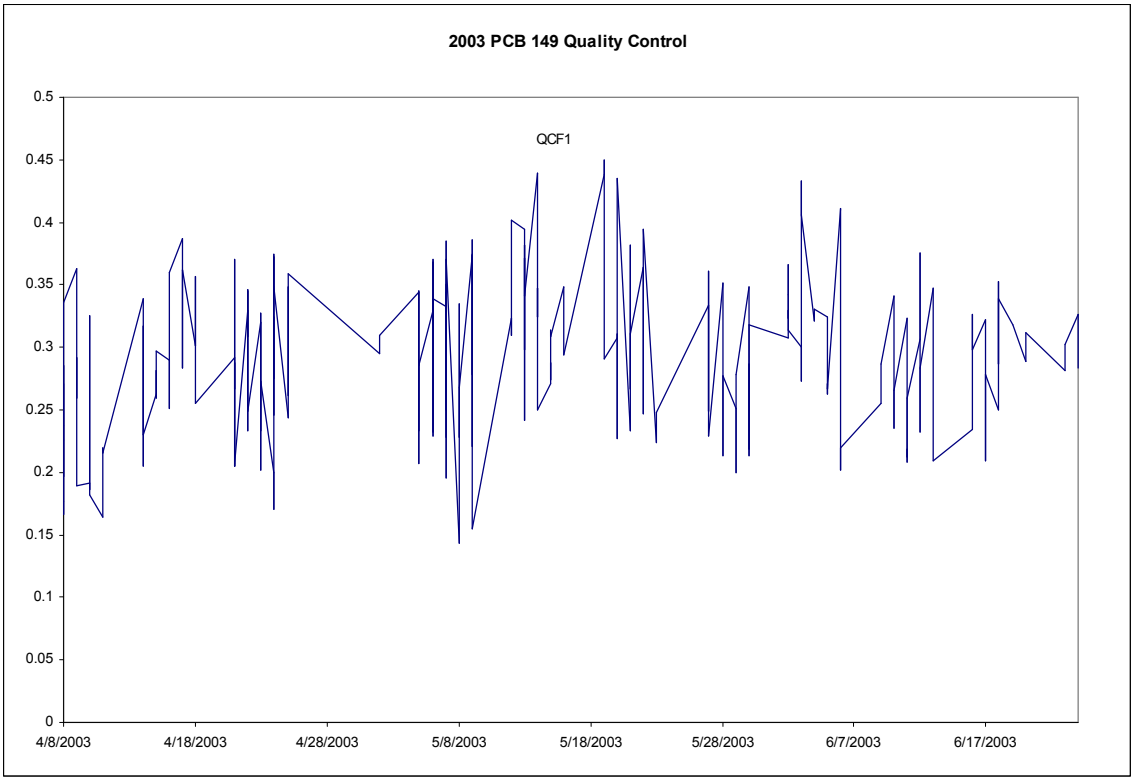
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	420	8/30/2001	9/30/2002	0.3321	0.0593	17.8
QCF1	269	4/8/2003	6/24/2003	0.3818	0.0333	8.7



N. PCB 149

Summary Statistics for PCB 149 by Lot

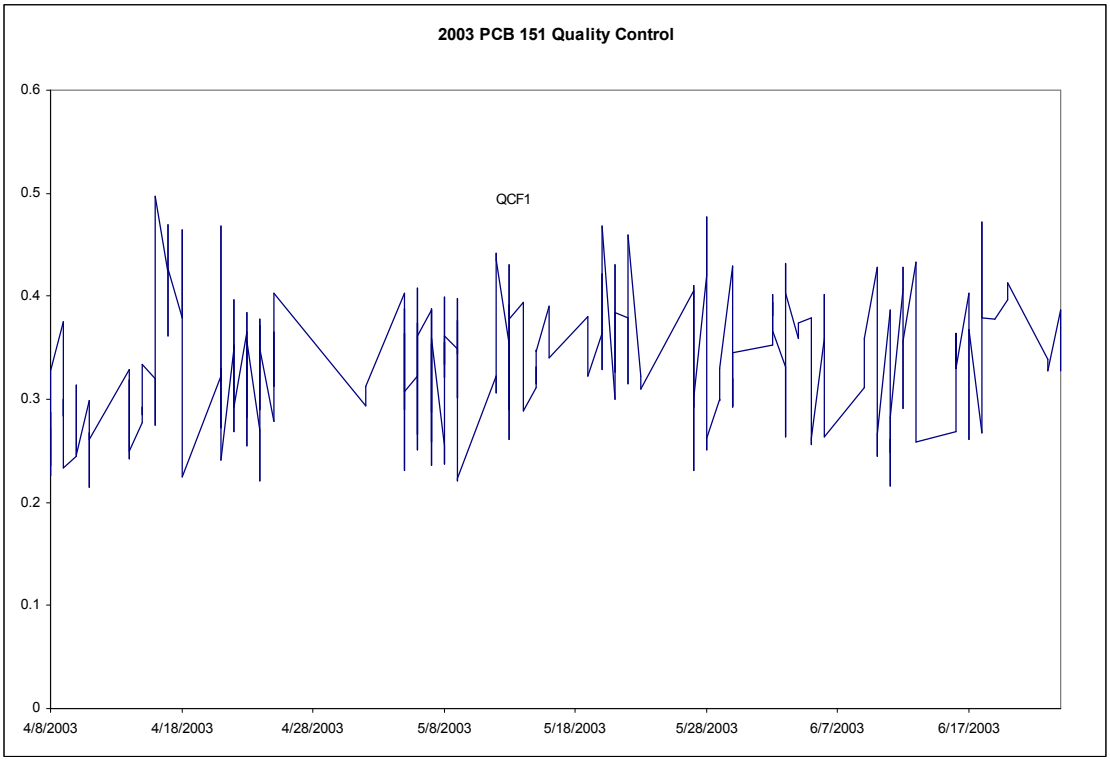
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF1	270	4/8/2003	6/24/2003	0.2913	0.0607	20.8



O. PCB 151

Summary Statistics for PCB 151 by Lot

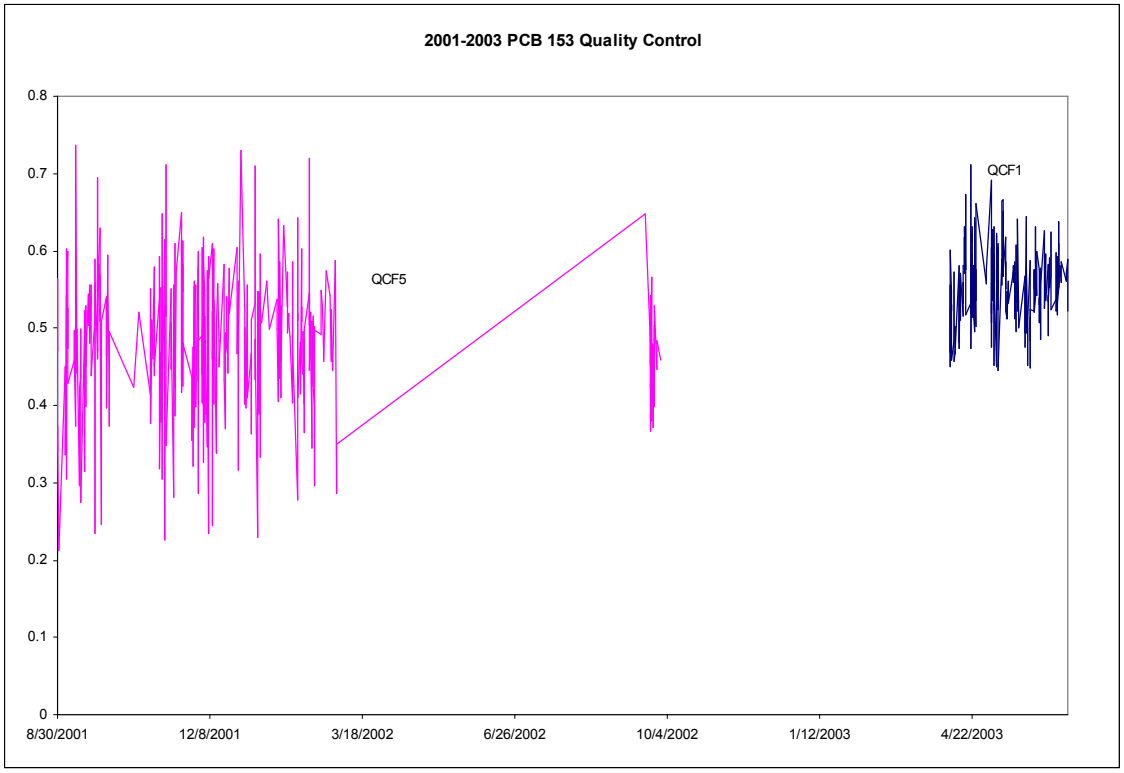
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF1	270	4/8/2003	6/24/2003	0.3329	0.0603	18.1



P. PCB 153

Summary Statistics for PCB 153 by Lot

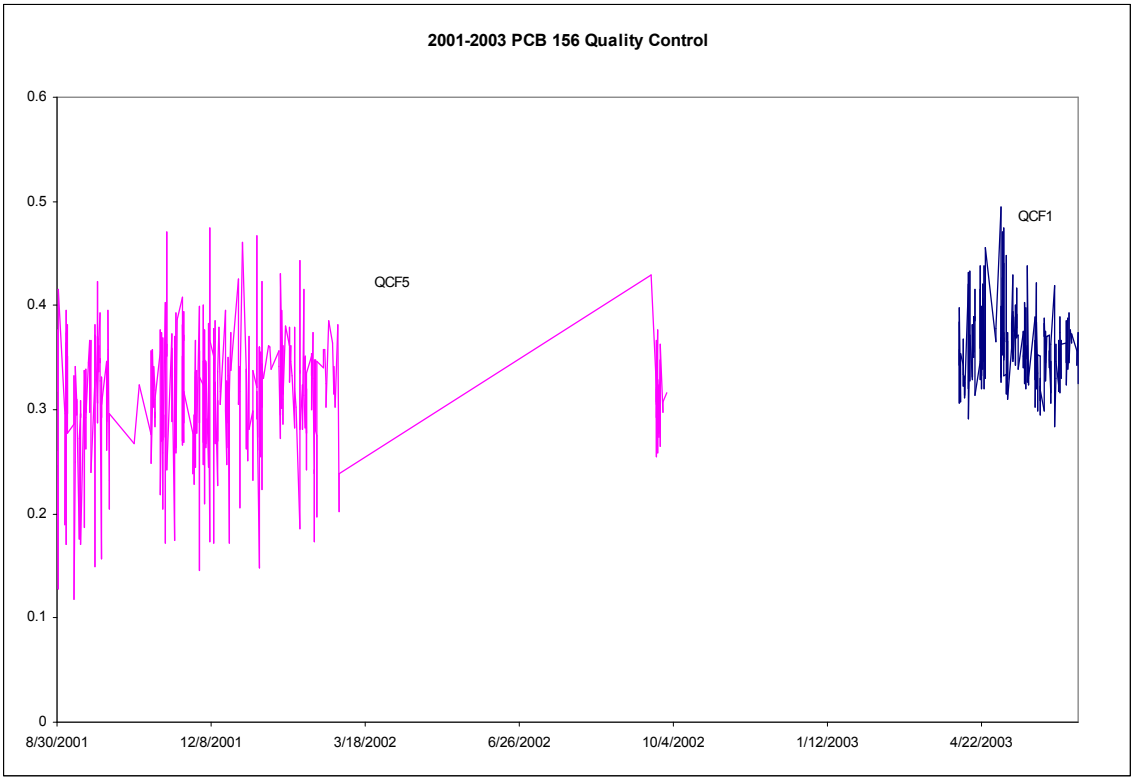
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	419	8/30/2001	9/30/2002	0.477	0.0874	18.3
QCF1	270	4/8/2003	6/24/2003	0.5546	0.048	8.7



Q. PCB 156

Summary Statistics for PCB 156 by Lot

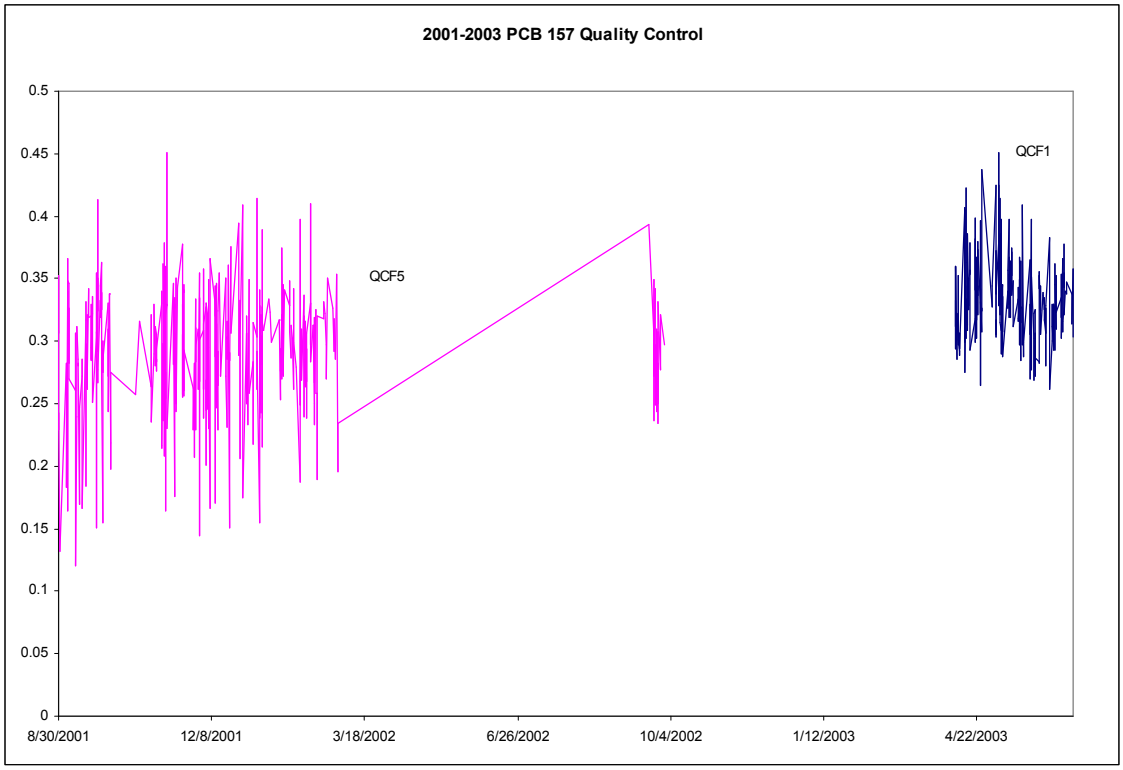
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	419	8/30/2001	9/30/2002	0.3126	0.0572	18.3
QCF1	269	4/8/2003	6/24/2003	0.3609	0.0349	9.7



R. PCB 157

Summary Statistics for PCB 157 by Lot

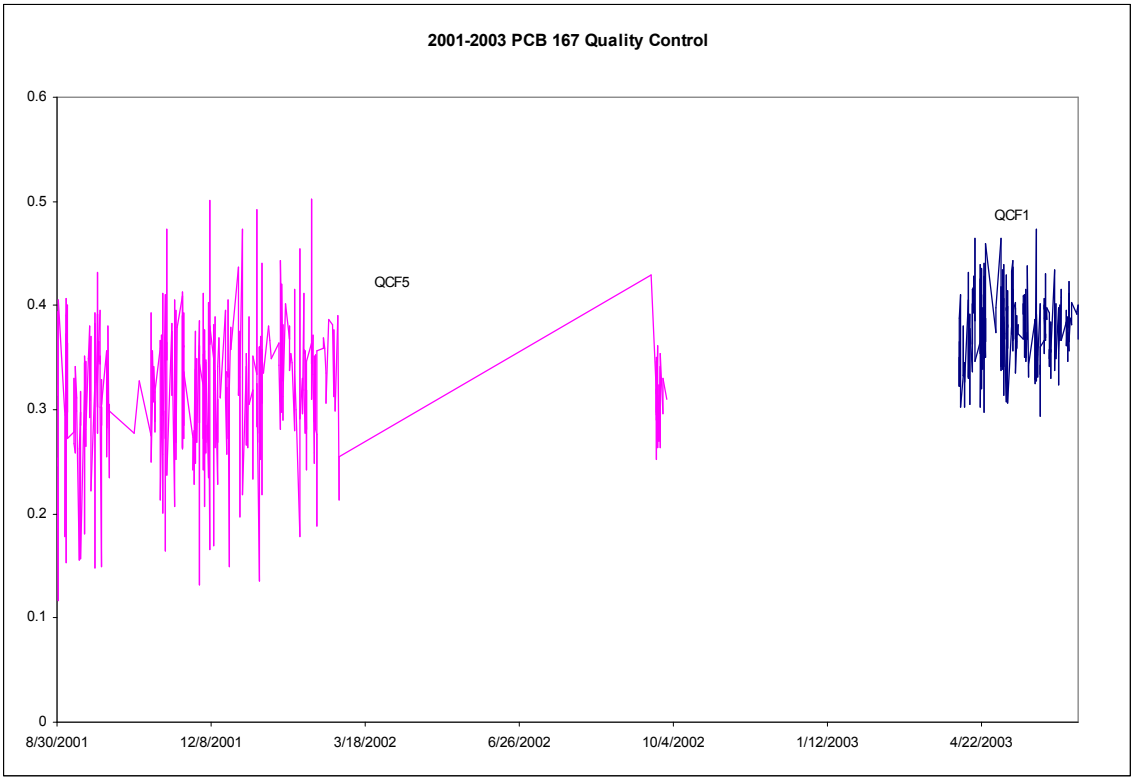
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	417	8/30/2001	9/30/2002	0.2906	0.0494	17
QCF1	269	4/8/2003	6/24/2003	0.3329	0.0323	9.7



S. PCB 167

Summary Statistics for PCB 167 by Lot

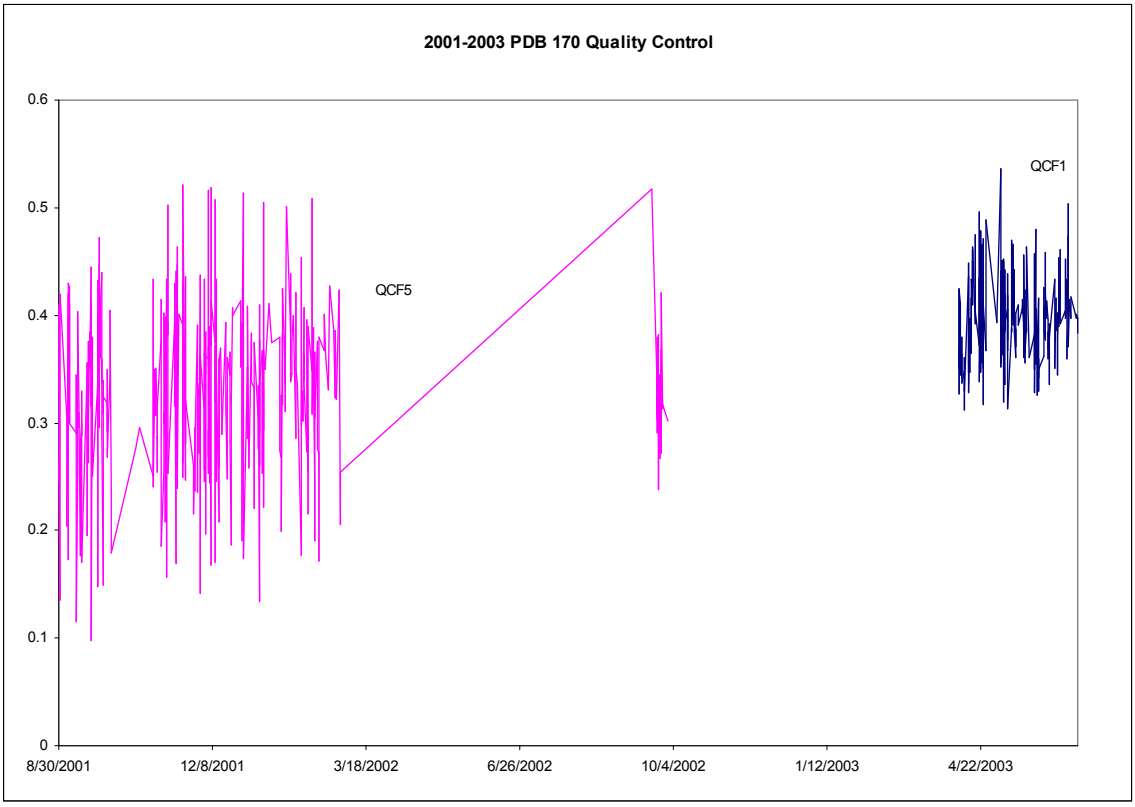
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	420	8/30/2001	9/30/2002	0.3172	0.061	19.2
QCF1	269	4/8/2003	6/24/2003	0.3759	0.0333	8.8



T. PCB170

Summary Statistics for PCB 170 by Lot

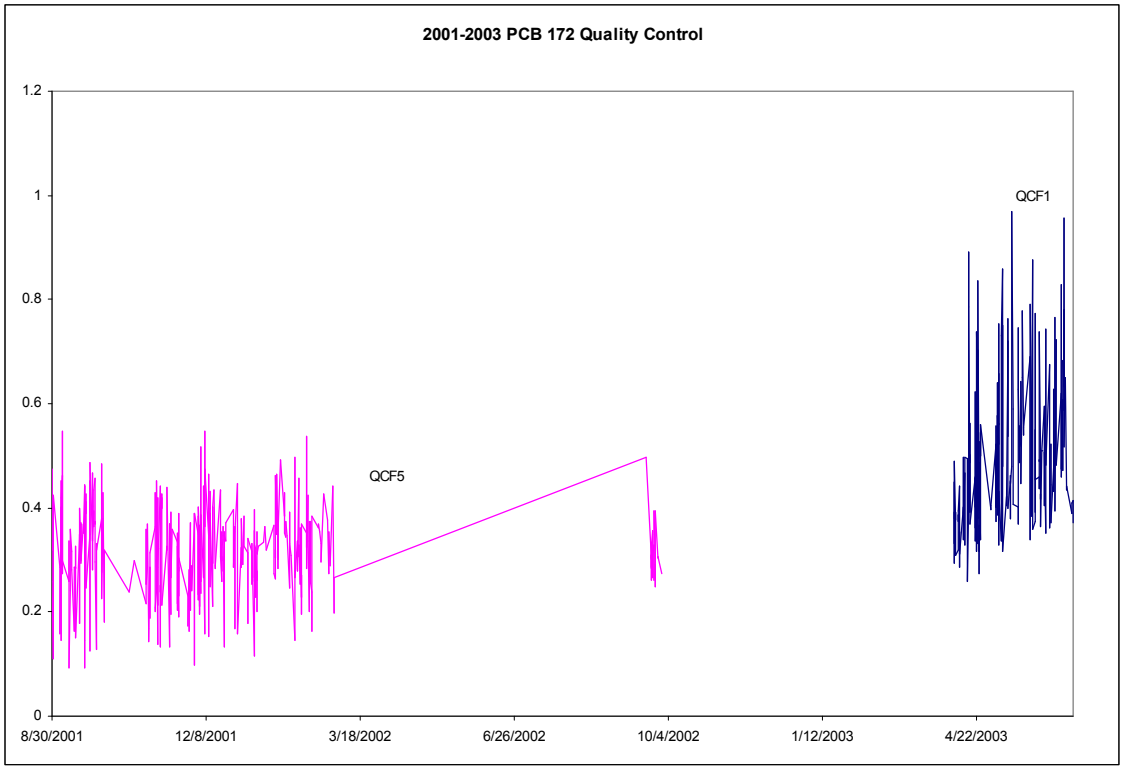
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	423	8/30/2001	9/30/2002	0.3259	0.0741	22.7
QCF1	270	4/8/2003	6/24/2003	0.3959	0.0384	9.7



U. PCB172

Summary Statistics for PCB 172 by Lot

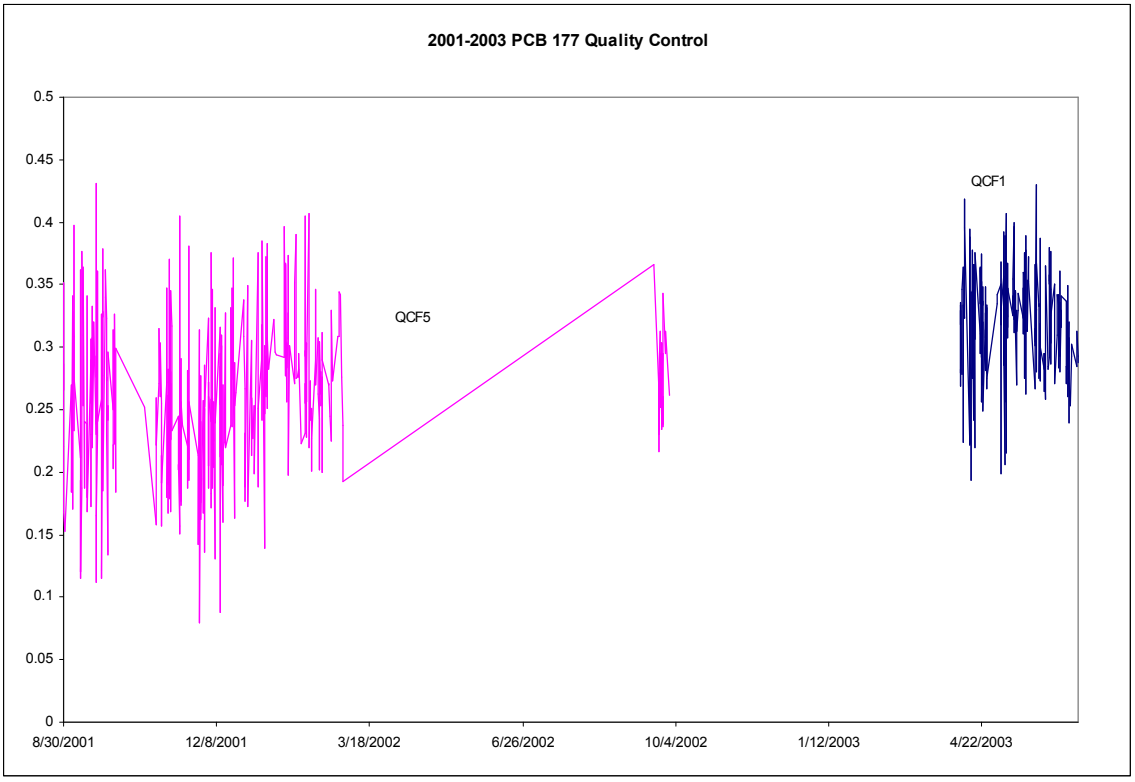
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	423	8/30/2001	9/30/2002	0.3117	0.0833	26.7
QCF1	258	4/8/2003	6/24/2003	0.5014	0.1403	28



V. PCB 177

Summary Statistics for PCB 177 by Lot

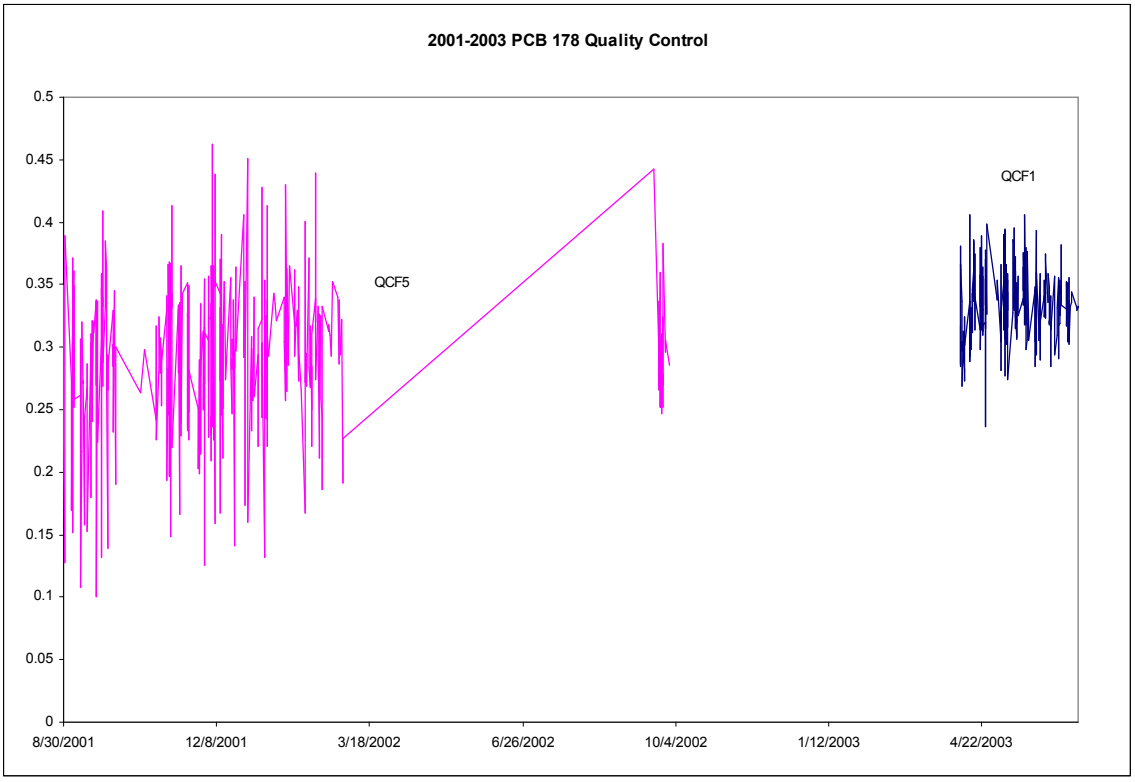
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	414	8/30/2001	9/30/2002	0.2595	0.0608	23.4
QCF1	268	4/8/2003	6/24/2003	0.3192	0.0406	12.7



W. PCB 178

Summary Statistics for PCB 178 by Lot

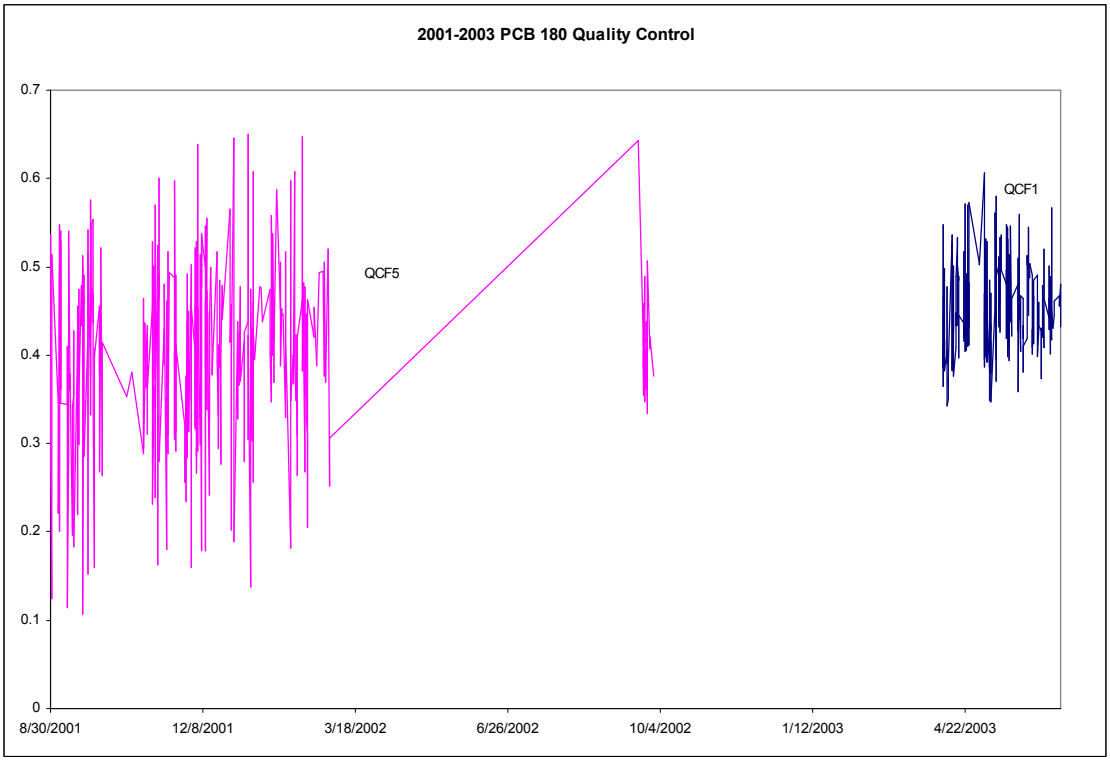
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	424	8/30/2001	9/30/2002	0.2899	0.0569	19.6
QCF1	269	4/8/2003	6/24/2003	0.3331	0.0274	8.2



X. PCB 180

Summary Statistics for PCB 180 by Lot

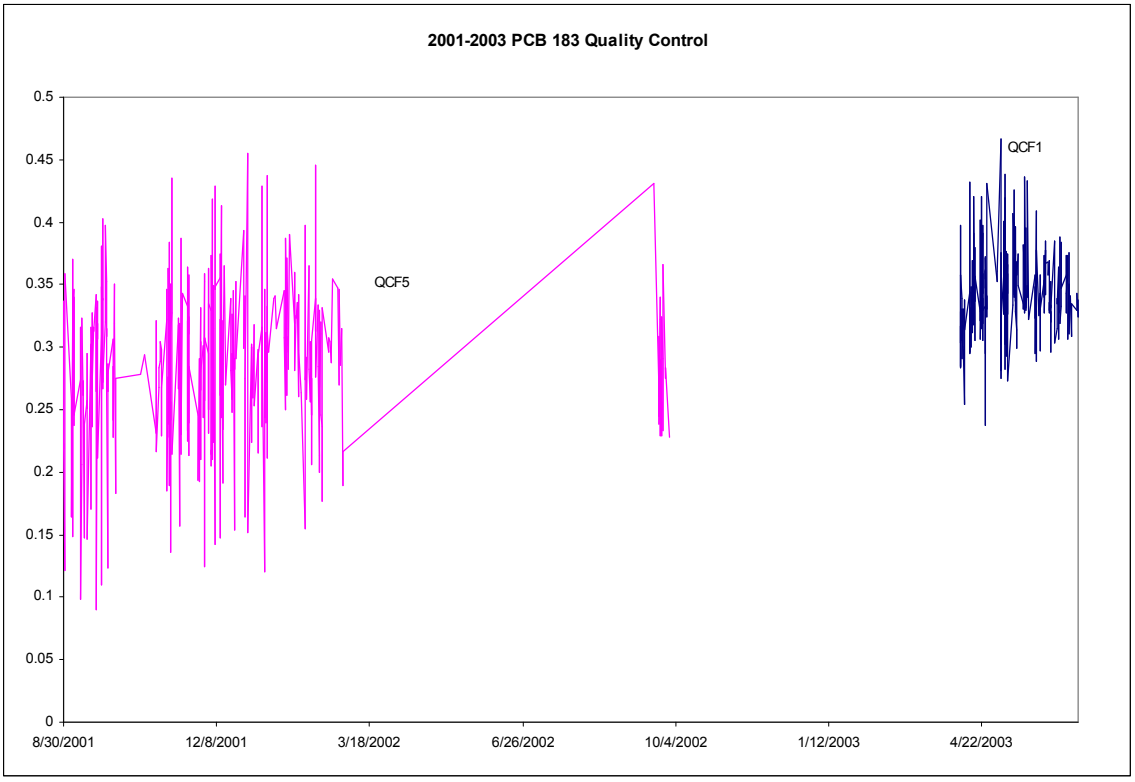
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	423	8/30/2001	9/30/2002	0.3969	0.0948	23.9
QCF1	270	4/8/2003	6/24/2003	0.4535	0.0496	10.9



Y. PCB 183

Summary Statistics for PCB 183 by Lot

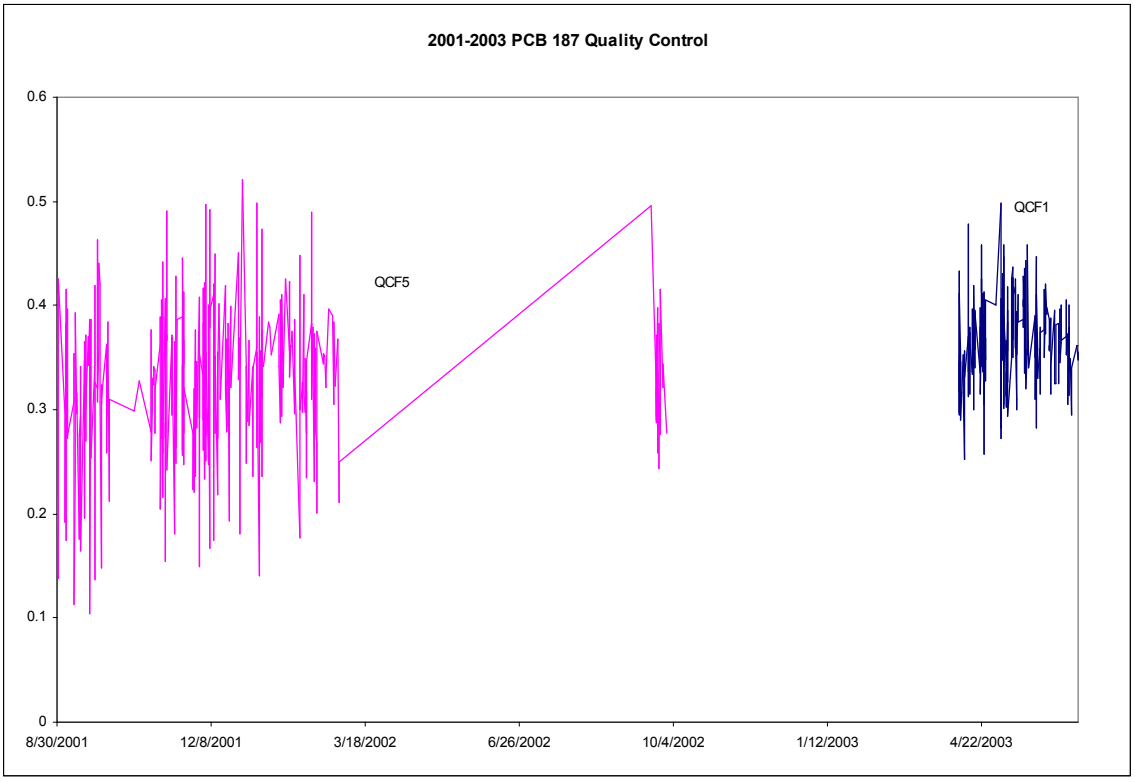
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	424	8/30/2001	9/30/2002	0.2836	0.0595	21
QCF1	270	4/8/2003	6/24/2003	0.3447	0.0337	9.8



Z. PCB 187

Summary Statistics for PCB 187 by Lot

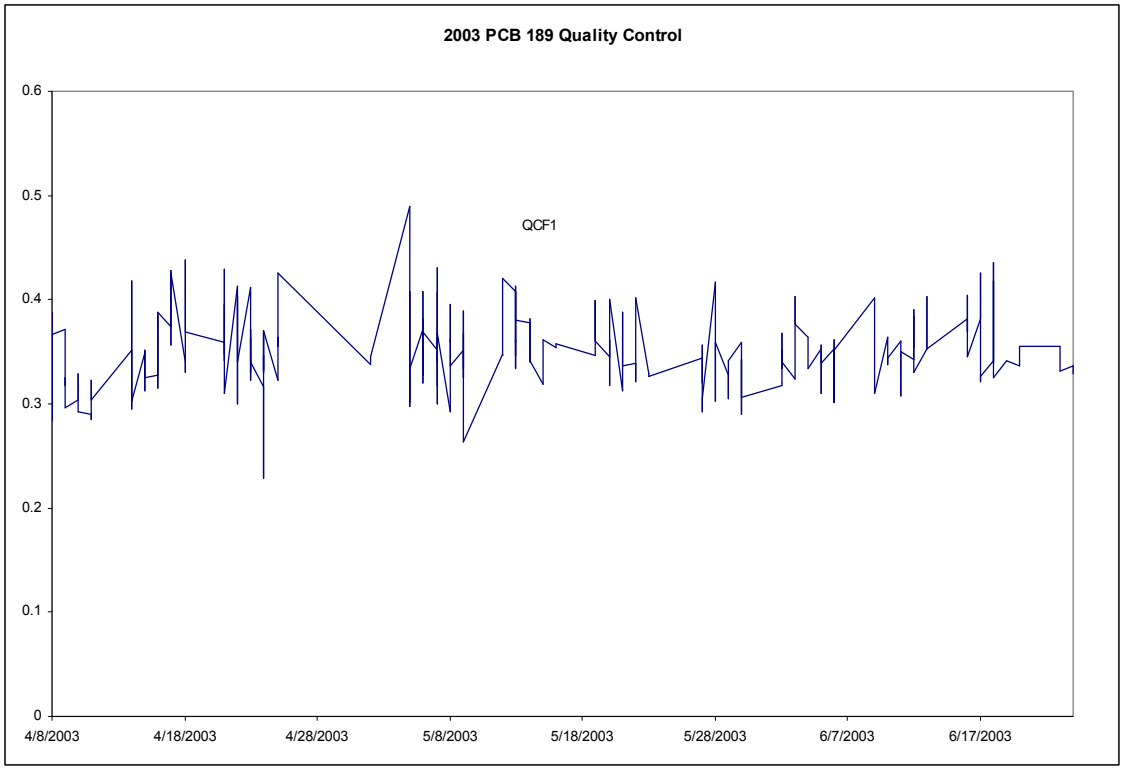
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	424	8/30/2001	9/30/2002	0.3233	0.0666	20.6
QCF1	270	4/8/2003	6/24/2003	0.3651	0.0388	10.6



AA. PCB 189

Summary Statistics for PCB 189 by Lot

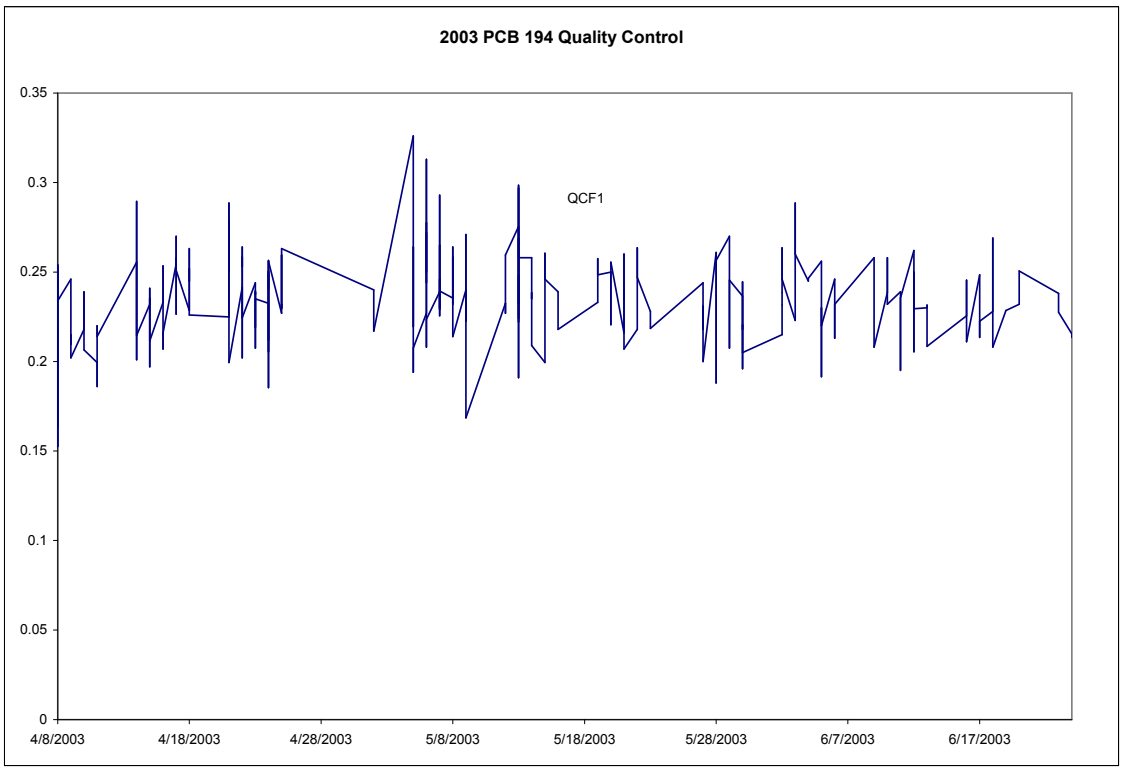
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF1	270	4/8/2003	6/24/2003	0.35	0.0358	10.2



BB. PCB 194

Summary Statistics for PCB 194 by Lot

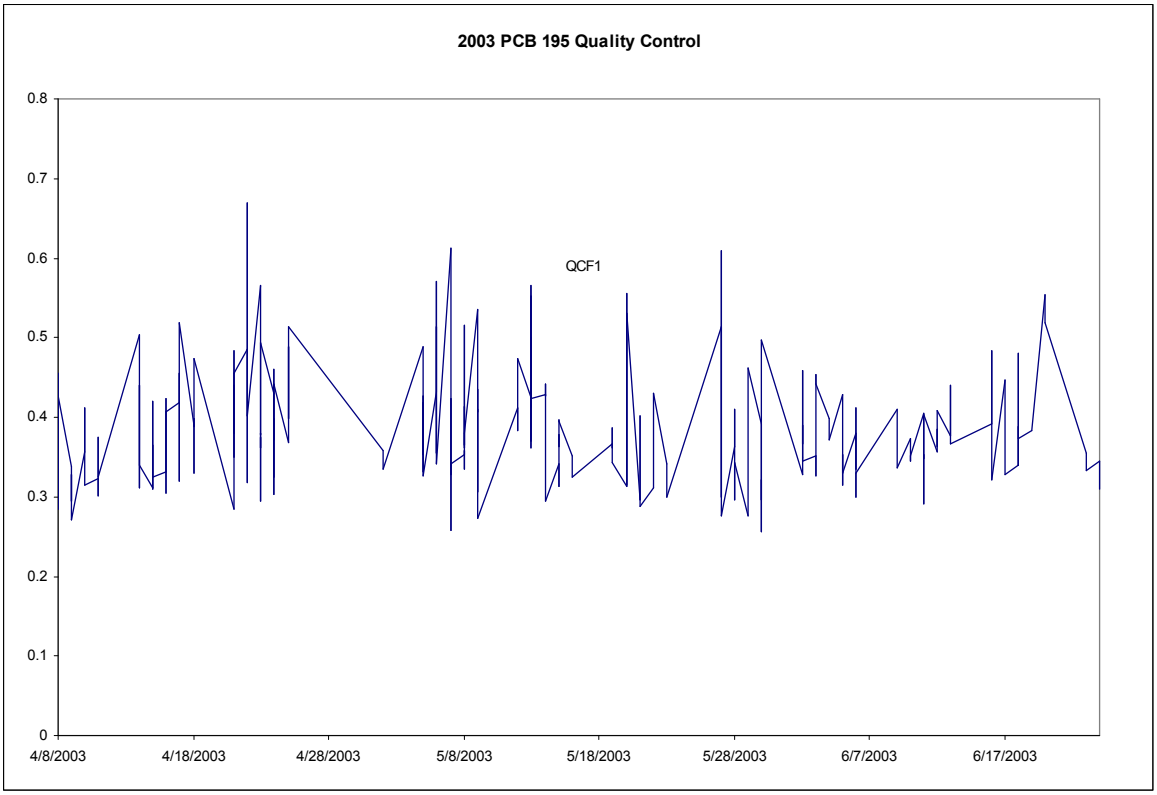
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF1	270	4/8/2003	6/24/2003	0.2339	0.024	10.3



CC. PCB195

Summary Statistics for PCB 195 by Lot

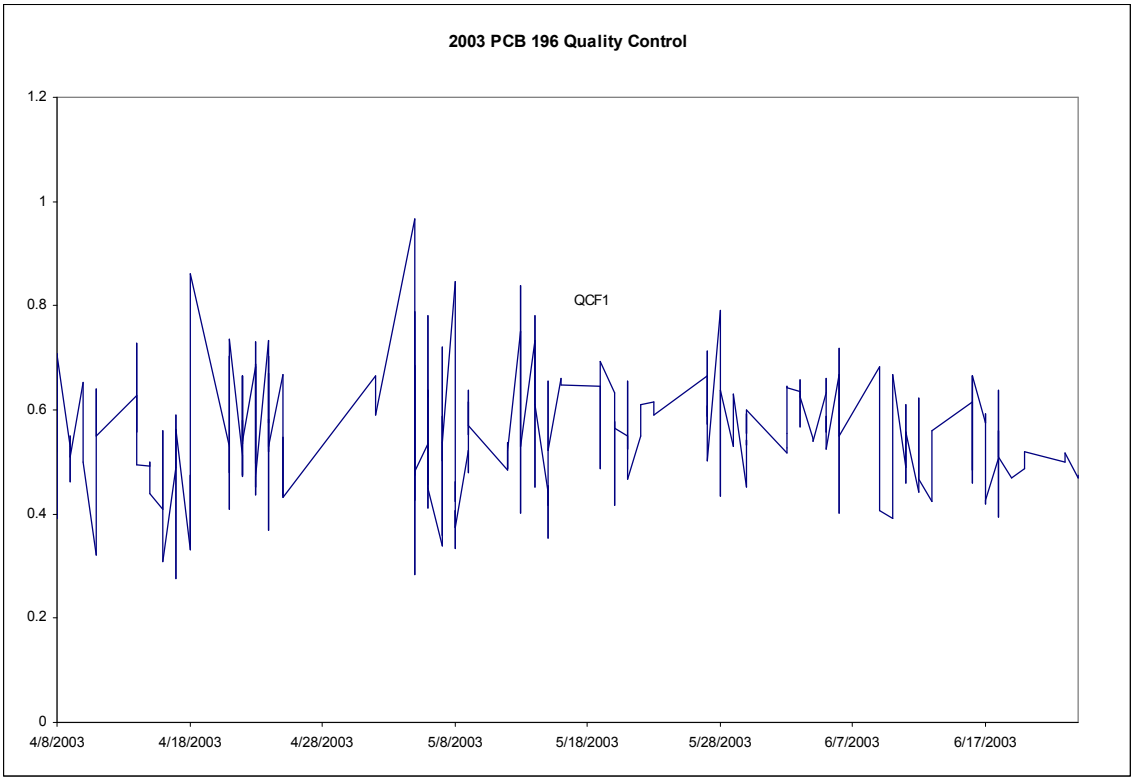
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF1	270	4/8/2003	6/24/2003	0.3853	0.0703	18.2



DD. PCB 196

Summary Statistics for PCB 196 by Lot

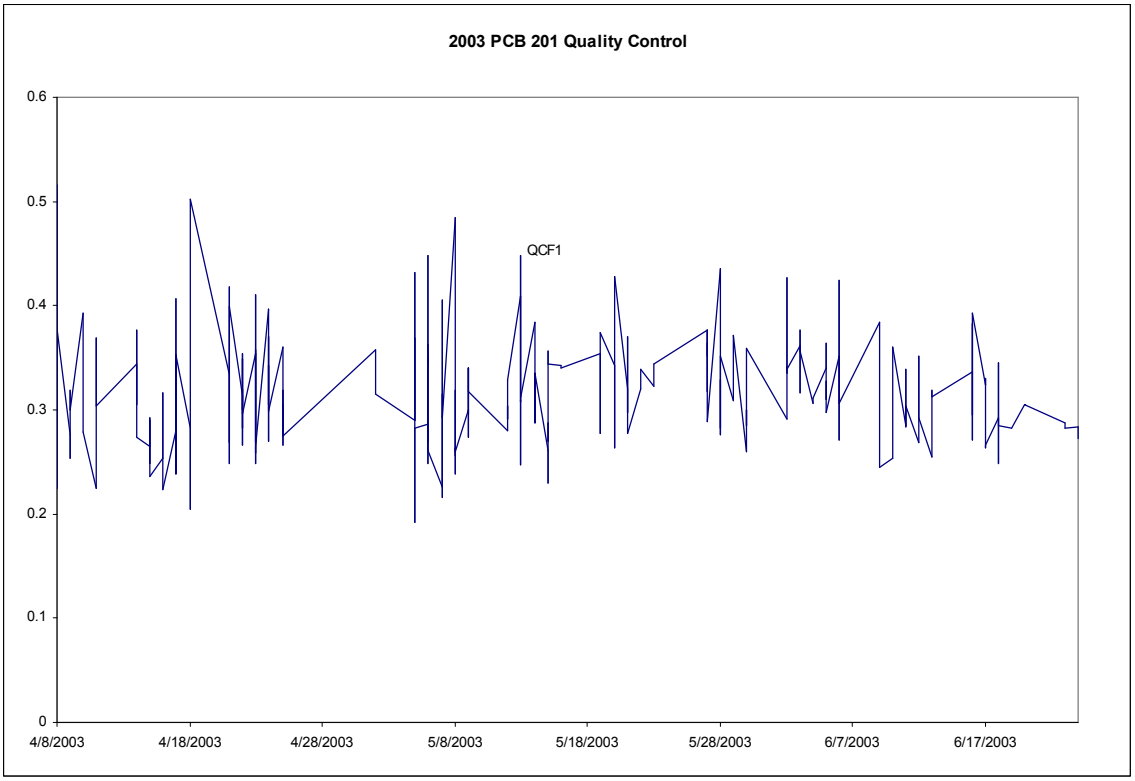
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF1	270	4/8/2003	6/24/2003	0.5503	0.1087	19.7



EE. PCB 201

Summary Statistics for PCB 201 by Lot

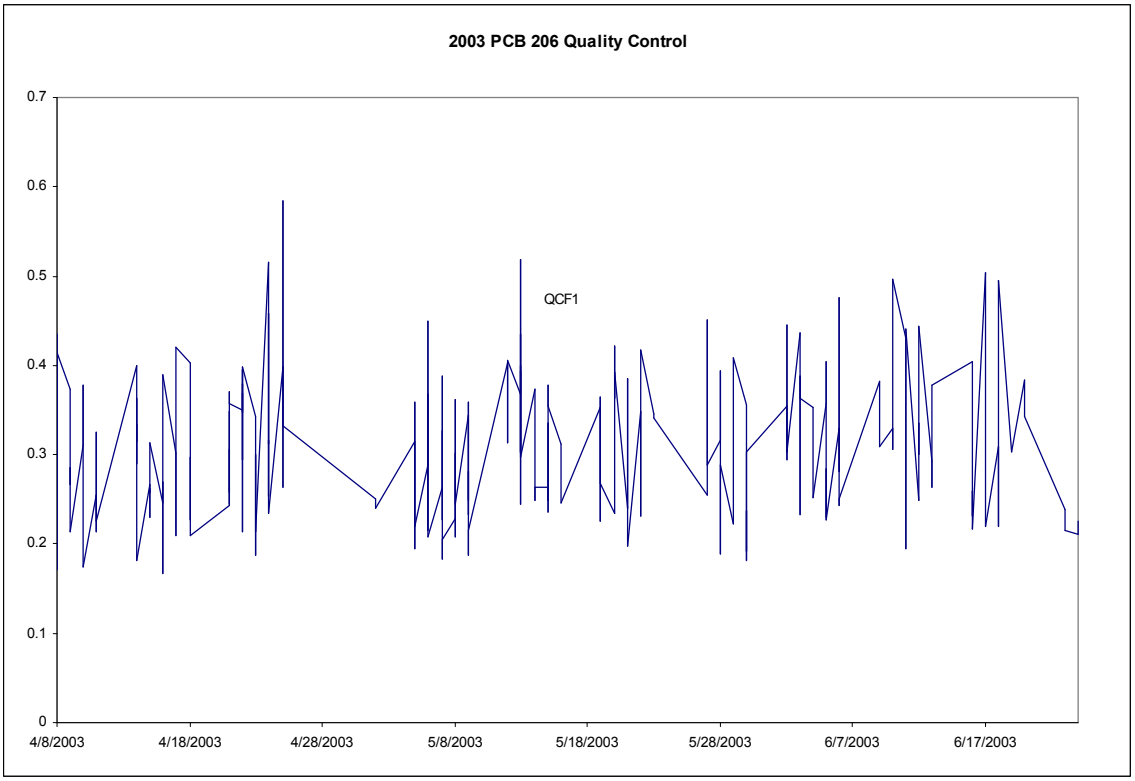
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF1	269	4/8/2003	6/24/2003	0.3158	0.0512	16.2



FF. PCB 206

Summary Statistics for PCB 206 by Lot

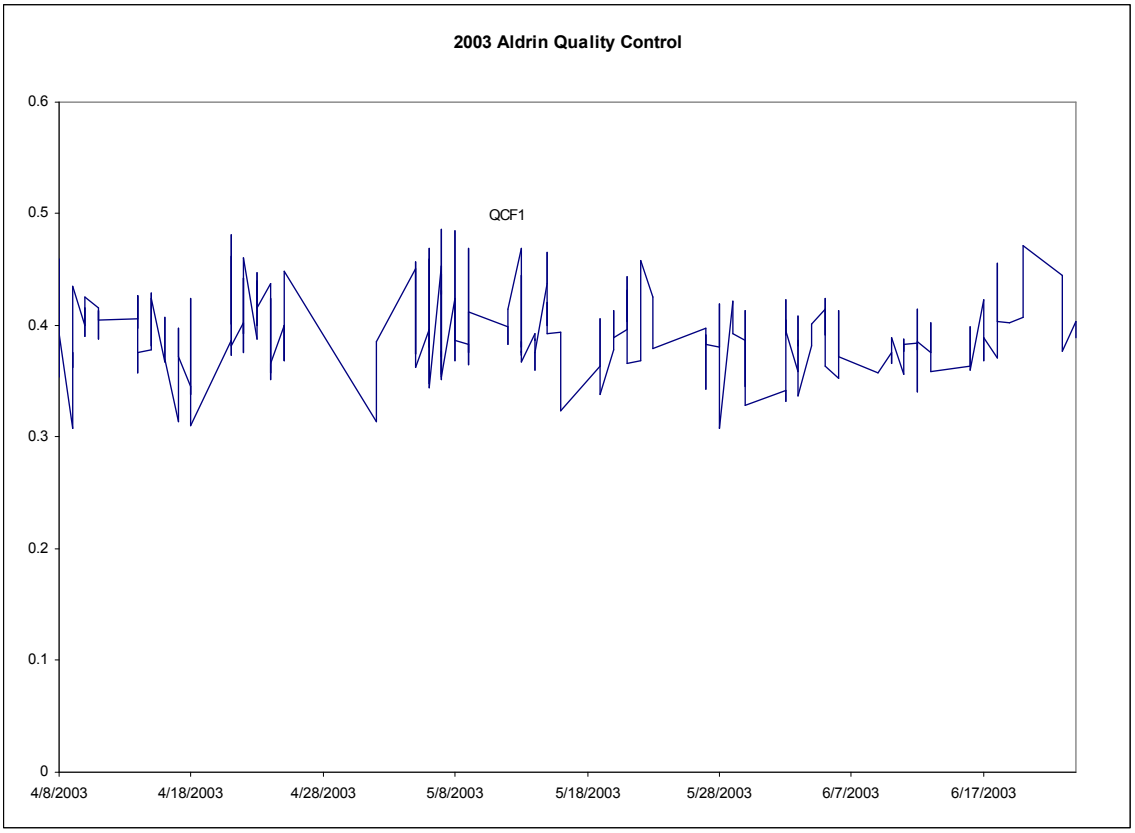
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF1	266	4/8/2003	6/24/2003	0.3065	0.0759	24.7



GG. Aldrin

Summary Statistics for Aldrin by Lot

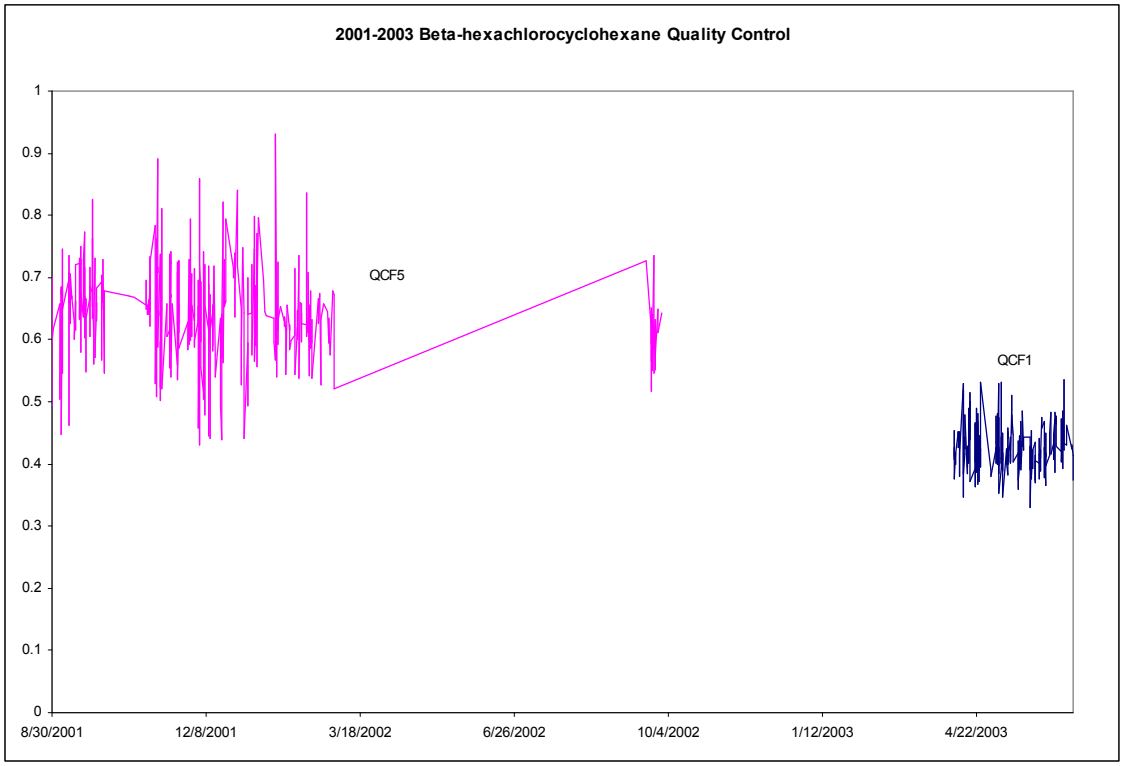
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF1	266	4/8/2003	6/24/2003	0.3946	0.0336	8.5



HH. **Beta-Hexachloro-Cyclohexane**

Summary Statistics for Beta-Hexachloro-Cyclohexane by Lot

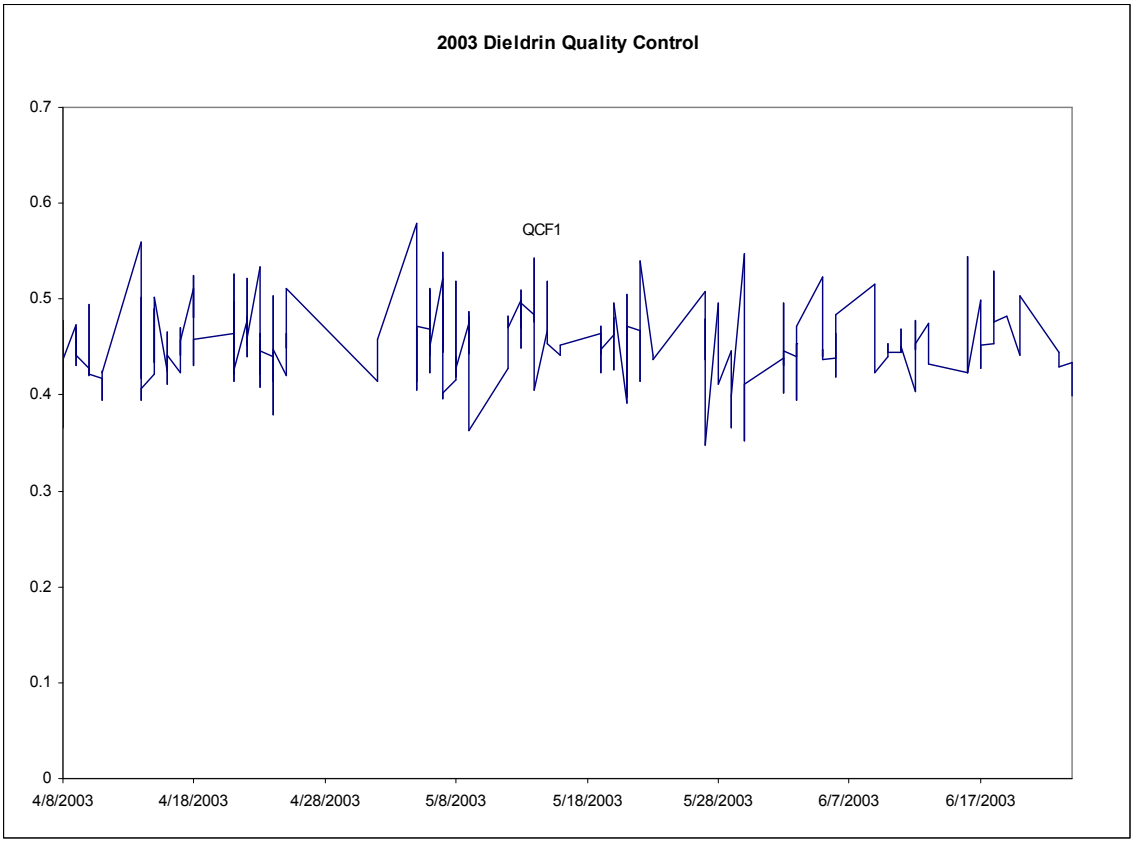
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	421	8/30/2001	9/30/2002	0.6433	0.0727	11.3
QCF1	269	4/8/2003	6/24/2003	0.4246	0.0355	8.4



II. Dieldrin

Summary Statistics for Dieldrin by Lot

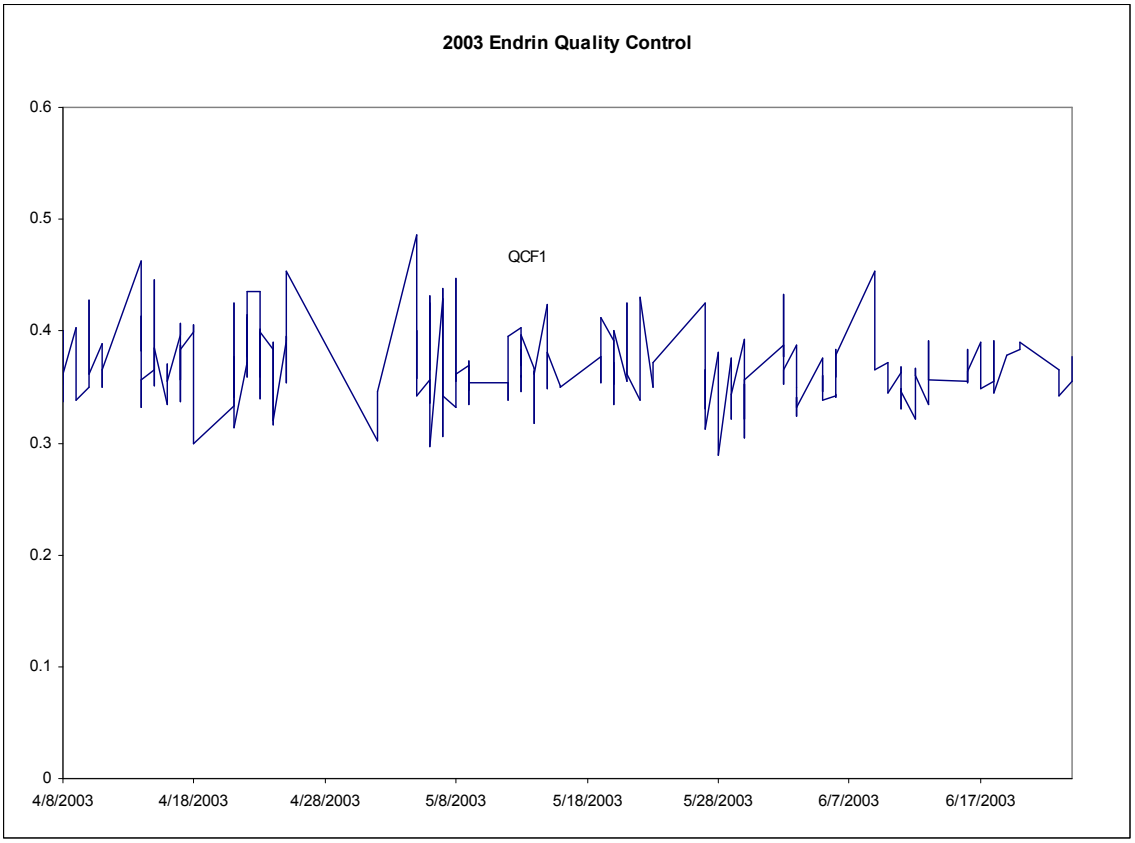
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF1	261	4/8/2003	6/24/2003	0.4551	0.0384	8.4



JJ. Endrin

Summary Statistics for Endrin by Lot

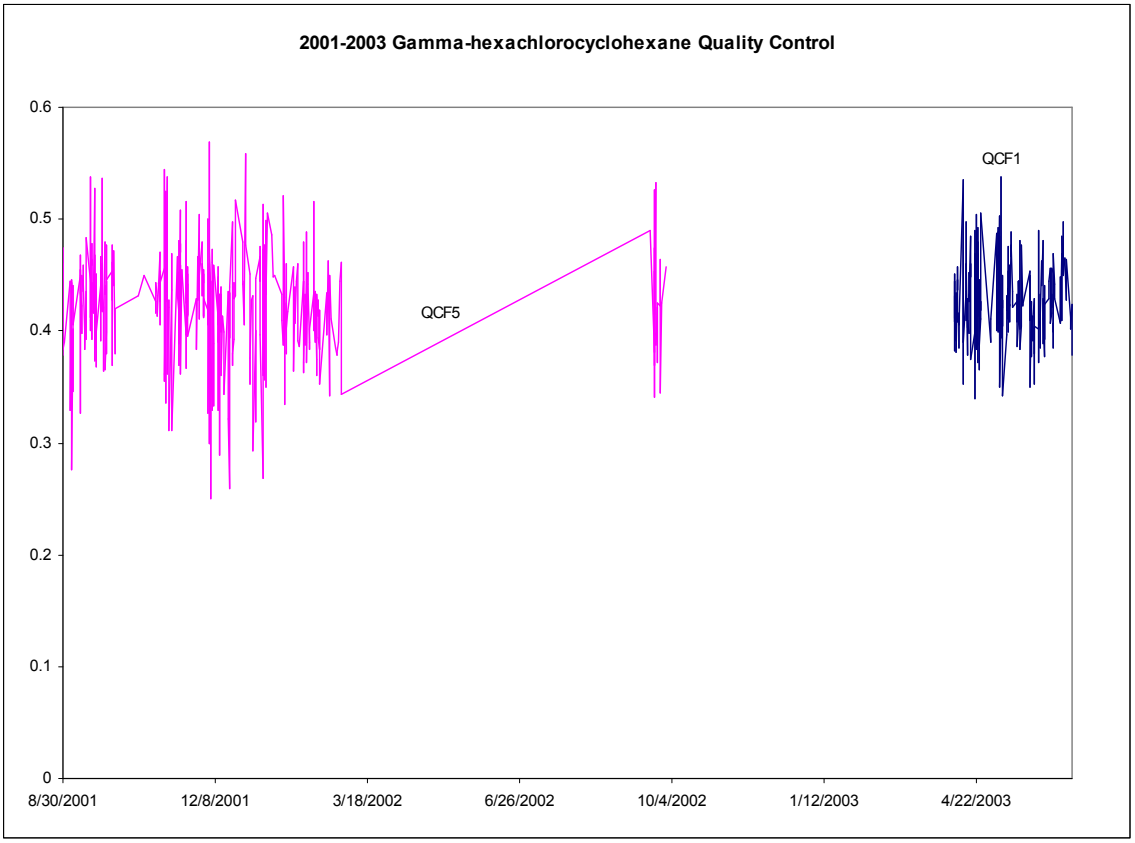
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF1	261	4/8/2003	6/24/2003	0.3668	0.031	8.4



KK. Gamma-Hexachloro-Cyclohexane

Summary Statistics for Gamma-Hexachloro-Cyclohexane by Lot

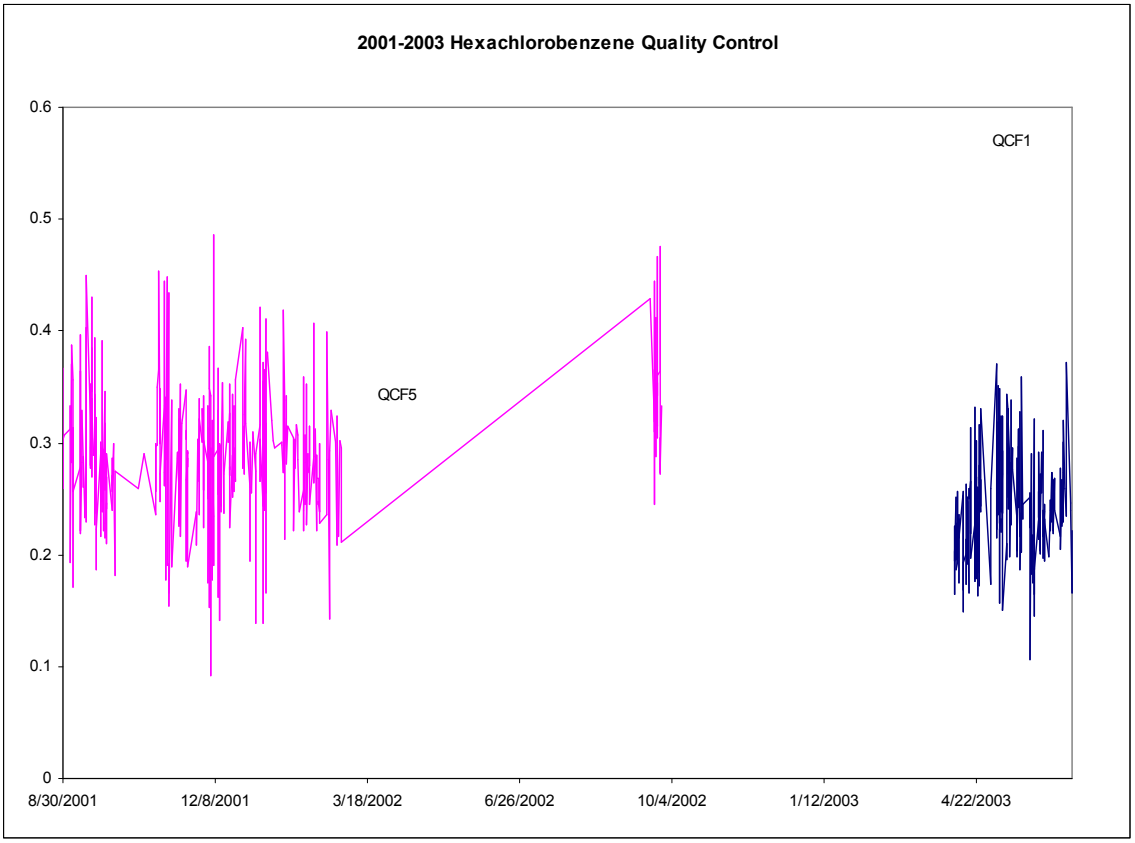
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	419	8/30/2001	9/30/2002	0.4215	0.0476	11.3
QCF1	268	4/8/2003	6/24/2003	0.4268	0.0341	8



LL. Hexachlorobenzene

Summary Statistics for Hexachlorobenzene by Lot

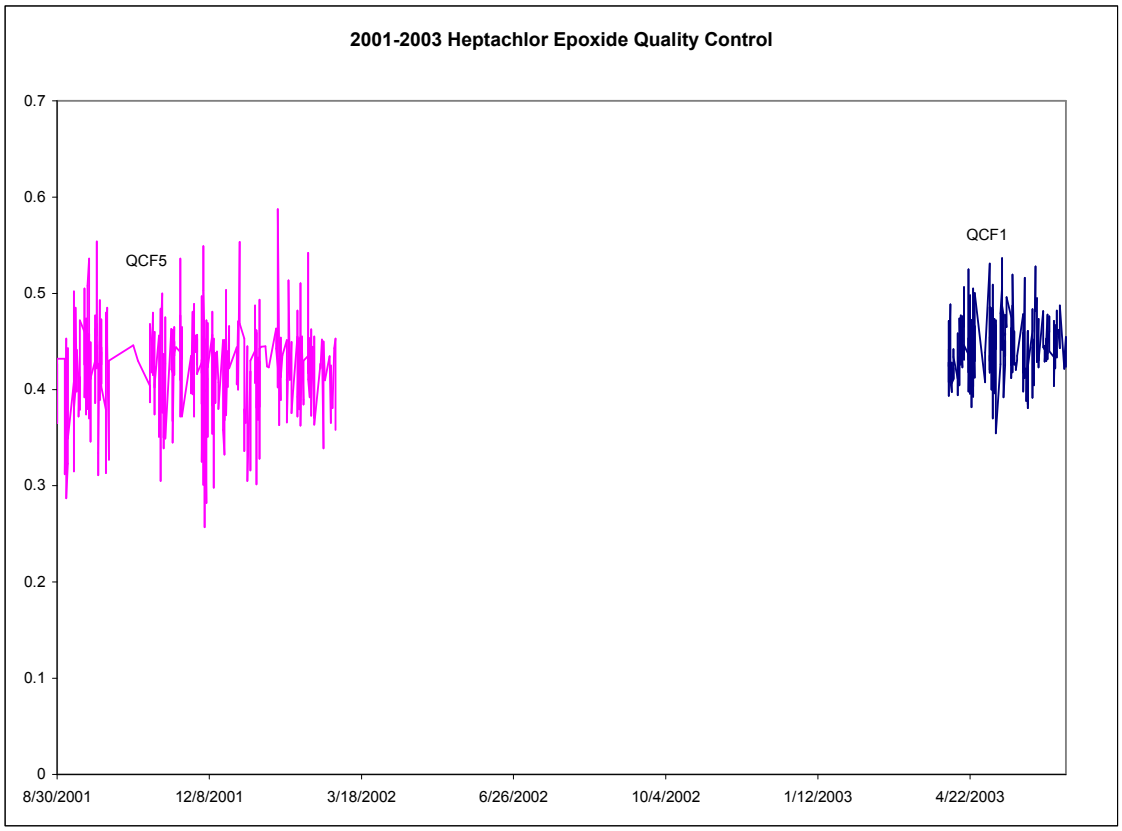
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	412	8/30/2001	9/27/2002	0.2906	0.0587	20.2
QCF1	268	4/8/2003	6/24/2003	0.2427	0.0455	18.7



MM. Heptachlor Epoxide

Summary Statistics for Heptachlor Epoxide by Lot

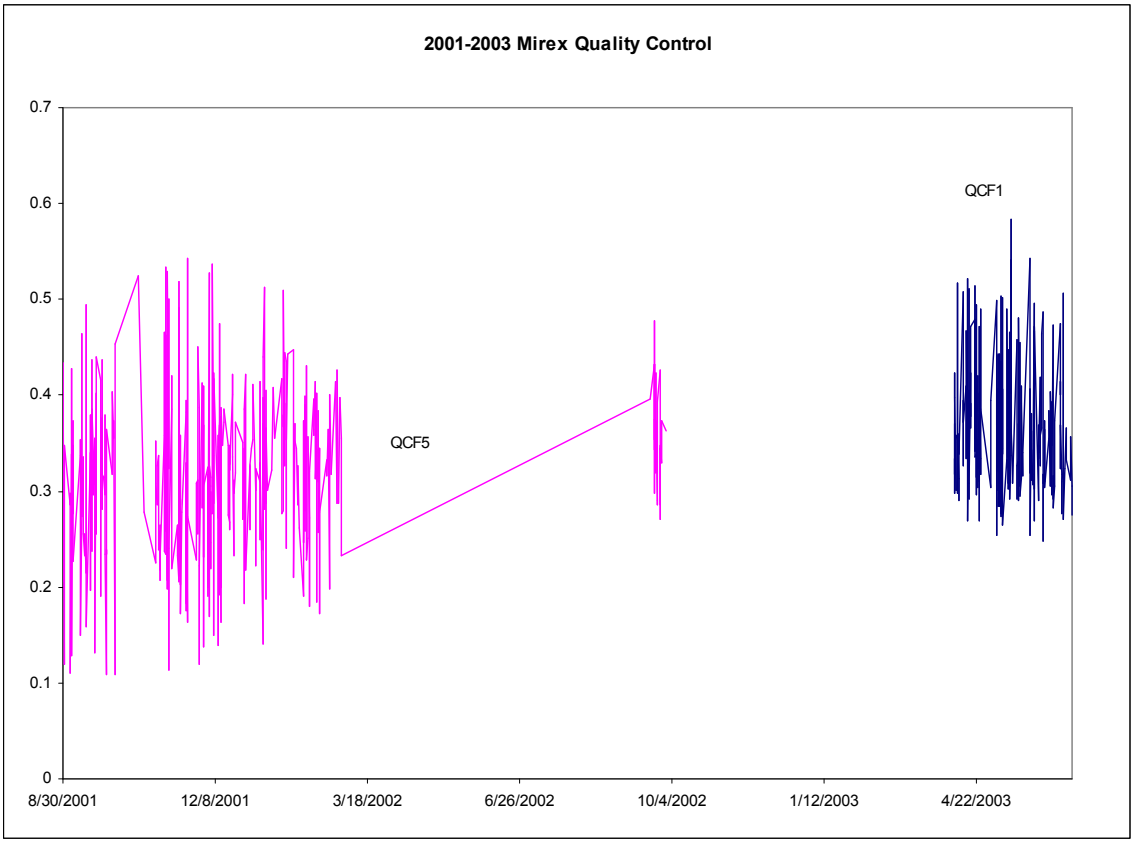
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	387	8/30/2001	3/1/2002	0.4213	0.047	11.2
QCF1	266	4/8/2003	6/24/2003	0.4455	0.0308	6.9



NN. Mirex

Summary Statistics for Mirex by Lot

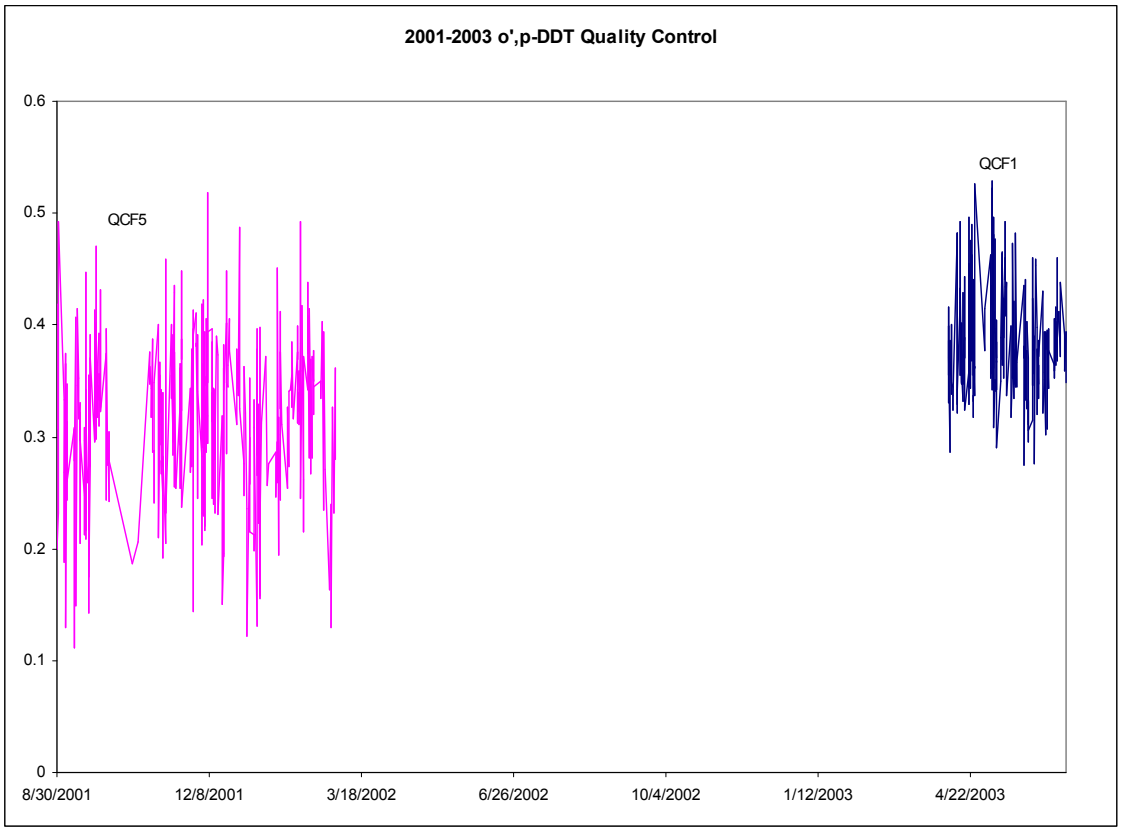
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	409	8/30/2001	9/30/2002	0.3171	0.0839	26.4
QCF1	266	4/8/2003	6/24/2003	0.3631	0.0637	17.5



OO. o,p'-DDT

Summary Statistics for o,p'-DDT by Lot

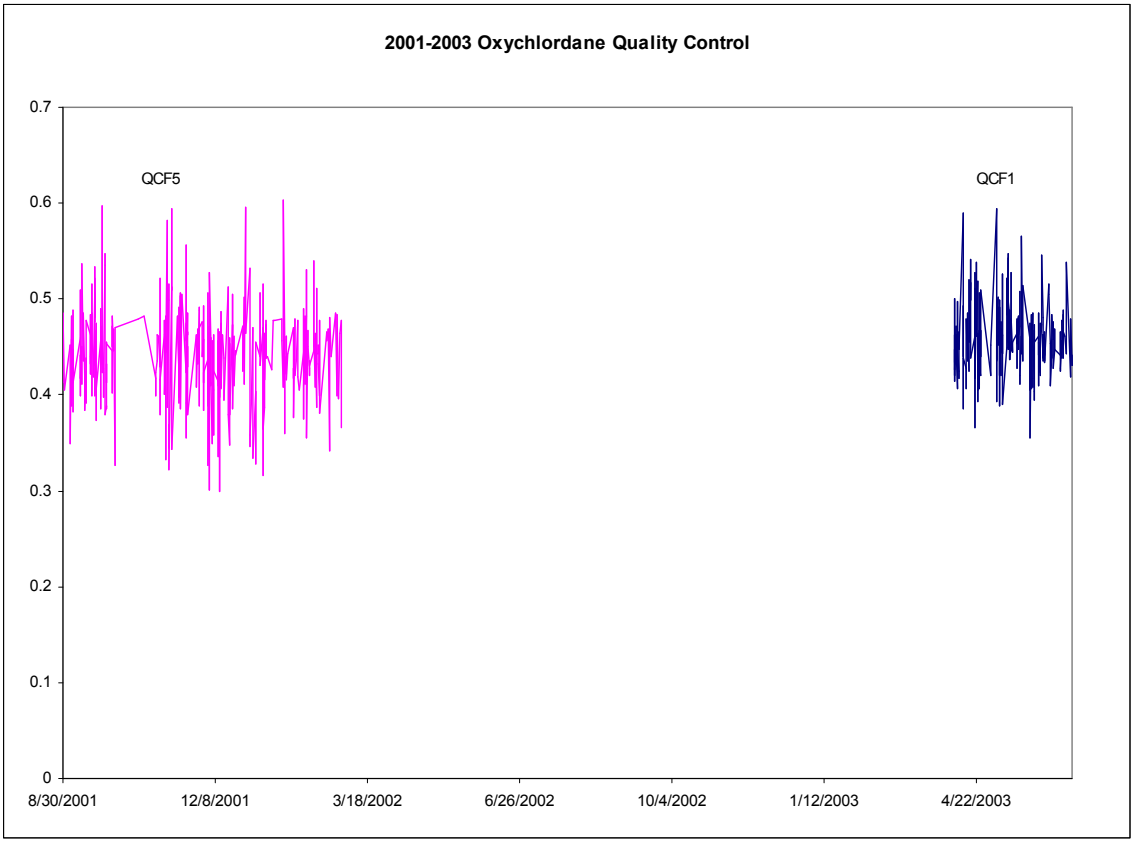
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	394	8/30/2001	3/1/2002	0.3172	0.0695	21.9
QCF1	269	4/8/2003	6/24/2003	0.3833	0.0474	12.4



PP. Oxychlordane

Summary Statistics for Oxychlordane by Lot

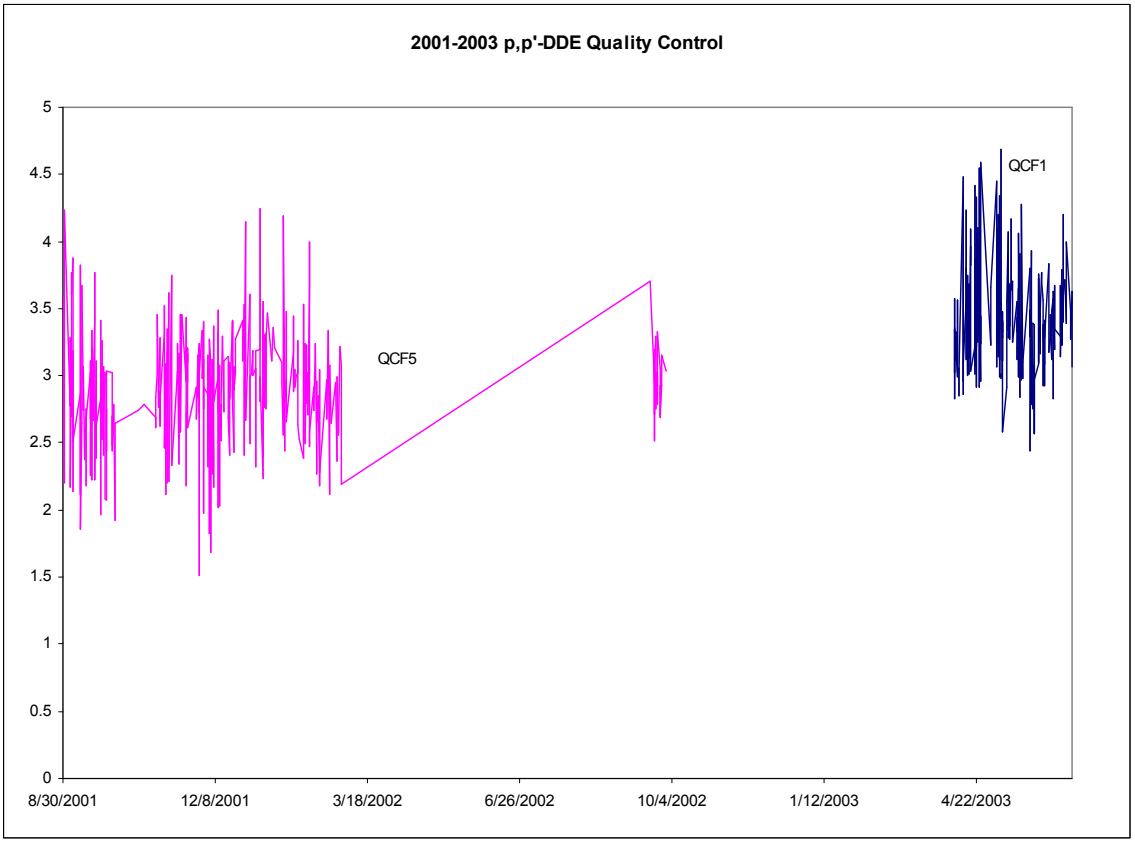
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	389	8/30/2001	3/1/2002	0.4414	0.0479	10.9
QCF1	268	4/8/2003	6/24/2003	0.4587	0.0357	7.8



QQ. p,p'-DDE

Summary Statistics for p,p'-DDE by Lot

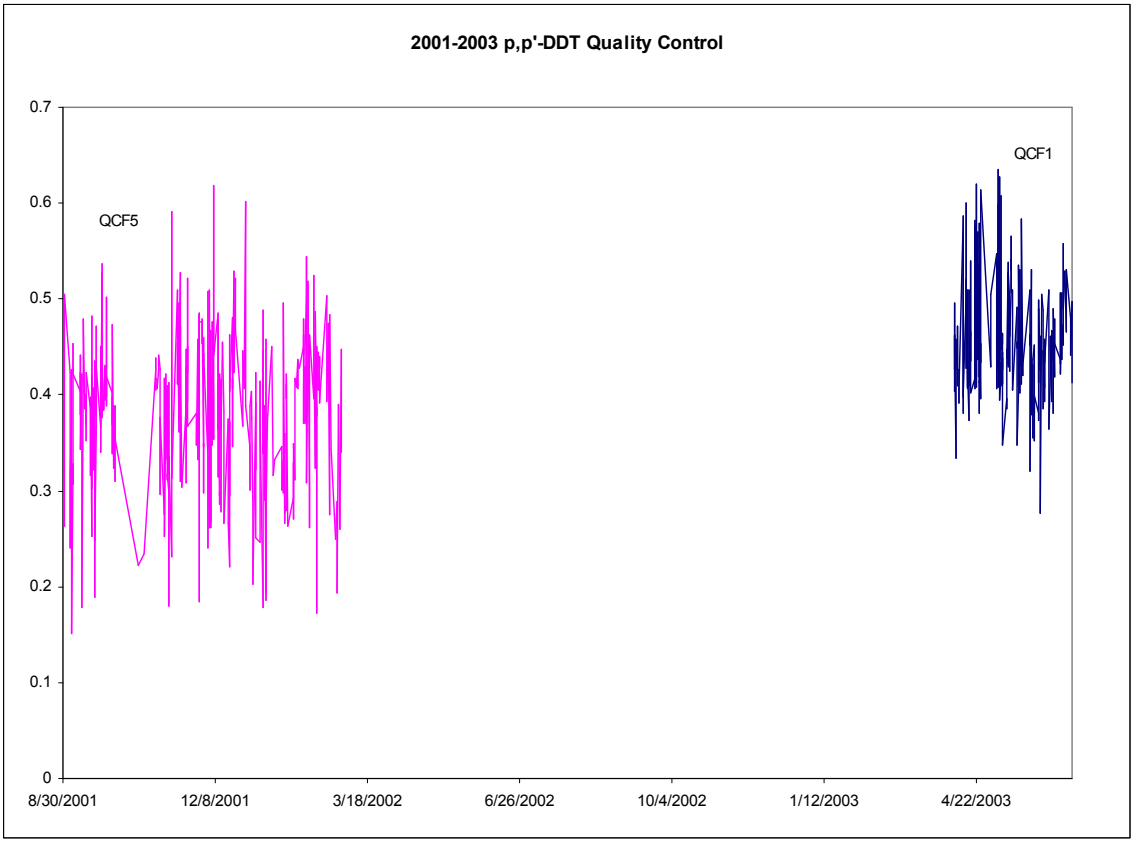
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	423	8/30/2001	9/30/2002	2.8861	0.4076	14.1
QCF1	270	4/8/2003	6/24/2003	3.426	0.3979	11.6



RR. p,p'-DDT

Summary Statistics for p,p'-DDT by Lot

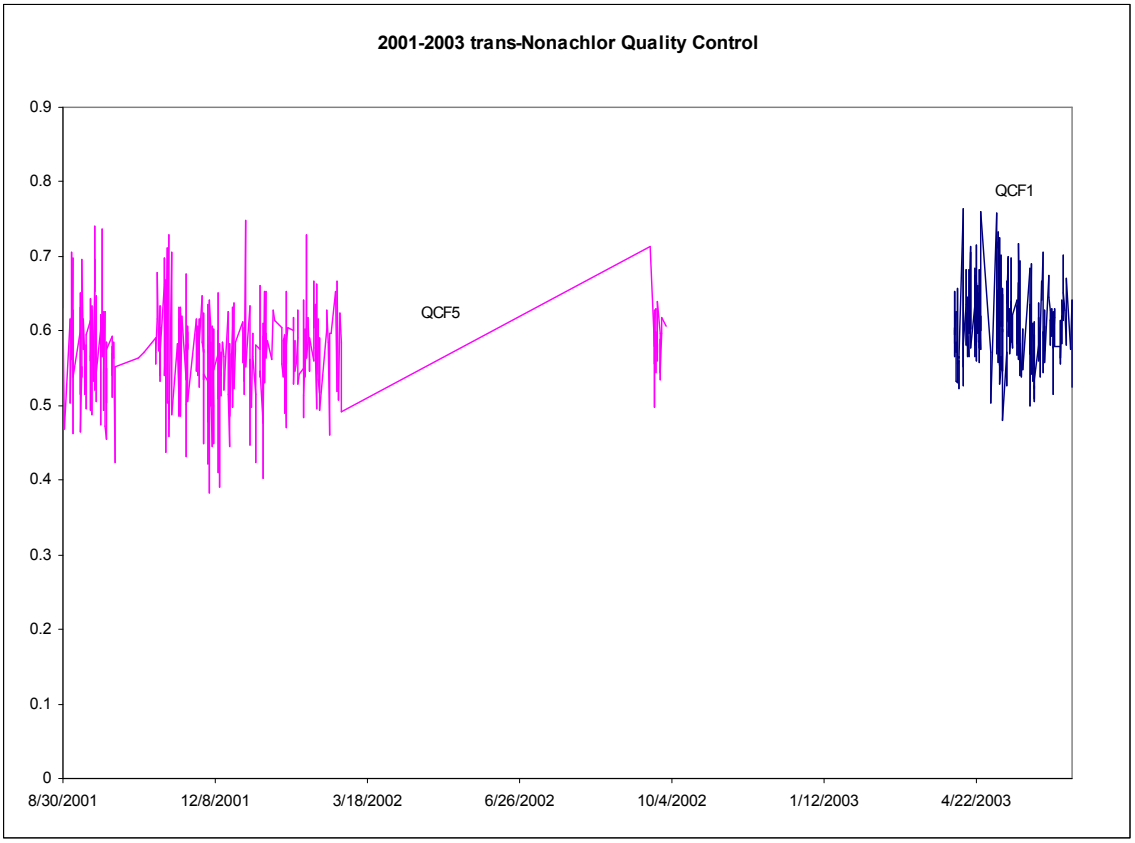
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	395	8/30/2001	3/1/2002	0.3842	0.0789	20.5
QCF1	271	4/8/2003	6/24/2003	0.4585	0.0584	12.7



SS. trans-Nonachlor

Summary Statistics for trans-Nonachlor by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
QCF5	417	8/30/2001	9/30/2002	0.5733	0.0591	10.3
QCF1	269	4/8/2003	6/24/2003	0.6098	0.0519	8.5



20. REFERENCES

Cleanup and Mass Spectrometry

1. Patterson D.G. Jr., Holler J.S., Lapeza C.R., *et al.* High-Resolution Gas Chromatographic/High-Resolution Mass Spectroscopic Analysis of Human Adipose Tissue for 2,3,7,8-TCDD. *Anal Chem*. 1986:58;705–713.

2. Lapeza C.R. Jr., Patterson D.G. Jr., Liddle J.A.. An Automated Apparatus for the Extraction and Enrichment of 2,3,7,8-TCDD in Human Adipose. *Anal Chem.* 1986:58;713–716.

3. Patterson D.G. Jr., Holler J.S., Belser W.T., Boozer E.L., Lapeza C.R. Jr., Needham L.L. Determination of 2,3,7,8-TCDD in Human Adipose Tissue on Whole Weight and Lipid Bases. *Chemosphere.* 1987:16;935–936.

4. Patterson D.G. Jr., Hampton L., Lapeza C.R. Jr., *et al.* High-Resolution Gas Chromatographic/High-Resolution Mass Spectrometric Analysis of Human Serum on a Whole-Weight and Lipid Basis for 2,3,7,8-TCDD. *Anal Chem*. 1987:59;2000–2005.

5. Patterson D.G. Jr., Turner W.E., Alexander L.R., Isaacs S.G., and Needham L.L. The Analytical Methodology and Method Performance for the Determination of 2,3,7,8-TCDD in Serum for the Vietnam Veteran Agent Orange Validation Study, The Ranch Hand Validation and Half-Life Studies, and Selected NIOSH Workers Studies. *Chemosphere.* 1989:18/1-6;875–882.

6. Patterson D.G. Jr., Fürst P., Henderson L.O., Isaacs S.G., Alexander L.R., Turner W.E., Needham L.L., and Hannon H. Partitioning of In Vivo Bound PCDDs/PCDFs among Various Compartments in Whole Blood. *Chemosphere.* 1989:19/1-6;135–142.

7. Patterson D.G. Jr., Alexander L.R., Turner W.E., Isaacs S.G., and Needham L.L. (1990). The Development and Application of a High Resolution Mass Spectrometry Method for Measuring Polychlorinated Dibenzo-p-dioxins and Dibenzofurans in Serum. Chapter 9 In: Instrumentation for Trace Organic Monitoring. Clement R.E., Sui K.M., and Hill H.H. Jr., eds, Lewis Publishers.

8. Patterson D.G. Jr., Isaacs S.G., Alexander L.R., Turner W.E., Hampton L., Bernert J.T., Needham L.L. (1990). Determination of Specific Polychlorinated Dibenzo-p-dioxins and Dibenzofurans in Blood and Adipose Tissue by Isotope-Dilution High Resolution Mass Spectrometry, Method 5 in "Environmental Carcinogens - Methods of Analysis and Exposure Measurement. Volume 11 - Polychlorinated Dibenzo-p-dioxins, Dibenzofurans, and Biphenyls," C. Rappe and H.R. Buser, Eds., WHO, International Association for Research on Cancer, Lyon, France.

9. Turner W., DiPietro E., Cash T.P., McClure P.C., Patterson, D.G Jr., and Shirkhan An Improved SPE Extraction and Automated Sample Cleanup Method for Serum PCDDs, PCDFs, and Coplanar PCBs. *Organohalogen Compounds.* 1994:19;31–35.

10. Burse V.B., Patterson D.G. Jr., Brock J.W., and Needham L.L. Selected Analytical Methods Used at the Centers for Disease Control and Prevention for Measuring Environmental Pollutants in Serum. *Toxicol Indust Health.* 1996:12(3/4);481–498.

11. Turner W., DiPietro E., Lapeza C., Green V., Gill J., Patterson, D.G. , Jr. A Fast Universal Automated Cleanup System for the Isotope-Dilution High-Resolution Mass Spectrometric Analysis of PCDDs, PCDFs, Coplanar PCBs, PCB Congeners, and Persistent Pesticides from the Same Serum Sample. *Organohalogen Compounds.* 1997:31;26–31.

12. Barr J.B., Maggio V.L., Barr D.B., Turner W.E., Sjodin A., Sandau C.D., Pirkle J.L., Needham L.L., and Patterson D.G. Jr. New High-Resolution Mass Spectrometric Approach for the Measurement of Polychlorinated Biphenyls and Organochlorine Pesticides in Human Serum. *J Chromatography B*. 2003:794;137–148.

Quality Control and Limit of Detection

1. Taylor J.K. Quality Assurance of Chemical Measurements. *Anal Chem*. 53: 1588A-1592A, 1596A (1981).

2. Keith H.K., Crummett W., Deegan J. Jr., *et al.* Principles of Environmental Analysis. *Anal Chem*. 55: 2210-2218 (1983).

3. Keith L.H . Report Results Right, Part I. *Chemtech.* June: 352-356 (1991).

4. Keith L.H . Report Results Right, Part II. *Chemtech.* August: 486-489 (1991).

Total Lipid Measurement

1. Akins J.R., Waldrep K., and Bernert J.T. Jr. The Estimation of Total Serum Lipids by a Completely Enzymatic 'Summation' Method. *Clin Chim Acta.* 184: 219-226 (1989).

2. Phillips, D.L., Pirkle, J.L., Burse V.W., Bernert, J.T., Henderson, L.O., and Needham, L.L. Chlorinated Hydrocarbon Levels in Humans Serum: Effects of Fasting and Feeding. *Arch Environ Contam Toxicol.* 18: 495-500 (1989).

Toxic Equivalency Factors (TEFs).

1. Van den Berg M, Birnbaum L, Bosveld ATC *et al*. Toxic Equivalency Factors (TEFs) for PCBs, PCDDs, and PCDFs for Humans and Wildlife. *Environ Health Perspec.*106: 775-792 (1998).

Universal Precautions

1. Universal Precautions. Recommendations for Prevention of HIV Transmission in Health-Care Settings. *MMWR* (Aug 21, 1987) Vol 36; No 2S: 2S–18S.