Laboratory Procedure Manual

Analyte:	**PCDDs,PCDFs,cPCBs and ortho-substituted PCBs.**
Matrix:	**Serum**
Method:	**HRGC/ID-HRMS**
Method No.:	**28**
Revised:	***October, 2006 [NHANES 2003-2004]***
as performed by:	***Organic Analytical Toxicology Branch Division of Laboratory Sciences National Center for Environmental Health, CDC***
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Important Information for Users

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

Notes regarding NHANES 2003-2004

There are two companion Lab 28 methods associated with NHANES 2003-2004. The Lab 28 PCB and Pesticide method is intended for small sample volumes (1-2 mL) and can be used to measure both ortho-substituted PCBs and Organohalogen Pesticides in the same serum sample simultaneously or PCBs and Pesticides in separate samples as individual methods. For NHANES 2003-2004 Pesticides were measured as an individual method. The Lab 28 PCDD/PCDF/cPCB method is intended for larger sample volumes (8-10 mL) to measure dioxins, furans and coplanar PCBs in serum. For NHANES 2003-2004 the PCDD/PCDF/cPCB method was modified by spiking the "dioxin" samples with both PCDD/PCDF/cPCB and PCB spiking solutions and collecting two analytical fractions. The "dioxin" fraction was analyzed for PCDDs/PCDFs/cPCBs as described in the PCDD/PCDF/cPCB method and the PCB fraction by the individual PCB method for ortho-substituted PCBs as described in the PCB method. This modification was made so we could obtain lower detection limits for the ortho-substituted PCBs.

PCDD, PCDF, cPCB Congener	CAS Number	SAS Label
2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)	1746-01-6	LBXTCD
1,2,3,7,8-pentachlorodibenzo-p-dioxin (PnDD)	40321-76-4	LBXD01
1,2,3,4,7,8-hexachlorodibenzo-p-dioxin (HxDD)	34465-46-8	LBXD02
1,2,3,6,7,8-hexachlorodibenzo-p-dioxin (HxDD)	57653-85-7	LBXD03
1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (HxDD)	19408-74-3	LBXD04
1,2,3,4,6,7,8-heptachlorodibenzo-p-dioxin (HpDD)	35822-46-9	LBXD05
1,2,3,4,6,7,9-heptachlorodibenzo-p-dioxin (HpDD)	58200-70-7	LBXD06
1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxin (OCDD)	3268-87-9	LBXD07
2,3,7,8-tetrachlorodibenzofuran (TCDF)	51207-31-9	LBXF01
1,2,3,7,8-pentachlorodibenzofuran (PnDF)	57117-41-6	LBXF02
2,3,4,7,8-pentachlorodibenzofuran (PnDF)	57117-31-4	LBXF03
1,2,3,4,7,8-hexachlorodibenzofuran (HxDF)	70648-26-9	LBXF04
1,2,3,6,7,8-hexachlorodibenzofuran (HxDF)	57117-26-9	LBXF05
1,2,3,7,8,9-hexachlorodibenzofuran (HxDF)	72918-21-9	LBXF06
2,3,4,6,7,8-hexachlorodibenzofuran (HxDFf)	60851-34-5	LBXF07
1,2,3,4,6,7,8-heptachlorodibenzofuran (HpDF)	67562-39-4	LBXF08
1,2,3,4,7,8,9-heptachlorodibenzofuran (HpDF)	55673-89-7	LBXF09
1,2,3,4,6,7,8,9-octachlorodibenzofuran (OCDF)	39001-02-0	LBXF10
3,3',4,4'-tetrachlorobiphenyl (TCB) PCB77	32598-13-3	LBXTC1
3,4,4',5-tetrachlorobiphenyl (TCB) PCB81	70362-50-4	LBXTC2
3,3',4,4',5-pentachlorobiphenyl (PnCB) PCB126	57465-28-8	LBXPCB
3,3',4,4',5,5'-hexachlorobiphenyl (HxCB) PCB169	32774-16-6	LBXHXC

Public Release Data Set Information

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

A tabular list of the released analytes follows:

Lab Number	Analyte	SAS Label
Lab28	LBXD01	1,2,3,7,8-(pncdd) (fg/g)
	LBXD02	1,2,3,4,7,8-(hxcdd)(fg/g)
	LBXD03	1,2,3,6,7,8-(hxcdd)(fg/g)
	LBXD04	1,2,3,7,8,9-(hxcdd)(fg/g)
	LBXD05	1,2,3,4,6,7,8-(hpcdd) (fg/g)
	LBXD07	1,2,3,4,6,7,8,9-(ocdd) (fg/g)
	LBXF01	2,3,7,8, (tcdf) (fg/g)
	LBXF02	1,2,3,7,8 (pncdf) (fg/g)
	LBXF03	2,3,4,7,8-(pncdf) (fg/g)
	LBXF04	1,2,3,4,7,8-(hcxdf) (fg/g)
	LBXF05	1,2,3,6,7,8-(hxcdf) (fg/g)
	LBXF06	1,2,3,7,8,9- (hxcdf) (fg/g)
	LBXF07	2,3,4,6,7,8,- (hxcdf) (fg/g)
	LBXF08	1,2,3,4,6,7,8- (hpcdf) (fg/g)
	LBXF09	1,2,3,4,7,8,9- (Hpcdf) (fg/g)
	LBXF10	1,2,3,4,6,7,8,9- (ocdf) (fg/g)
	LBXPCB	3,3',4,4',5-(pncb) (fg/g)
	LBXTC2	3,4,4',5- (tcb)
	LBXTCD	2,3,7,8- (tcdd) (fg/g)
	LBXHXC	3,3',4,4',5,5' (hxcb) (fg/g)

1. **SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE**

Seven polychlorinated dibenzo-***p***-dioxins (PCDDs), 10 dibenzofurans (PCDFs), 4 non-ortho-substituted or coplanar polychlorinated biphenyls (cPCBs)and 38 ortho-substituted polychlorinated biphenyls (PCBs)are measured in serum by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry (HRGC/ID-HRMS).

Serum specimens (5-10 mL) to be analyzed for PCDDs/PCDFs/cPCBs and PCBs are spiked with carbon-13 labeled ($^{13}C_{12}$) internal standards and the analytes of interest are isolated in hexane using a C_{18} solid phase extraction (SPE) procedure followed by a Power-Prep/6 (Fluid Management Systems) automated cleanup and enrichment procedure using multi-layered silica gel (acidic, basic, and neutral silica) and alumina columns coupled to an AX-21 carbon column. An analytical run consists of eight unknown specimens, two method blanks, and two quality control samples. PCB fractions are isolated in the forward direction from AX-21 columns, while PCDD/PCDF/cPCB fractions are isolated in the reverse direction from AX-21 columns with toluene.

Following collection of PCDD/PCDF/cPCB factions, excess solvent is evaporated to 350 µL using a TurboVap II (Caliper Life Sciences) and the remaining solvent is transferred to silanized auto sampler vials containing 1µL of dodecane "keeper" and allowed to evaporate to "dryness". Before quantification, each vial is reconstituted with 5µL $^{13}C_{12}$-labeled external standard. Sample extracts are then analyzed for PCDDs/PCDFs/cPCBs by HRGC/ID-HRMS where 2µL are injected, using a GC Pal (Leap Technology) auto sampler, into a Agilent Technologies 6890 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 a Thermo Electron MAT95 XP (5kV) magnetic sector mass spectrometer operated in EI mode at 40 eV, using selected ion monitoring (SIM) at 10,000 resolving power (10% valley). Two ion current responses corresponding to two masses are monitored for each native (carbon-12 or $^{12}C_{12}$) compound and its corresponding $^{13}C_{12}$-internal standard. The instrumental response factor for each analyte is calculated as the sum of the two $^{12}C_{12}$-isomers divided by the sum of two $^{13}C_{12}$-isomers

Following collection of PCB fractions, excess solvent is evaporated to 350 µL using a TurboVap II (Caliper Life Sciences) and the remaining solvent is transferred to silanized auto sampler vials containing 1µL of dodecane "keeper" and allowed to evaporate to "dryness". Before quantification, the vials are reconstituted with 10µL ^{13}C-labeled external standard. Sample extracts are then analyzed PCBs 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 (30m x 0.25 mm x 0.25 µm film thickness) where analytes are separated prior to entering a Thermo Electron

MAT95 XP (5kV) magnetic sector mass spectrometer operated in EI mode at 40 eV, using selected ion monitoring (SIM) at 10,000 resolving power (10% valley). Two ion current responses corresponding to two masses are monitored for each native (carbon-12 or $^{12}C_{12}$) compound and its corresponding $^{13}C_{12}$-internal standard. The instrumental response factor for each analyte is calculated as the sum of the two $^{12}C_{12}$- isomers divided by the sum of two $^{13}C_{12}$-isomers

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

2. SAFETY PRECAUTIONS

All serum specimens are handled using ***Universal Precautions.*** Specimens received for analysis must be considered potentially positive for infectious agents including HIV and hepatitis B viruses. Universal Precautions must be observed; laboratory coats, safety glasses and protective gloves should be worn during all steps of this method. The Hepatitis B vaccination series is recommended for all analysts working with whole blood and/or serum samples. Laboratory personnel should abide by common safety practices: no eating, drinking, or smoking in the laboratory. Protective clothing should not be worn out of the laboratory; and hands should be washed with soap and water before leaving the area. When organic solvents are being used, all operations should be performed under a fume hood. As an added precaution, laboratory staff should also wear solvent-resistant nitrile gloves during all phases of the sample enrichment procedure, including glassware washing. 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 □g of TCDD toxic equivalents should not be stored in sample preparation or GC/MS laboratories.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

Relational databases have been set up on PC network using R:BASE version 7.5 (R:BASE Technologies, Murryville, PA). The databases are used for storage, retrieval, and analysis of data from projects of the Dioxin and Persistent Organic Pollutants Laboratory. Data entries are made into four tables containing: 1) Demographic information; 2) Information from the clean-up section; 3) Mass spec data; and 4) Lipid results. Each section has access only to the information that it entered. However, after the information from each section has been entered, the data sets can be merged for a complete report on each sample. Data sets can be sent to SAS, Statistical Analysis System, on the PC network. Entry forms and reports can be changed to fit the needs of each section.

The statistical analysis of the results are performed using the software package SAS, Statistical Analysis System. The data from the each of the sections is brought together by specimen identification number, the notebook number of the clean-up section, and the mass spec run number. Only the project supervisor and the database manager will have access to the whole database.

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 RBASE 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. Initially, the amount of serum specimen used for dioxin measurements was the amount collected from a unit of whole blood (about 180 mL). With more sensitive mass

spectrometers, the volumes of serum are now routinely between 5 mL and 25 mL. The minimum amount is about 5 5mL of serum.

Specimen Collection Materials for Each Participant. Up to 50 g Serum sample.

(1) Gauze sponges, sterile, individually wrapped 2"x2" (2 ea).
(2) Alcohol wipes
(3) Band-aid
(4) 15 mL red-top Vacutainers) or 20 mL red top Vacutainers
(5) 21 gauge multi sample needle, sterile
(6) Pre-printed labels
(7) Tourniquet
(8) Vacutainers holder
(9) Freezer
(10) Pasteur pipette (1 each*)
(11) Qorpak bottle (1 each*)
(12) Teflon-lined stoppers for above Qorpak bottle (2 ea.*)
(13) Aluminum seals (2 each)
(14) Pre-printed labels
(15) Pipette bulb
(16) Test tube racks
(17) Centrifuge
(18) Freezer (-20 ^{0}C)

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

Collection of 5-50 mL 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, pipet the serum from each participant's red-top tubes into the Qorpak 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 ^{0}C freezer and store at the same temperature until shipment to CDC. The time between collecting blood and freezing serum should not be more than 1 1/2 hours. Note on the sample log if a sample is turbid or hemolyzed, or if the serum was left in contact with red cells for more than 1 hour or left at room temperature for more than 90 minutes before freezing.

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

For all shipments, do not pack shippers with frozen specimens and dry ice until just before shipment. Telephone the laboratory at CDC the day the shipment is transported. For each shipment, fill out a blank Specimen Shipping List provided by CDC. When packing the shippers, use gloves to handle the dry ice to avoid burning the hands. Glasses or an eye shield should also be worn if the dry ice cakes are to be broken into small pieces. Place the frozen serum specimens from each participant in one 4"x7" bubble bag and seal. Pack 1 set of filled bubble bags upright in the bottom of the shipper. If necessary, use sheets of bubble-pack, packing material to ensure the specimens are in a vertical position. Fill the shipper with dry ice. Insert the completed "Specimen Shipping List" in a 12x12" zip-lock bag and secure to the top of the Polyfoam lid with filament tape. Secure the outer carton lid on the shipper with EPA seal tape and complete the appropriate information. Attach pre-addressed "FEDERAL EXPRESS" shipping label, the HUMAN BLOOD - THIS SIDE UP label, and the DRY ICE label.

Specimen stability has been demonstrated for analytes measured by this method for at least 10 years at -60 ^{0}C or below. However, due to the chemical inertness of these compounds, they can be assumed to be stabile indefinitely if specimens are maintained in a frozen state.

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

Not applicable for this procedure.

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

6.1. Reagent Preparation

6.1.1 50% dichloromethane/hexane solution (v/v).
With a 2L graduated cylinder, measure 1.5L dichloromethane, and pour into a clean labeled 4L bottle. Measure 1.5L hexane with the same graduated cylinder and pour into the same 4L bottle. Gently swirl to mix.

6.1.2 2% dichloromethane/hexane solution (v/v).
In a 2 L graduated cylinder, measure 1960 mL of hexane and 40 mL dichloromethane. Pour into a clean, labeled 4L bottle. Gently swirl to mix.

6.1.3 10% dichloromethane/hexane (v/v).
In a 2 L graduated cylinder measure 200mL dichloromethane and 1800 mL hexane. Transfer to a 4L bottle. Gently swirl to mix

6.1.4 50% ethyl acetate/benzene (v/v).
In a 1 L graduated cylinder, measure 500 mL ethyl acetate and 500 mL benzene. Transfer to a labeled 2L bottle. Gently swirl to mix.

6.1.5 10% Dimethyldichlorosilane (DMCS) silanizing solution.
A 10% DMCS in toluene solution (v/v) is prepared for silanizing glass Vessels and Turbo-Vap tubes. The silane solution is stored in a glass reagent bottle at 4 ^{0}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 ^{0}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 calibration standards were purchased from Cambridge Isotopes Laboratory (CIL, Woburn, MA). They were prepared in nonane according to CDC specifications and contain the 7 PCDDs, 10 PCDFs, and 4 cPCBs or 38 ortho-substituted PCBs found in humans. Standards were prepared from individual stock solutions of labeled $^{13}C_{12}$-PCDDs, $^{13}C_{12}$-PCDFs, $^{13}C_{12}$-cPCBs, $^{13}C_{12}$-PCBs and native $^{12}C_{12}$-PCDDs, $^{12}C_{12}$-PCDFs, $^{12}C_{12}$-cPCBs or $^{12}C_{12}$-PCBs 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. Table 1 describes the composition of the isotope dilution standards.

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, 48 pg/mL $^{13}C_{12}$-3355P and 62.5 pg/mL $^{13}C_{12}$-123478F in nonane. This standard is used to reconstitute sample extracts before mass spectral analysis for dioxins, furans and coplanar PCBs. 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($^{13}C_{12}$-PCDDs/PCDFs) and the recovery standard ($^{13}C_6$-1,2,3,4-TCDD) can be used to calculate the absolute percent recovery of the carbon-13-labeled internal standards for each 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 -20 ^{0}C or below. The analytical standards used for day-to-day analyses are stored at room temperature.

Table 1. High Resolution Calibration Solutions for PCDDs, PCDFs, and cPCBs in Human Serum.

Standard Concentration (pg/µL)

Congener	CDC 9	CDC 8	CDC 7	CDC 6	CDC 5	CDC 4	CDC 3	CDC 2	CDC 1	$^{13}C_{12}$ in Each Standard	$^{13}C_{12}$ in Spiking Standard	Recovery Standard
2378D	50	35	20	7	2	1	0.2	0.1	0.04	50	25	- -
12378D	20	10	5	2	1	0.5	0.2	0.1	0.04	50	25	- -
123478D	20	10	5	2	1	0.5	0.2	0.1	0.04	120	60	- -
123678D	50	25	12.5	5	2.50	1.25	0.5	0.25	0.10	120	60	- -
123789D	30	25	20	10	5	2	1	0.5	0.2	120	60	- -
1234678D	200	100	50	25	20	10	5	2	1	120	60	- -
1234679D	20	10	5	2	1	0.5	0.2	0.1	0.04	-	-	
OCDD	600	500	400	300	200	100	50	20	10	250	125	- -
2378F	10	7.5	5	2	1	0.5	0.2	0.1	0.04	50	25	- -
12378F	10	7.5	5	2	1	0.5	0.2	0.1	0.04	50	25	- -
23478F	10	7.5	5	2	1	0.5	0.2	0.1	0.04	50	25	- -
23478F	10	7.5	5	2	1	0.5	0.2	0.1	0.04	50	25	- -
123478F	10	7.5	5	2	1	0.5	0.2	0.1	0.04	125	62.5	- -
123678F	10	7.5	5	2	1	0.5	0.2	0.1	0.04	125	62.5	- -
123789F	10	7.5	5	2	1	0.5	0.2	0.1	0.04	125	62.5	- -
234678F	10	7.5	5	2	1	0.5	0.2	0.1	0.04	125	62.5	- -
1234678F	30	25	20	10	5	2	1	0.5	0.2	125	62.5	- -
1234789F	10	7.5	5	2	1	0.5	0.2	0.1	0.04	-	-	- -
OCDF	10	7.5	5	2	1	0.5	0.2	0.1	0.04	250	125	- -
3344P	160	80	40	20	16	8	4	1.6	0.80	48	24	- -
3445P	160	80	40	20	16	8	4	1.6	0.80	48	24	- -
33445P	160	80	40	20	16	8	4	1.6	0.80	72	36	- -
334455P	160	80	40	20	16	8	4	1.6	0.80	96	48	- -
3355P-$^{13}C_{12}$	48	48	48	48	48	48	48	48	48	48	-	48
1234789F-$^{13}C_{12}$	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	62.5	-	62.5
1234D-$^{13}C_6$	25	25	25	25	25	25	25	25	25	25	-	25

6.3 Other Materials

6.3.1 Nitrogen gas, PEAK NM180L High Purity Nitrogen Generator (PEAK Scientific Instruments Ltd, Boston, MA).

6.3.2 Detergent, Micro liquid laboratory cleaner (Cole-Parmer, Chicago, IL). [A 5% solution of Micro in deionized water (v/v), is used for washing glassware]

6.3.3 ICN Silica (PRP-SIL-061) [1 g neutral, 2g base, 1g neutral, 4g acid, 1g neutral],ICN Alumina (PRP-ALU-071) [12g basic alumina], and Carbon (PRP-CAR-091) [0.275 g AX-21/Celite mixture; which is 0.022g carbon per columns; ICN neutral Silica (NEUSIL) [9g neutral]; ICN acid silica ((SILACID) [8g acid silica] (Fluid Management Systems, Inc., Watertown, MA).

6.3.4 TurboVap tubes: 200 mL with 0.5 mL stems [42527] (Caliper Life Sciences, Hopkinton, MA).

6.3.5 Solvent rinsed 20x125 and 20 x 150 mm disposable glass tubes with Teflon lined caps size 18.

6.3.6 Viton 3-stop tubing, 2.79 mm I.D. (Cole-Parmer Instrument Co., Niles, IL).

6.3.7 1 mL screw top vial [186000384DV] with Teflon faced silicone septa cap Total Recovery Vial [12x32mm] (Waters, Milfors, MA)

6.2.8 DB-5ms 30m, 0.25 mm I.D., 0.25 µm film thickness gas chromatography column (J&W Scientific, Folsom, CA).

6.2.9 GC Syringe, 10 µL [019390](SGE Incorporated, Austin, Texas).

6.2.10 TurboVap II Concentration Workstation [ZW8001] for 200 mL tubes with 0.5 mL stem (Caliper Life Sciences, Hopkinton, MA).

6.2.11 Master Flex variable-speed motor with controller [07553-80] and Ismatic minicartridge pump head with 8 cartridges [07623-10] (Cole-Parmer Instrument Co., Niles, IL).

6.2.12 Microman M25, M50, and M250 positive-displacement pipettes with capillaries and pistons. Rainin EDP 10 µL and 100 µL; EDP PLUS 10 µL and 100 µL; EDP2 100µL, 1000µL, and 2.5 mL Motorized Pipettes (Rainin Instrument Co., Woburn, MA).

6.2.13 Eppendorf 1000µL adjustable pipette (Brinkman Instrument Company, Westbury, NJ).

6.3.14 Wrist action shaker, Model 75 (Burrell, Pittsburg, PA).

6.3.15 Sample rotation device (Glas-Col Instrument Co).

6.3.16 SPE vacuum manifold (J.T. Baker, Phillipsburg, NJ).

6.3.17 Balance model BP310S (Sartorius, Goettinger, Germany).

6.3.18 Solvents: glass-distilled dichloromethane, toluene, ethyl acetate, acetone, HPLC grade water, and hexane, methanol, dichloromethane (glass-distilled grade) ethanol (anhydrous reagent), benzene, and ACS grade formic acid (Tedia, Fairfield, OH). Dodecane (Aldrich Chemical Co., Milwaukee, WI).

6.3.19 Dimethyldichlorosilane (Aldrich, Chemical Co., Milwaukee, WI).

6.3.20 Water, deionized (Culligan Water Systems, Inc., Marietta, GA).

6.3.21 SPE cartridges, (United Chemical Technologies, Inc., Bristol, PA.) 5g/25mL [XRODH5M25] C_{18} SPE cartridges.

6.3 Instrumentation

6.3.1 Power-Prep/6 (Fluid Management Systems, Inc., Watertown, MA). Figure 1 shows the 6-carbon column automated systems respectively. Each apparatus consists of a PC computer, controller, management module, valve module, piston pump, and a system pressure-protection gauge. All fluid interconnections of the system are made using 1/8" o.d. Teflon tubing and 1/4-in Delrin end fittings with 28 threads per inch (Rainin Instrument Co., Woburn, MA.

6.3.2 High-resolution gas chromatograph/high-resolution mass spectrometry systems: Thermo Electron MAT95 XP, with X-caliber data systems (Thermo Electron, 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 PCDDs/PCDFs/cPCBs by HRGC/ID-HRMS where 2µL are injected using an auto sampler, into a 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).

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

7.1 Isotope-Dilution Calibration

7.1.1 Slope and intercept.

Calibration of mass spectrometer response factor vs. concentration is performed using quantitative analytical standards containing known concentrations of each native ($^{12}C_{12}$) compound and its corresponding $^{13}C_{12}$-internal standard. The quantitative analytical standards are listed in Table 1 for the 2,3,7,8-substituted PCDDs, PCDFs and coplanar PCBs. The standards are analyzed in ascending and descending order for several days. At least 6 analyses for each standard is 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 $\log_{10}$ 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 -2 to -3.

7.1.2 Blank Correction and Background Correction for PCDDs, PCDFs, and coplanar PCBs.

Blank Corrections and background corrections for congeners having analytical blanks are made using the respective 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 and seventh samples in every clean-up run are the analytical blanks. Blanks consist of $^{13}C_{12}$-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). 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.

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

Table 2A Ion Ratios for Analysis of cPCBs

Compound	Ions Monitored	Theoretical Ratio	Acceptable Range[1] 99% Confidence
Tetra-CB	289.9224/291.9194	0.762	(0.561-0.9163)
$^{13}C_{12}$-Tetra-CB	301.9626/303.9597	0.762	(0.611-0.912)
Penta-CB	323.8834/325.8804	0.610	(0.449-0.770)
$^{13}C_{12}$-Penta-CB	335.9237/337.9207	0.610	(0.489-0.730)
Hexa-CB	359.8415/361.8385	1.219	(0.898-1.540)
$^{13}C_{12}$-Hexa-CB	371.8817/373.8788	1.219	(0.978-1.460)

[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 2B Ion Ratios for Analysis of PCDDs and PCDFs. **I)**

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Compound	Ions Monitored	Theoretical Ratio	Acceptable Range[1] 99% Confidence
TCDD	319.8965/321.8936	0.76	(0.576-0.987)
$^{13}C_{12}$-TCDD	331.9368/333.9338	0.76	(0.628-0.936)
TCDF	303.9016/305.8987	0.76	(0.576-0.987)
$^{13}C_{12}$-TCDF	315.9419/317.9389	0.76	(0.628-0.936)
PeCDD	355.8546/357.8517	0.625	(0.460-0.789)
$^{13}C_{12}$-PeCDD	367.8949/369.8919	0.625	(0.501-0.748)
PeCDF	339.8597/341.8568	0.625	(0.460-0.789)
$^{13}C_{12}$-PeCDF	351.9000/353.8970	0.625	(0.501-0.748)
HxCDD	389.8156/391.8127	1.251	(0.921-1.580)
$^{13}C_{12}$-HxCDD	401.8559/403.8530	1.251	(1.004-1.498)
HxCDF	373.8207/375.8178	1.251	(0.921-1.580)
$^{13}C_{12}$-HxCDF	385.8610/387.8580	1.251	(1.004-1.498)
HpCDD	423.7769/425.7737	1.042	(0.768-1.316)
$^{13}C_{12}$-HpCDD	435.8169/437.8140	1.042	(0.836-1.248)
HpCDF	407.7818/409.7788	1.042	(0.768-1.316)
$^{13}C_{12}$-HpCDF	419.8220/421.8191	1.042	(0.836-1.248)
OCDD	457.7377/459.7348	0.893	(0.658-1.128)
$^{13}C_{12}$-OCDD	469.7780/471.7750	0.893	(0.717-1.069)
OCDF	441.7428/443.7398	0.893	(0.658-1.128)
$^{13}C_{12}$-OCDF	453.7831/455.7800	0.893	(0.717-1.069)

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

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. For PCDD/PCDF standards, this ratio can be calculated for each standard concentration. 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 PCDD/PCDF 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 +0.003 in order for the data to be reportable.

Table 3 contains a list of all the mass ions used for the determination of 2378-substituted dibenzo-p-dioxins, dibenzofurans and coplanar PCBs.

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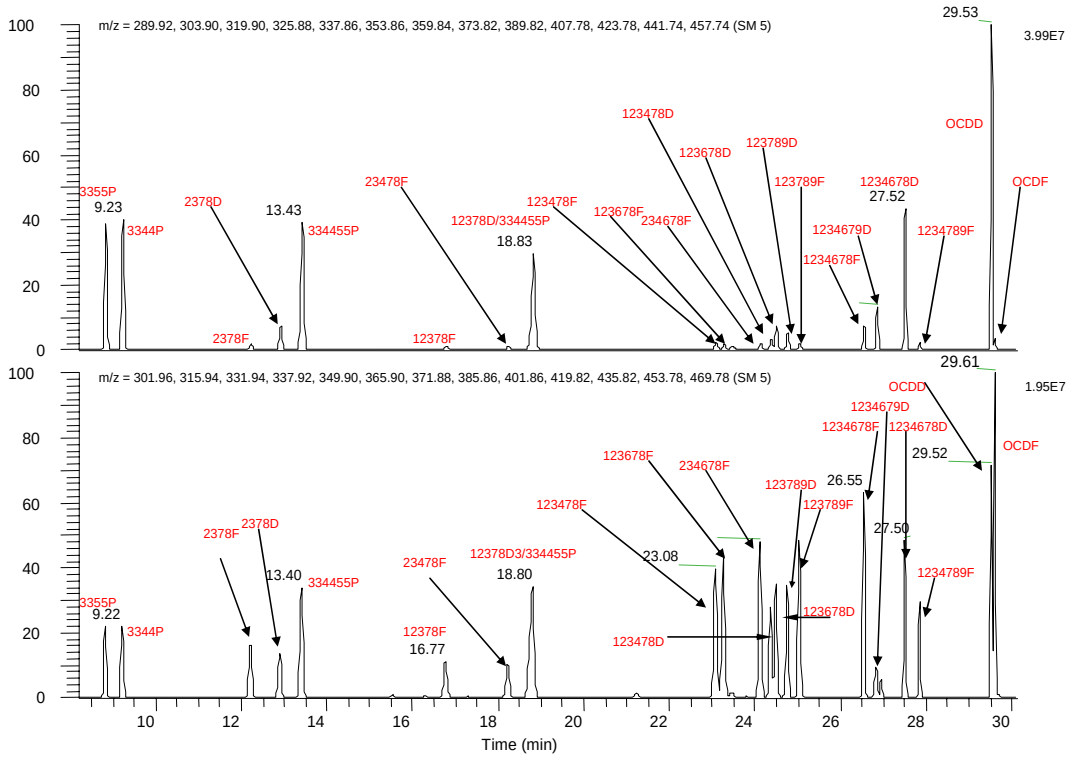
Table 3 Ions Monitored for High-Resolution Mass Spectrometric Analysis of 2378-Substituted PCDDs, PCDFs, and CPCBs on a Thermo Electron MAT95 XP Mass Spectrometer.

Descriptor	Analyte	Ion	Accurate Mass
1	Lockmass	-	292.982
	TCB	M	289.9223
	TCB	M+2	291.9194
	$^{13}C_{12}$-TCB	M	301.9625
	$^{13}C_{12}$-TCB	M+2	303.9596
2	Lockmass	-	316.982
	TCDF	M	303.9016
	TCDF	M+2	305.8987
	$^{13}C_{12}$-TCDF	M	315.9418
	$^{13}C_{12}$-TCDF	M+2	317.9389
	TCDD	M	319.8965
	TCDD	M+2	321.8935
	$^{13}C_{12}$-TCDD	M	331.9367
	$^{13}C_{12}$-TCDD	M+2	333.9338
	$^{13}C_{6}$-1234-TCDD	M+4	331.9078
	PnCB	M+2	325.8804
	PnCB	M+4	327.8775
	$^{13}C_{12}$-PnCB	M+2	337.9206
	$^{13}C_{12}$-PnCB	M+4	339.9176
3	Lockmass	-	366.979
	PnCDF	M	337.8627
	PnCDF	M+2	339.8597

	$^{13}C_{12}$-PnCDF	M	349.9028
	$^{13}C_{12}$-PnCDF	M+2	351.8999
	PnCDD	M	353.8576
	PnCDD	M+2	355.8546
	$^{13}C_{12}$-PnCDD	M	365.8977
	$^{13}C_{12}$-PnCDD	M+2	367.8948
	HxCB	M+2	359.8414
	HxCB	M+4	361.8385
	$^{13}C_{12}$-HxCB	M+2	371.8817
	$^{13}C_{12}$-HxCB	M+4	373.8788
4	Lockmass	-	380.976
	HxCDF	M+2	373.8107
	HxCDF	M+4	375.8178
	$^{13}C_{12}$-HxCDF	M+2	385.8609
	$^{13}C_{12}$-HxCDF	M+4	387.8580
	HxCDD	M+2	389.8156
	HxCDD	M+4	391.8127
	$^{13}C_{12}$-HxCDD	M+2	401.8558
	$^{13}C_{12}$-HxCDD	M+4	403.8529
5	Lockmass	-	430.973
	HpCDF	M+2	407.7817
	HpCDF	M+4	409.7788
	$^{13}C_{12}$-HpCDF	M+2	419.8219
	$^{13}C_{12}$-HpCDF	M+4	421.8190
	$^{13}C_{12}$-1234789-HpCDF	M+2	419.8219
5	$^{13}C_{12}$-1234789-HpCDF	M+4	421.8190
	HpCDD	M+2	423.7767
	HpCDD	M+4	425.7737
	$^{13}C_{12}$-HpCDD	M+2	435.8168
	$^{13}C_{12}$-HpCDD	M+4	437.8139
6	Lockmass	-	466.973
	OCDD	M+2	457.7377

Descriptor	Analyte	Ion	Accurate Mass
6	OCDD	M+4	459.7349
	$^{13}C_{12}$-OCDD	M+2	469.7780
	$^{13}C_{12}$-OCDD	M+4	471.7749
	OCDF	M+2	441.7427
	OCDF	M+4	443.7398
	$^{13}C_{12}$-OCDF	M+2	453.7800
	$^{13}C_{12}$-OCDF	M+4	455.7800

Figure 3 Ion Chromatogram of PCDDs, PCDFs and cPCBs



Top: native DFP

Bottom: ^{13}C-labeled DFP

7.2 Calibration Verification

7.2.1 Daily Function Check.

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

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. PCDD, PCDF, and cPCB: Analyze the 1, 10, 100, and 250 pg/5 µL calibration standards (Table 1) within a three 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.

7.2.3 Calibration verification -- among instruments.

At least once every six months, an among instrument calibration verification will be performed over the reportable range of the method to ensure that the accuracy of the measurement process among instruments, over the reportable range, is maintained over time. Analyze the 1, 10, 100, 175, and 250 pg/5µL calibration standards (Table 1) within a three day time period on each mass spectrometer. The calculated Pearson correlation coefficient of the results for each instrument pair and congener must be greater than 0.95. The Supervisor will compute correlations of the among instrument calibration verification and maintain the results in the respective instrument QC manuals.

8. OPERATING INSTRUCTIONS FOR SAMPLE CLEANUP

8.1 An Analytical Run

An analytical run consists of twelve samples: the first and seventh is a method blank and the sixth and twelfth samples are a quality control sample that has and 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 specimens.

8.2 Serum -- (C_{18}) Solid Phase Extraction (SPE) Method for 5 g C_{18} Cartridges and Sample Weights 5-12 g.

Rinse all Teflon and glassware before use. The solvent rinse order is 1) acetone; 2) toluene; 3) 50:50 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

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 database. Add 10 µL of the two internal standards (PCDDs/PCDFs/cPCBs and PCBs), check the pipette for correct dispensing amount. Sonicate the standard for 2 minutes before spiking). Vortex for 15-20 seconds and equilibrate for 15 minutes.

With a graduated cylinder or adjustable dispenser, add 5 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 15 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.) With the same graduated cylinder used for formic acid, measure a volume of high purity water equal to the volume of formic acid and add it to the serum formic acid mixture. (If the final volume is less than 5 mL, add enough high purity water to make the final volume 5 mL.) Vortex and then allow gas to escape from the solution. Wait 15 minutes.

Activation of the C_{18} SPE cartridges.

Attach one 5g 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 C_{18} cartridge followed by 2 volumes of high purity water. Discard methanol in appropriate waste container.

C_{18} Extraction of PCDDs, PCDFs, cPCBs and ortho-substituted PCBs.

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 5 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 5 mL high purity water. Dry the SPE cartridge under vacuum (15 psi) for about 60 minutes on the vacuum manifold.

Using the SPE vacuum manifold, elute PCDDs/PCDFs/CPCBs into labeled solvent rinsed 20x125 mm glass tube. Measure hexane elution solvent with a hexane dispenser, apply to cartridge and collect eluate as follows:

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

Collect all aliquots from one cartridge in same tube. Remove any water extracted from cartridge with Pasteur pipette and cap tubes with screw caps until ready to start FMS Power-Prep/6 System.

8.3 The Power-Prep/6 Sample Cleanup Procedure.

The Power-Prep/6 system is used to remove additional interferences present in the serum eluates from the C_{18} SPE cartridges. The eluates are pumped through acid/neutral/base silica columns, alumina columns and AX-21 carbon columns using hexane and 10% DCM/hexane solvents. The PCDDs, PCDFs, cPCBs and PCBs are eluted on to the carbon columns with 50% dichlomethane/hexane. The PCDDs, PCDFs, and cPCBs are retained by the carbon while PCBs compounds are washed off by the 50% DCM/hexane. The PCB fractions are collected in forward direction off the carbon column. The PCDD, PCDF, and cPCB fractions are removed from the carbon columns with toluene in the reverse direction. The toluene extract is collected in a TurboVap tube and evaporated to 0.35 mL at 50°C, 10 psi in TurboVap II. The sample is transferred to an auto sampler vial with 1µL dodecane 'keeper' where the toluene is evaporated to "dryness" at room temperature. Table 4 describes the Power Prep/6 program.

Table 4 TEQ**SIX Program for Clean-up of a Sample for PCDDs, PCDFs, and cPCBs Through the 6-Carbon Column POWER-Prep/6 System.**

Batch	Flow	Volume Rate (mL)	Modules 1 2 3 4 5 6 7 8	Comments
1	13	60	0 3 1 2 2 0 0 6	50% wet silica
2	15	15	0 1 2 2 2 0 0 6	change to hexane
3	13	60	0 1 1 2 2 0 0 5	hex wet silica
4	15	15	0 1 2 2 2 0 0 5	hex bypass to waste
5	13	35	0 1 2 1 2 0 0 5	wet alumina
6	15	15	0 5 2 2 2 0 0 6	change to toluene
7	8	40	0 5 2 2 1 1 1 6	toluene carbon rev
8	15	15	0 4 2 2 2 0 0 6	change to etac benz
9	8	10	0 4 2 2 1 1 1 6	etac benz carbon rev
10	15	15	0 3 2 2 2 0 0 6	change to 50%
11	8	10	0 3 2 2 1 1 1 6	50% carbon rev
12	15	15	0 1 2 2 2 0 0 6	change to hexane
13	8	25	0 1 2 2 1 1 1 5	hexane carbon rev
14	6	30	0 6 1 1 1 2 2 1	add sample PCB
15	6	10	0 1 1 1 1 2 2 1	hex sil/alum/car PCB
16	6	30	0 2 1 1 1 2 2 1	10% sil/alum/car PCB
17	6	130	0 3 1 1 1 2 2 1	50% sil/alum/car PCB
18	15	15	0 4 2 2 2 0 0 6	change to etac benz
19	8	4	0 4 2 2 1 2 2 6	etac benz carbon
20	15	15	0 1 2 2 2 0 0 6	change to hexane
21	8	10	0 1 2 2 1 2 2 6	hexane carbon
22	15	15	0 5 2 2 2 0 0 6	change to toluene
23	6	45	0 5 2 2 1 1 1 2	toluene PCDD/PCDF/cPCB
24	6	2	0 1 2 2 1 1 1 2	hexane purge carbon
25	1	1	0 0 0 0 0 0 0 0	valves off

8.3.1Operating Power-Prep/6 System for sample cleanup for PCDDs, PCDFs, cPCBs and ortho-substituted PCBs.

Daily Function Checks -- perform with 50% dichloromethane/hexane (file = HANRIN1) and hexane (file = HANSRIN2), place collection lines in round deep dish for waste.

Check valves, fittings, and all columns for leaks by filling the solvent reservoirs and flushing the lines. Record results of this RINSE function check daily in Notebook. If applicable (e.g., change valve), also record in Equipment Maintenance Log. Check pump flow rate by collecting hexane eluant for 1 minute during HANSRIN2. The results of both RINSE and FLOW function checks must be satisfactory before proceeding.

Install one acid/neutral/base silica, one alumina, and one carbon column per sample on Power-Prep/6 system. Insert manifold solvent lines into their appropriate solvent bottles: hexane, 50% dichloromethane in hexane, 10% dichloromethane in hexane, 50% ethyl acetate in benzene, and toluene. Attach sample test tube to the manifold in their proper position according to notebook number and insert intake tubing into sample. Place

correct line in 200 mL TurboVap collection tube. Start a run (file =TEQSIX). Check all fittings for possible leaks as the program wets the columns. To stop the program to tighten any leaks press the "Halt" switch. Collect 200mL for PCBs in 200mL TurboVap tube. Collect 45 mL for PCDDs/PCDFs/cPCBs in 200 mL TurboVap tube. Evaporate solvent to 0.35 mL at 50 °C using TurboVap with automatic sensor shutoff. Add 1µL dodecane 'keeper' to 1 mL silanized conical glass vial and transfer 0.35 mL toluene sample to vial. Rinse TurboVap tube with 0.5 mL dichloromethane. Transfer rinse to vial. Allow remaining solvent to evaporate at room temperature (overnight) in plastic box ~1µL dodecane 'keeper' will be retained in the vial. Seal vial using Teflon-faced silicone septa and plastic screw cap. Place run and transfer sheet in rack for Mass Spec Lab.

8.4 HIGH RESOLUTION MASS SPECTROMETRY ANALYSIS OF PCDDs, PCDFs and cPCBs

8.4.1 GC Conditions:

30 m x 0.25mm i.d. x 0.25µm thickness DB-5MS

Injection port temperature	275°C
Oven temperature program	150 °C, hold 0.6 min; 50 °C/min to 250 °C, hold 4 min; 4°C/min to 270 °C; 50°C/min to 310 °C, hold 4 min.
Carrier gas	Helium flow rate 1 cc/min

Splitless injection
Constant flow mode; vacuum correct off; pressure correct off

8.4.2 MS 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 all 2,3,7,8-substituted PCDDs, and PCDFs on the MAT95 XP mass spectrometers consist of six groups. Table 3 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. Sixty-five ion fragments are monitored for the 20 PCDD and PCDF analytes and 5 PCBs. 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 carbon-13-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 PCDDs and PCDFs

After installation of a new GC column, inject a PCDD and PCDF calibration and determine the retention time windows for all the congeners. Verify the GC column specificity for each 2,3,7,8- substituted PCDD and PCDF. For each congener, determine the retention times relative to the interfering compounds $^{13}C_{12}$-labeled isomer present for each congener group.

Daily Instrument Function Checks.

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

Inject 2µL of a 0.25 pg/µL 2,3,7,8-TCDD S/N ratio check standard. Begin the run by programming the 30m 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 °C at 50 °C/min. The column temperature is held at 310 °C for 3 min. Check the sensitivity of the instrument by verifying that the S/N ratio for the unlabeled 2,3,7,8-TCDD (m/z 319.8965)is greater than 30:1 in order to begin analyzing specimen. If the S/N ratio is unsatisfactory: 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 (Table 2) and compute the Slope Checks for 2378D, 123478F, OCDD, and 344P which serve as surrogates for checking the calibration of all PCDDs, PCDFs and CPCBs and as recovery standard for the day:

Slope Check = R_factor of Standard/Concentration of Standard

where 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 $^{13}C_{12}$ 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 $^{13}C_{12}$-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

Note: See section 8.4 in PCB and Pesticide method for details of mass spectral analysis of ortho-substituted PCBs.

Reconstitute samples from cleanup with diluent (25 ppq $^{13}C_6$-1,2,3,4 TCDD)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 whole weight concentration , the absolute recovery of the primary internal standard, the isotope ratio (Table 4) for the two native ions and the two $^{13}C_{12}$-labeled internal standard ions; and the retention time of each analyte peak relative to its associated carbon-^{13}-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, or once the setting is greater than 2.3. The outer source is replaced annually. GC column is replaced as needed usually every two months. Reference inlet septum and auto sampler 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 PCDDs, PCDFs and cPCBs, the units are ppq(fg/g).

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

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

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) = (2.27xTCHOL+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 samples, 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

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

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

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

$C_{average}$= the average lipid adjusted concentration from the two analyses.

9. REPORTABLE RANGE OF RESULTS

9.1 Criterion for Calibration Standards.

The ion current responses for each mass of a particular analyte or $^{13}C_{12}$ 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 2]. These confidence intervals are periodically updated. The recovery of the internal quantization 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 $^{13}C_{12}$ internal standard must maximize to within ± 1 second of each other. The ion current intensities for a particular analyte must be three 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 2]. 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 specimens 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 eight unknown samples must first give valid results. If one or more of the requirements are not met for the blank or both QC samples, then the seven unknown sample results cannot be reported. The ion current responses for each mass of a particular analyte or $^{13}C_{12}$ internal standard must maximize to within ± 1 second of each other. The ion current intensities for a

particular analyte must be at least 3.0 times the noise level (S/N ≥ 3.0). The isotope ratio of the analyte and the $^{13}C_{12}$ internal standard must fall within the confidence intervals established for each analyte [see Table 2] 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 three-parts-per-thousand 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

The Division of Laboratory Sciences has 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

Initial calibration. 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.

10.2 Blanks (Bench Controls).

A laboratory method blank is prepared along with every eight unknown samples and inserted into positions A and G 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.

10.3 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 through all of the Power-Prep/6 systems and analyzed on each GC/MS. One QC sample is inserted into position F and

position L of an analytical run of eight 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: (1) 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 (2) 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. Further, every measurement of a set of samples must fulfill the following criteria to be considered a valid measurement: (1) the ratio of the two ions monitored for every analyte and 13C-labelled internal standard, must not deviate more then 20% from the theoretical value, (2) the ratio of the retention time of the analyte over its corresponding $^{13}C_{12}$-labeled internal standard must be within the range 0.99 – 1.01. For analytes that do not have an identical $^{13}C_{12}$ - labeled internal standards, their ratio to the $^{13}C_{12}$-internal standard 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 (3) the measured recovery of the $^{13}C_{12}$-internal standard must be within the range 10-120%.

10.4 Duplicate sample analysis.

If the study protocol requires external blind duplicate samples on a subset of study samples, they are inserted "blind" into different analytical runs. The identity of this sample is "blind" to the laboratory and analyst. The precision is calculated as described in 8.8.5.

10.5 External or Round Robin samples.

Various types of samples may be submitted from a source external to the laboratory (e.g., a study coordinator). These samples, submitted as "blind" samples, may be performance solutions, spiked samples, or duplicate study samples.

10.6 Proficiency Testing.

A HCFA-approved PT program is not available. Depending on the type of studies being performed in the laboratory, either single or multiple congeners are selected for analysis.

When available, two aliquots of the same specimen or duplicate specimens collected from the same person are submitted blind to the laboratory for cleanup and analysis. Duplicate specimens are then assigned to separate analytical runs by the laboratory supervisor to insure that analysis is done on different days. Results from the analysis of the two samples are statistically evaluated to determine if the difference is within the overall long-term variability of the method. Confidence intervals for duplicate results vary depending on concentration levels and/or the amount of sample available for analysis.

10.7 Absolute recoveries of the internal quantitation standards.

The absolute recoveries of the $^{13}C_{12}$-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 10,000 serum samples, we have observed that absolute recoveries of the $^{13}C_{12}$-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. Recoveries above 120% (100% + coefficient of variability (CV)) may indicate potential interferences or an error in spiking the internal standards.

10.8 Mass spectrometer resolving power (RP).

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 13C6-1,2,3,4-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.

10.9 Isotope ratios.

The analytical standards (Table 2) can be used to determine the isotope ratios for the $^{13}C_{12}$-labeled internal standards as well as for the $^{12}C_{12}$ 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.)

10.10 Summary of Quality Assurance Functions.

All the QA functions outlined above have options that allow each PCDD, PCDF and PCB congener 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.

Smith, Stalling, and Johnson (1987) published a detailed study of much possible interference in the analysis for PCDDs and PCDFs. These authors provided us with a sample of this interference mixture, and the following compounds were spiked into blank pork fat at the 10 ppb level and carried through the cleanup procedure: Halowax 1014, 3,3',4,4'-PCB; 3,3',4,4',5-PCB; 3,3',4,4',5,5'-PCB; 2,3,4',5',3-PCB-2-methoxy; 2,4,3',4'-PCB-3-methoxy; 3,5,3',4'-PCB-4-methoxy; 3,3',4,4'-DPE; 2,2',4,4',5-DPE; 2,3'4,4',5,5'-DPE; 2,2',3,3'4,4', 5-DPE; 2,2',3,3',4,4',5,6-DPE; decachloro-DPE; 3,4,5,2',4'-DPE-2-hydroxy; nonachloro-DPE- 2-hydroxy; nonachloro-DPE-3-hydroxy; 3,4,5,2',4'-DPE-2-methoxy; nonachlor-DPE-2-methoxy; nonachlor-DPE-3-methoxy; 2,3,2',4',5'-PCB-4-hydroxy; and 2,2',3',4',5'-PCB-2-hydroxy. In addition, the CDC laboratory formulated mixtures of the following compounds, which were spiked into blank pork fat at the 10-ppm level and carried through the cleanup procedure: Aroclor 1254; Aroclor 1260; hexachloro-benzene; .-hexachlorocyclohexane; p,p'-DDT; o,p'-DDT; p,p'-DDE; o,p'-DDE; t-nonachlor; oxychlordane; heptachlor epoxide; .-hexachlorocyclohexane; dieldrin; and mirex. The results of these experiments demonstrated that potentially interfering compounds present in these mixtures, at 103- to 106-fold excess, were effectively removed during the multi-column cleanup of the sample.

12.2 Potential Method Contamination.

We have observed contamination of adsorbents by PCDDs and PCDFs that originate from commonly used phenolic-based floor cleaning solutions and observed that these contaminants can be formed during the activation of

the alumina which is used during sample cleanup. These contaminants include: (major: 123478HxF, 123678HxF, 1234678HpF) and (minor: 12378PnF, 23478PnF, 123789HxF, 234678HpF, 1234678HpD).

Many laboratories use white chemical tissue wipes for a number of laboratory functions. We have analyzed weighed portions of several types of these tissue wipes and quantified the following 2378-substituted PCDDs and PCDFs: (major: 2378F, 1234678HpD, 1234679HpD, OCDD, MS co-eluting peaks with carbon-13 standards) and (minor: 2378D, 12378PnD, 1234678HpD, 12378PnF, 23478PnF,TCDDs and HxCDDs). These tissue-wipes can cause laboratory contamination of some of the important PCDDs and PCDFs found in human samples.

PCDDs have been reported in cigarettes, cigarette smoke and ash. While 2378-TCDD was not present, other TCDDs were detected along with PnCDDs, HxCDDs, and OCDD.

13. REFERENCE RANGES (NORMAL VALUES)

Reference ranges for PCDDs, PCDFs, and CPCBs had 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 the 18 congeners measured in NHANES 1999-2000. The concentrations of many of the congeners were below their detection limits in most samples. NHANES 2001-2002 was done with a larger sample size, which results in lower the detection limits. The next national exposure report, containing the 2001-2002 NHANES data, will be released in 2005.

14. CRITICAL CALL RESULTS

The health effects resulting from exposure to PCDDs, PCDFs, and cPCBs 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 analysis of PCDDs, PCDFs, and CPCBs in serum and adipose tissue at the parts-per-quadrillion (ppq) levels is an extremely complex measurement. There are no acceptable alternative methods for this analysis. 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 10 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

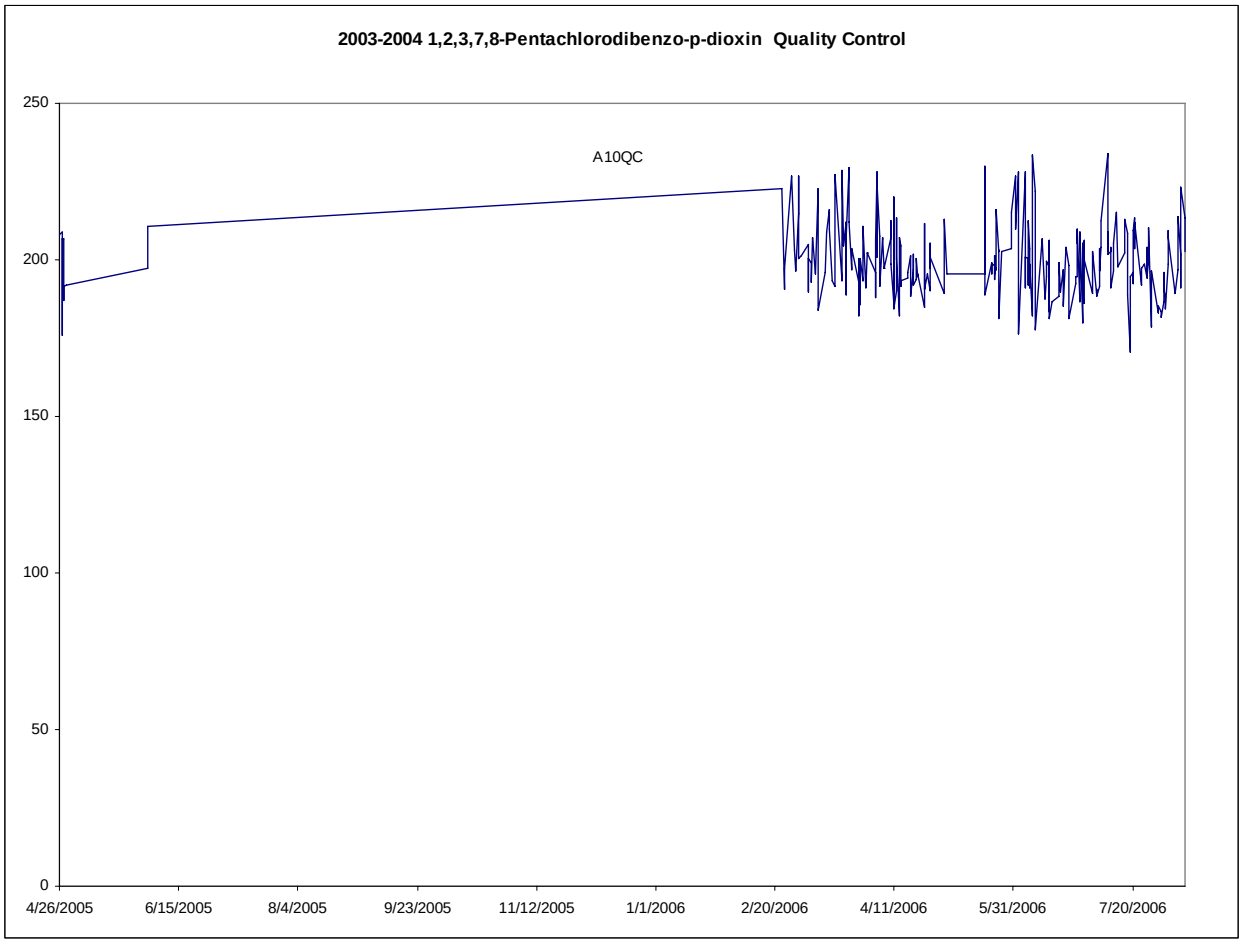
The sample remaining after the test should be returned to storage at -30 °C. Standard record keeping means (database, sample logs, optical disc files) are used to track specimens. Records are maintained for three (3) years, including related QA/QC data; duplicate records are kept in electronic format.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

19. SUMMARY STATISTICS AND QC GRAPHS

A. 1,2,3,7,8-Pentachlorodibenzo-p-dioxin

Summary Statistics for 1,2,3,7,8-Pentachlorodibenzo-p-dioxin by Lot

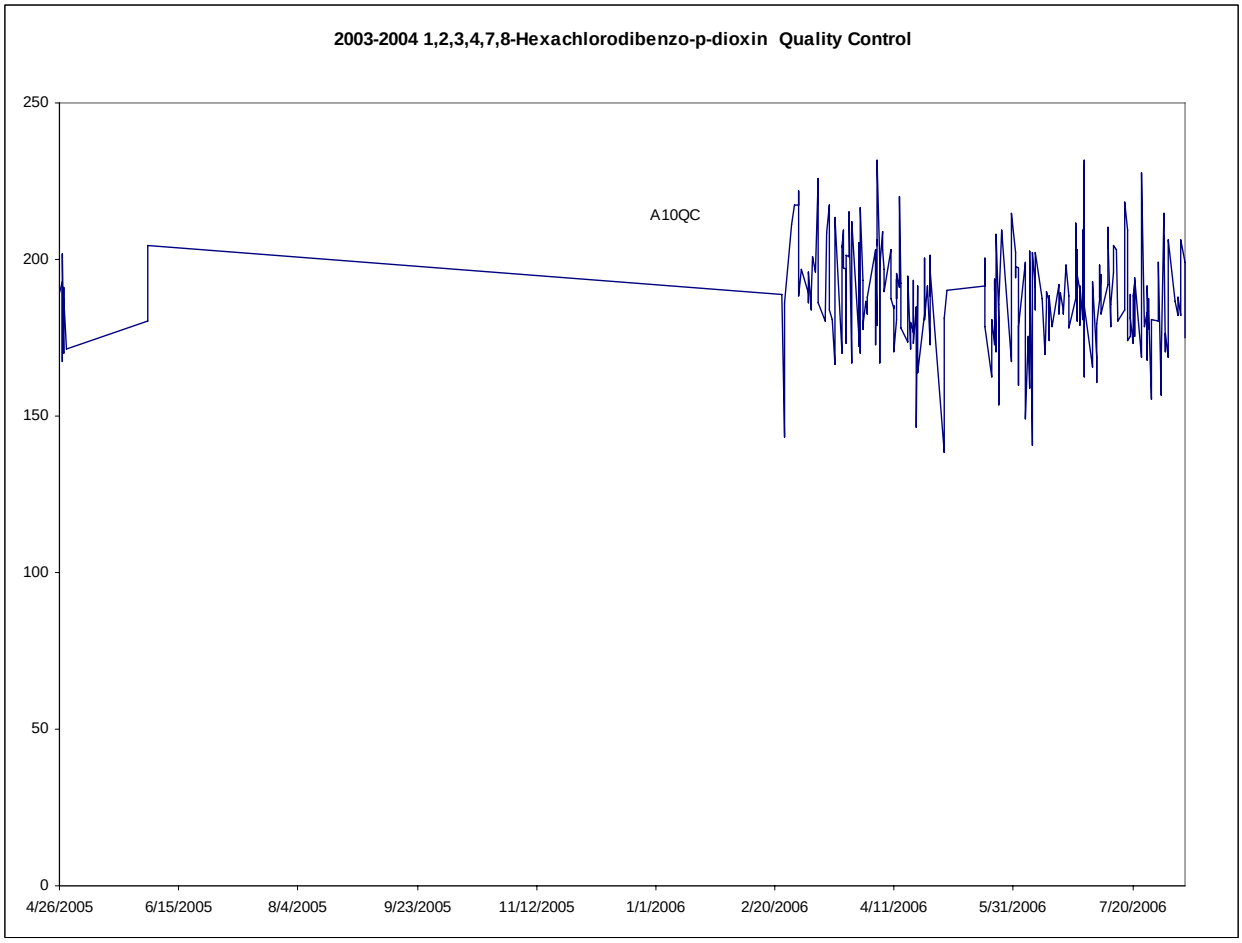
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	257	4/26/2005	8/11/2006	199.9348	11.7846	5.9



B. 1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin

Summary Statistics for 1,2,3,4,7,8-Hexachlorodibenzo-p-dioxin by Lot

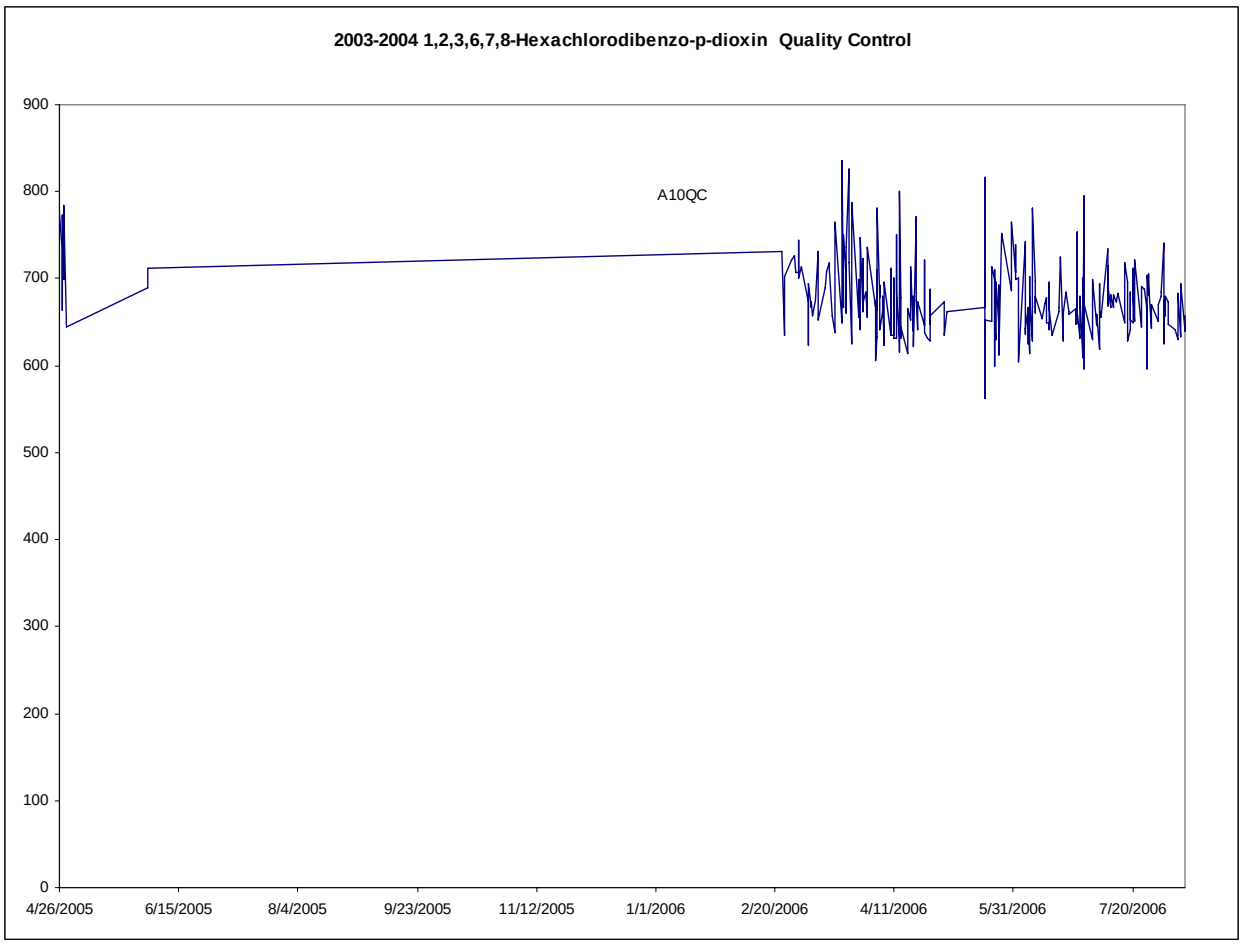
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	255	4/26/2005	8/11/2006	187.7414	15.9697	8.5



C. 1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin

Summary Statistics for 1,2,3,6,7,8-Hexachlorodibenzo-p-dioxin by Lot

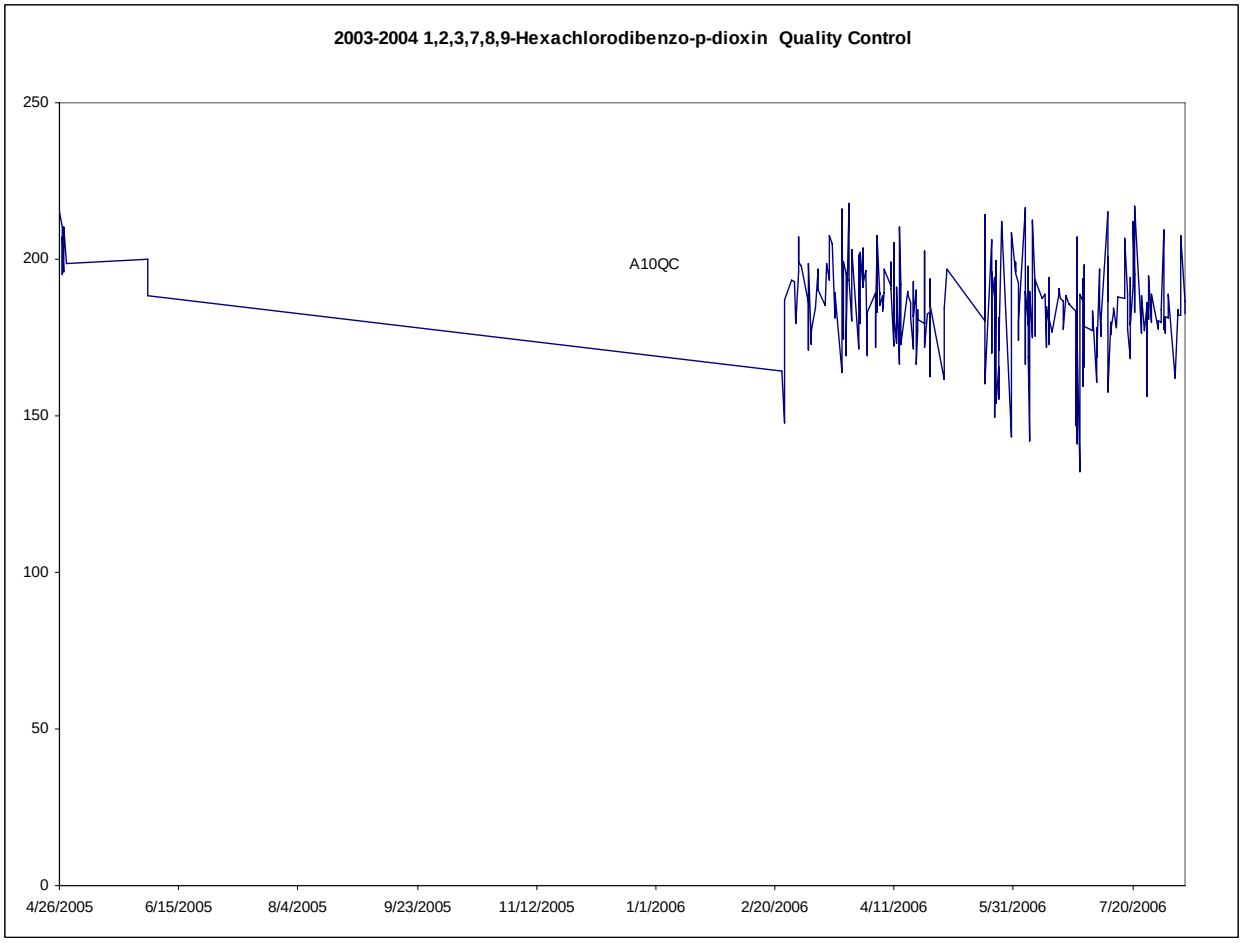
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	256	4/26/2005	8/11/2006	677.4805	44.0518	6.5



D. 1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin

Summary Statistics for 1,2,3,7,8,9-Hexachlorodibenzo-p-dioxin by Lot

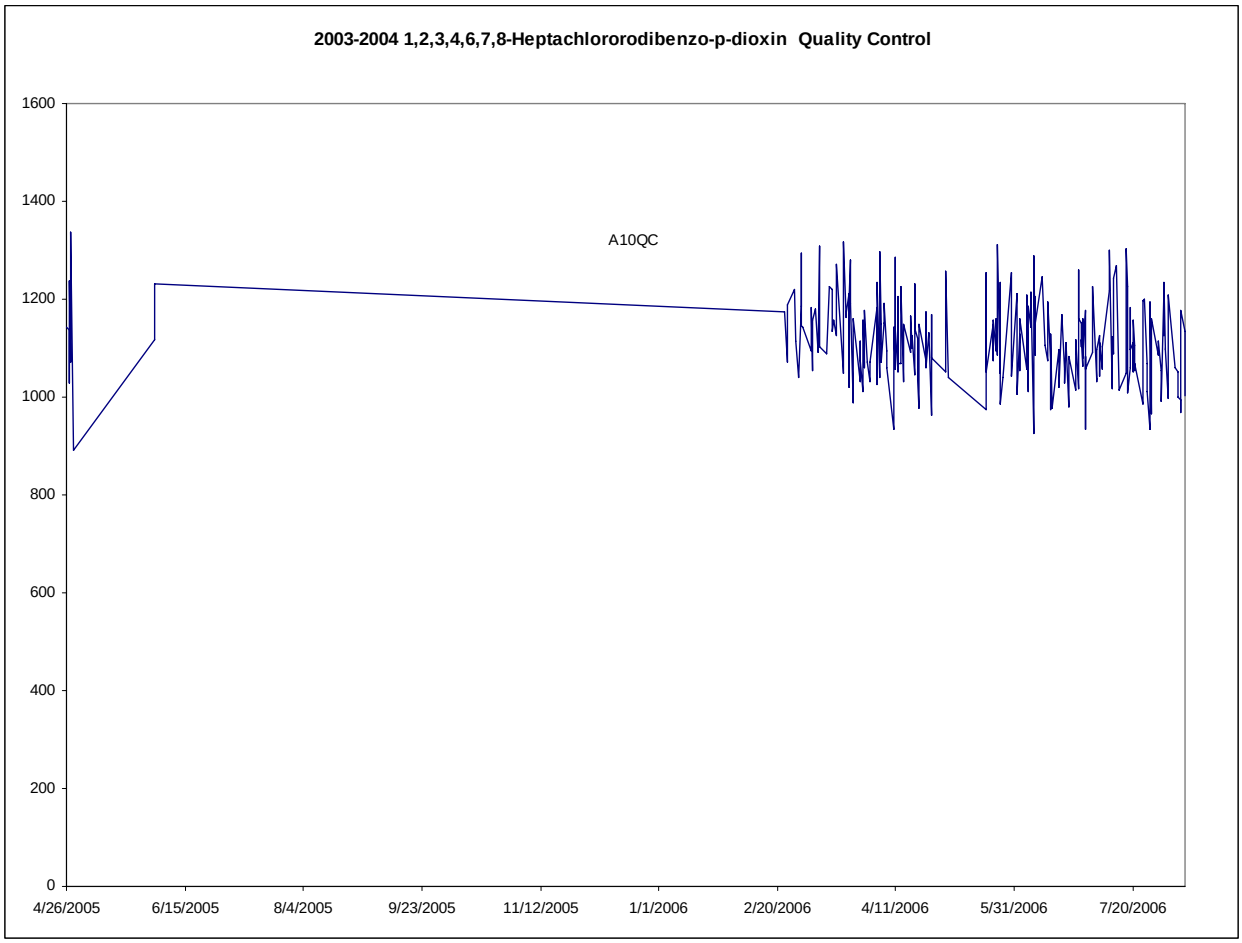
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	256	4/26/2005	8/11/2006	185.5535	14.7124	7.9



E. 1,2,3,4,6,7,8-Heptachlororodibenzo-p-dioxin

Summary Statistics for 1,2,3,4,6,7,8-Heptachlororodibenzo-p-dioxin by Lot

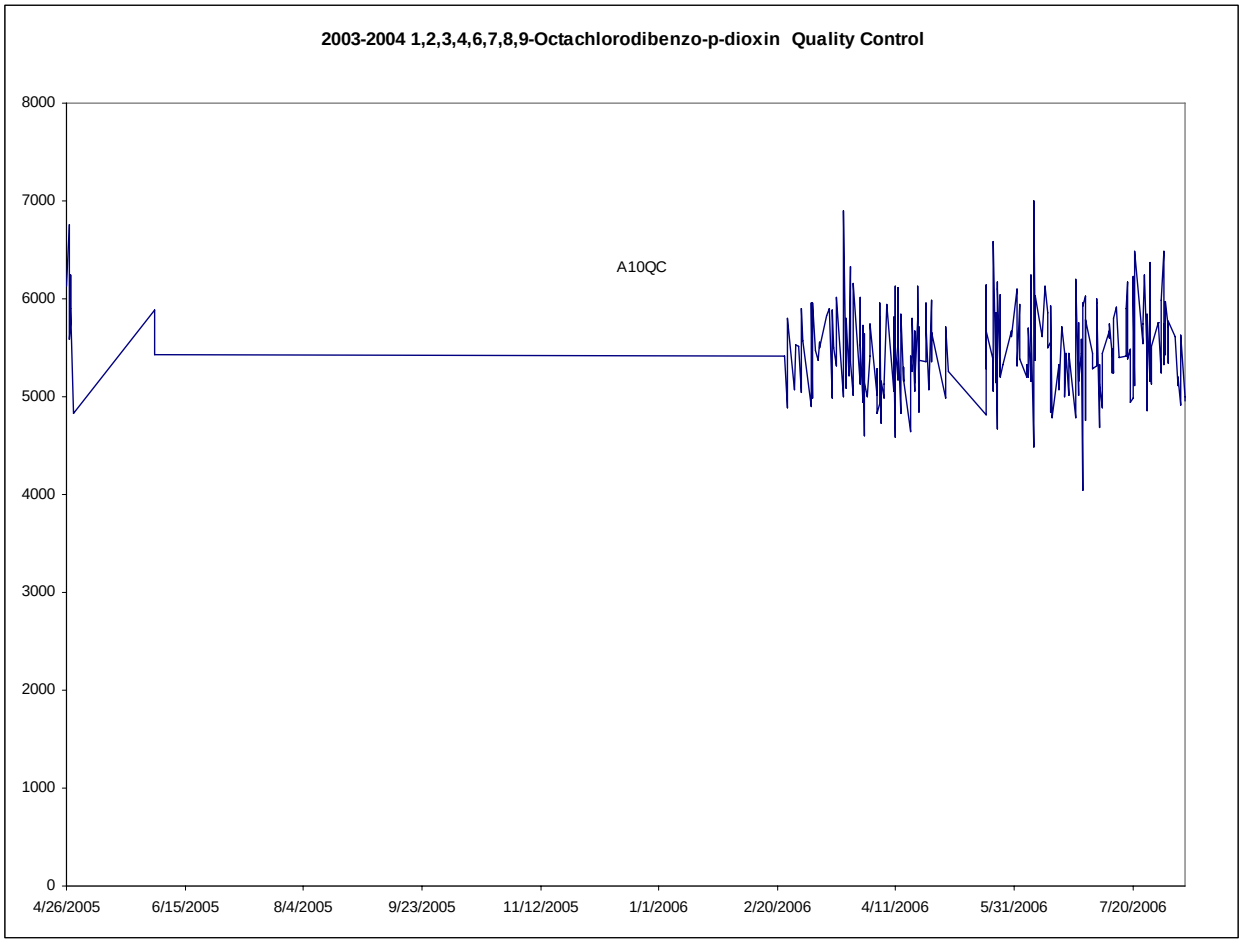
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	256	4/26/2005	8/11/2006	1118.503	86.01	7.7



F. 1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin

Summary Statistics for 1,2,3,4,6,7,8,9-Octachlorodibenzo-p-dioxin by Lot

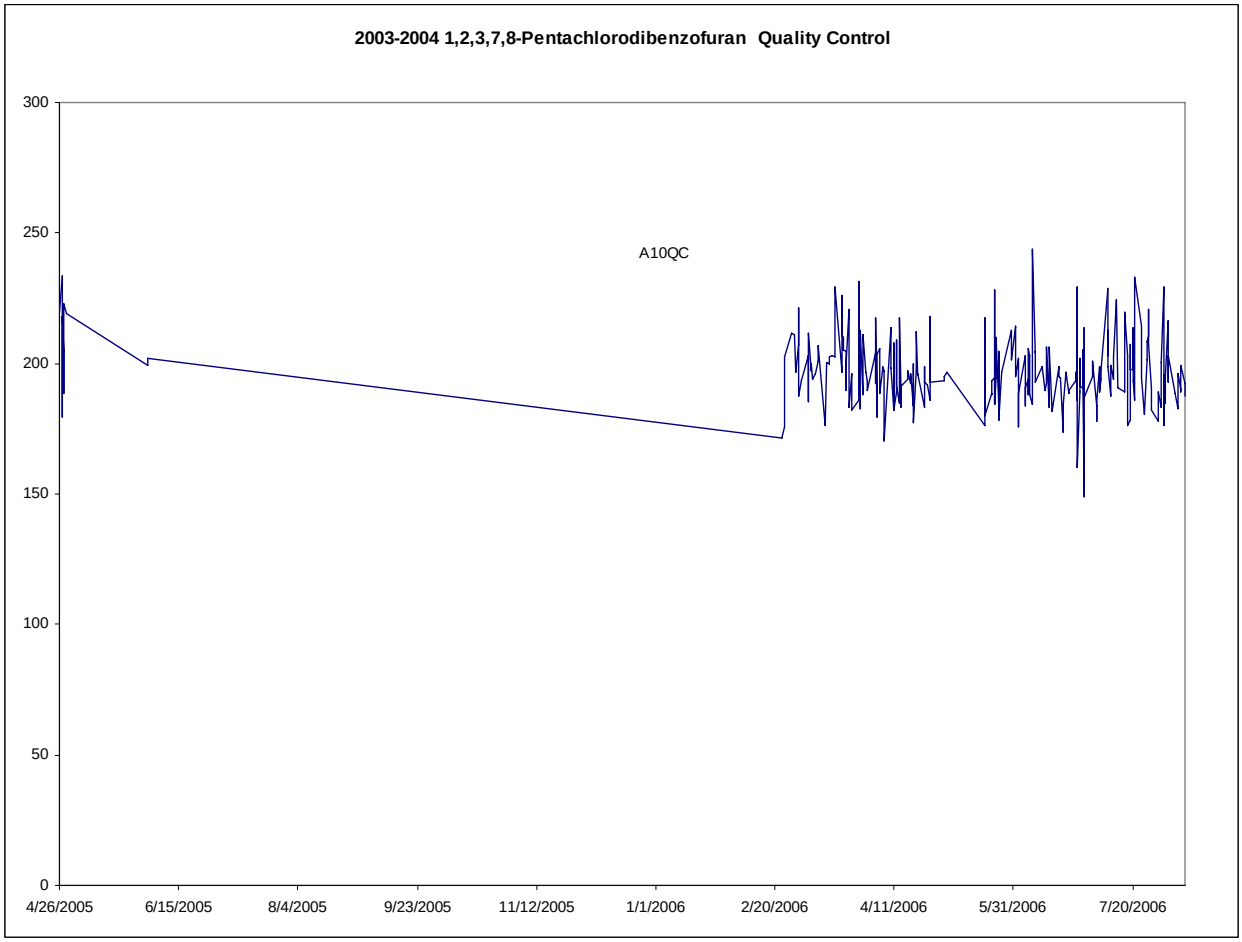
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	254	4/26/2005	8/11/2006	5501.348	449.682	8.2



G. 1,2,3,7,8-Pentachlorodibenzofuran

Summary Statistics for 1,2,3,7,8-Pentachlorodibenzofuran by Lot

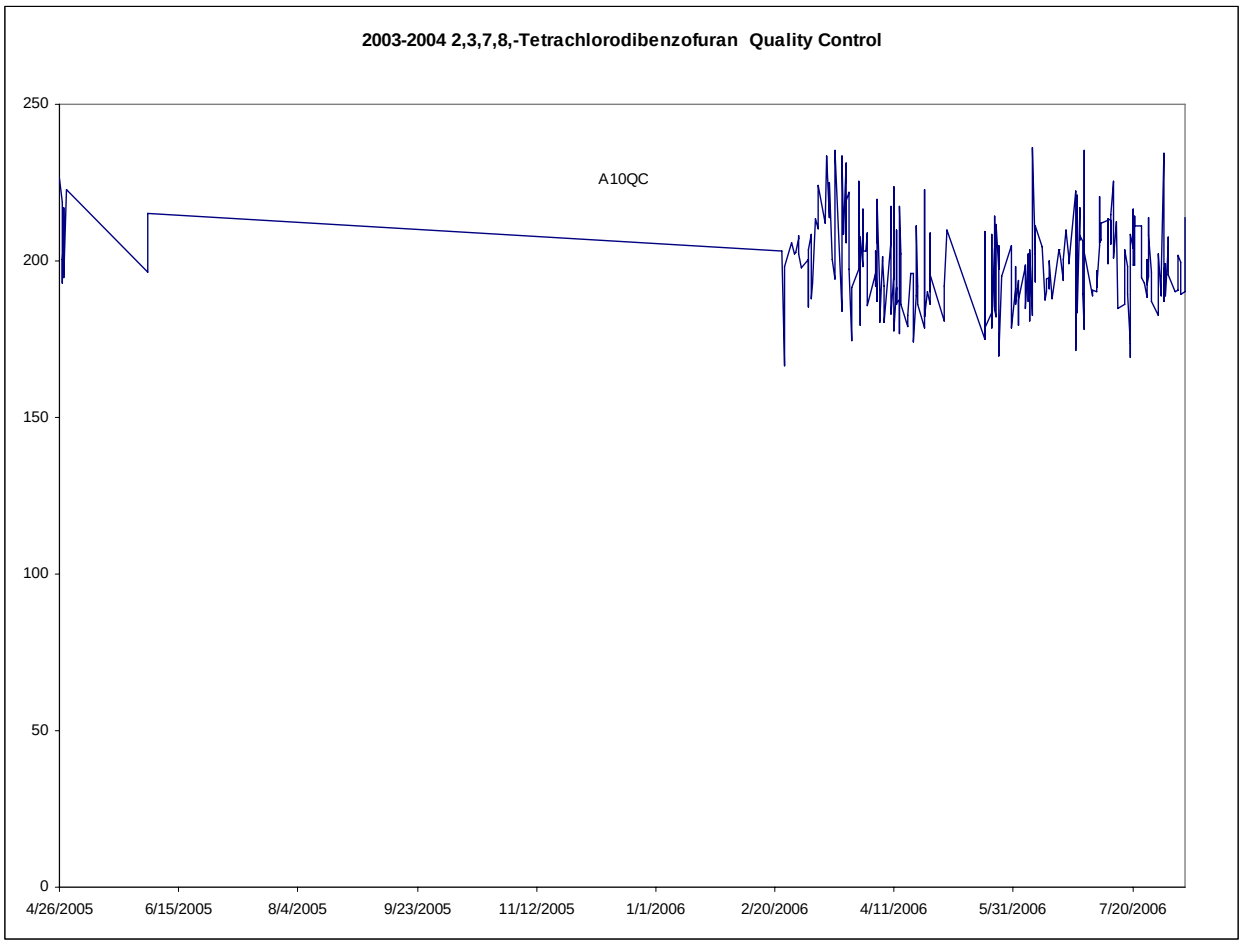
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	256	4/26/2005	8/11/2006	197.002	13.4786	6.8



H. 2,3,7,8,-Tetrachlorodibenzofuran

Summary Statistics for 2,3,7,8,-Tetrachlorodibenzofuran by Lot

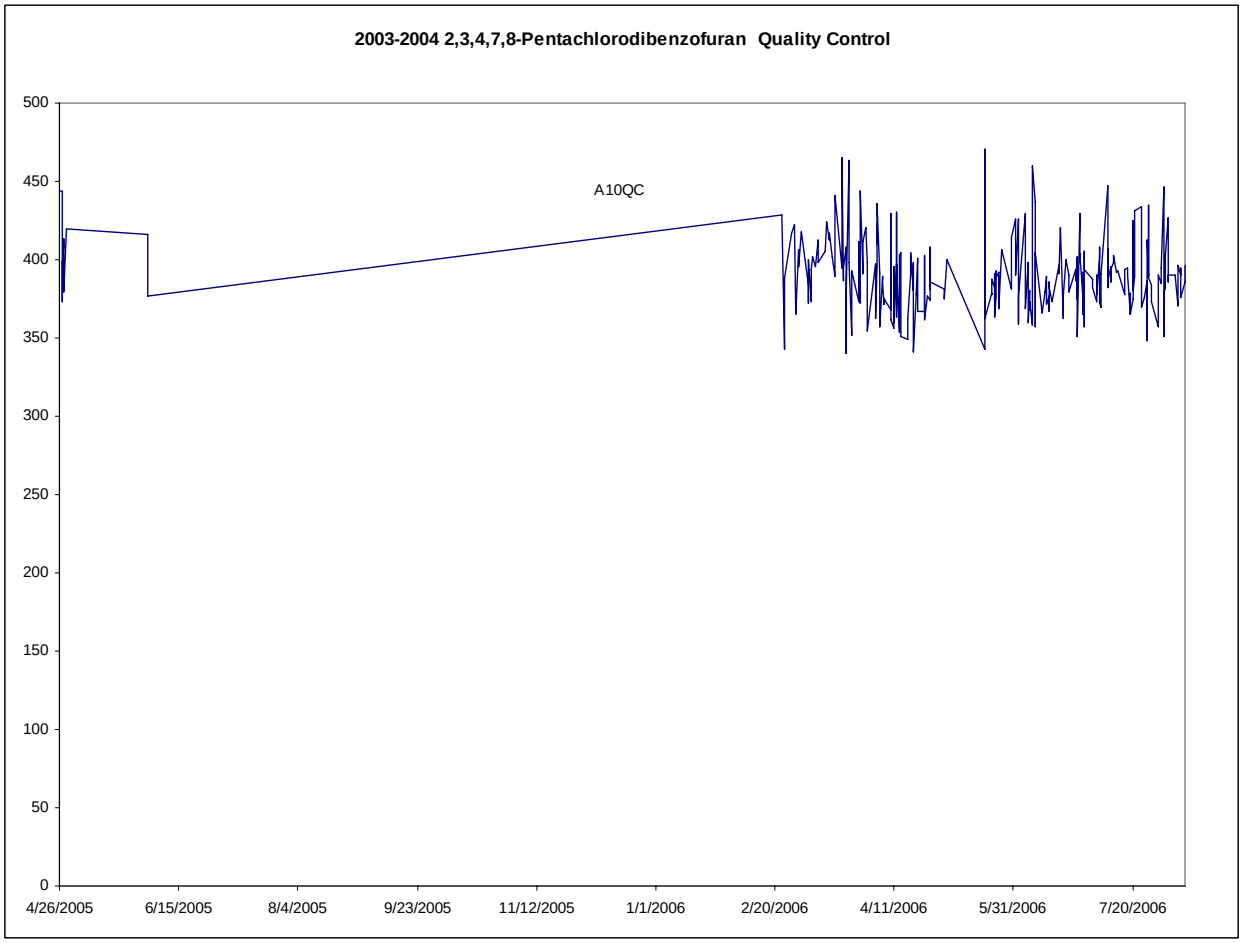
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	256	4/26/2005	8/11/2006	199.2	13.8063	6.9



I. 2,3,4,7,8-Pentachlorodibenzofuran

Summary Statistics for 2,3,4,7,8-Pentachlorodibenzofuran by Lot

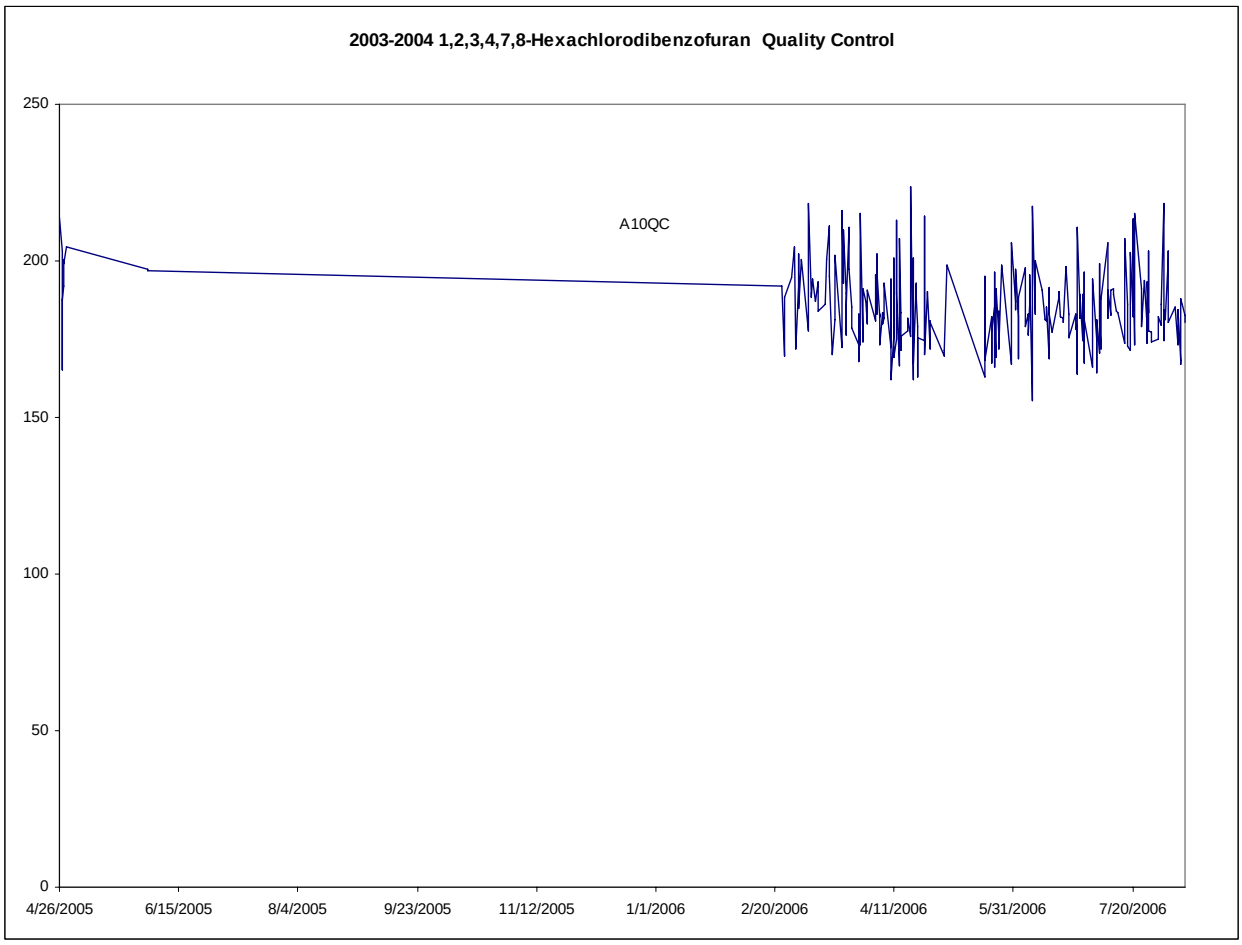
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	256	4/26/2005	8/11/2006	390.1434	23.591	6.0



J. 1,2,3,4,7,8-Hexachlorodibenzofuran

Summary Statistics for 1,2,3,4,7,8-Hexachlorodibenzofuran by Lot

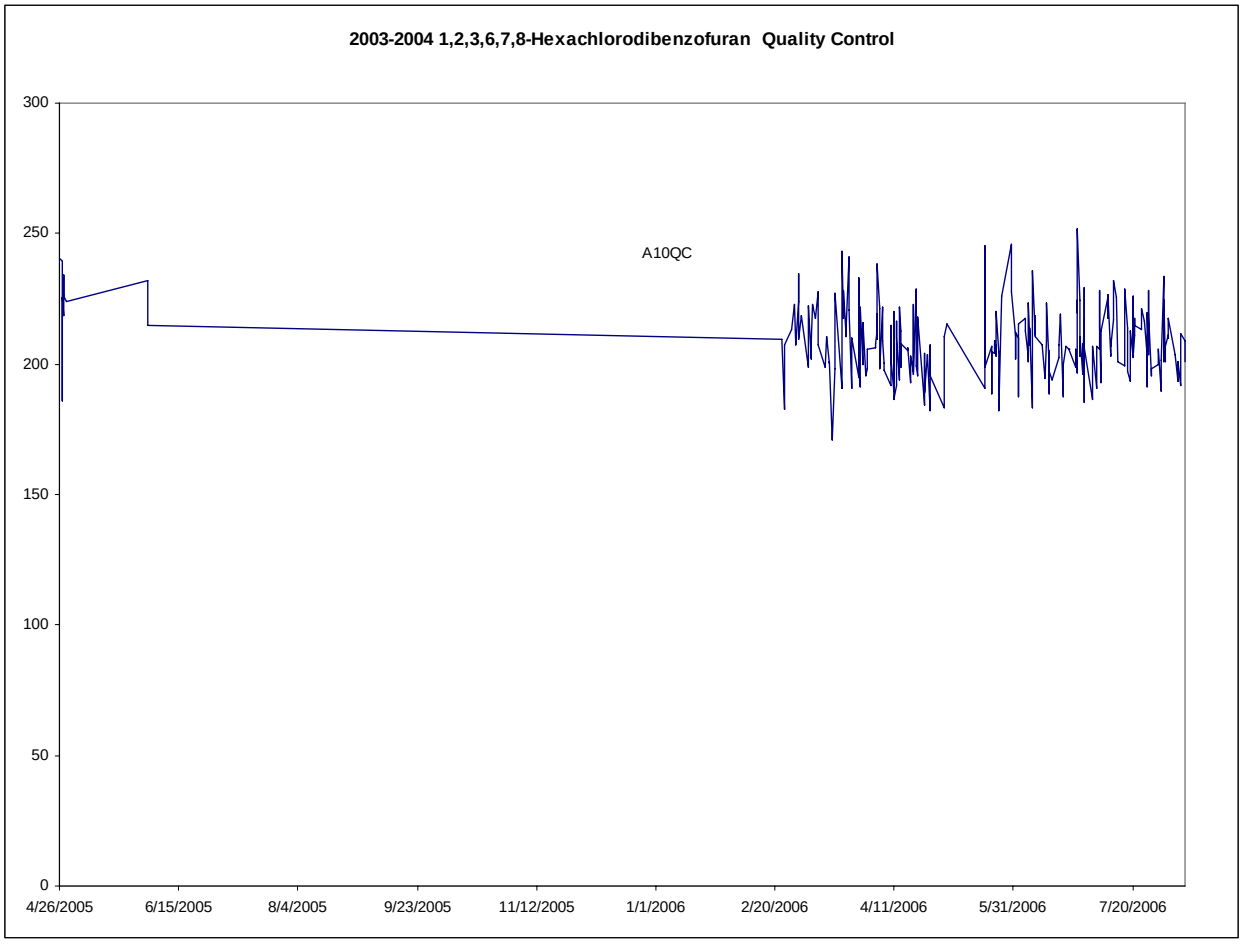
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	256	4/26/2005	8/11/2006	185.648	12.8061	6.9



K. 1,2,3,6,7,8-Hexachlorodibenzofuran

Summary Statistics for 1,2,3,6,7,8-Hexachlorodibenzofuran by Lot

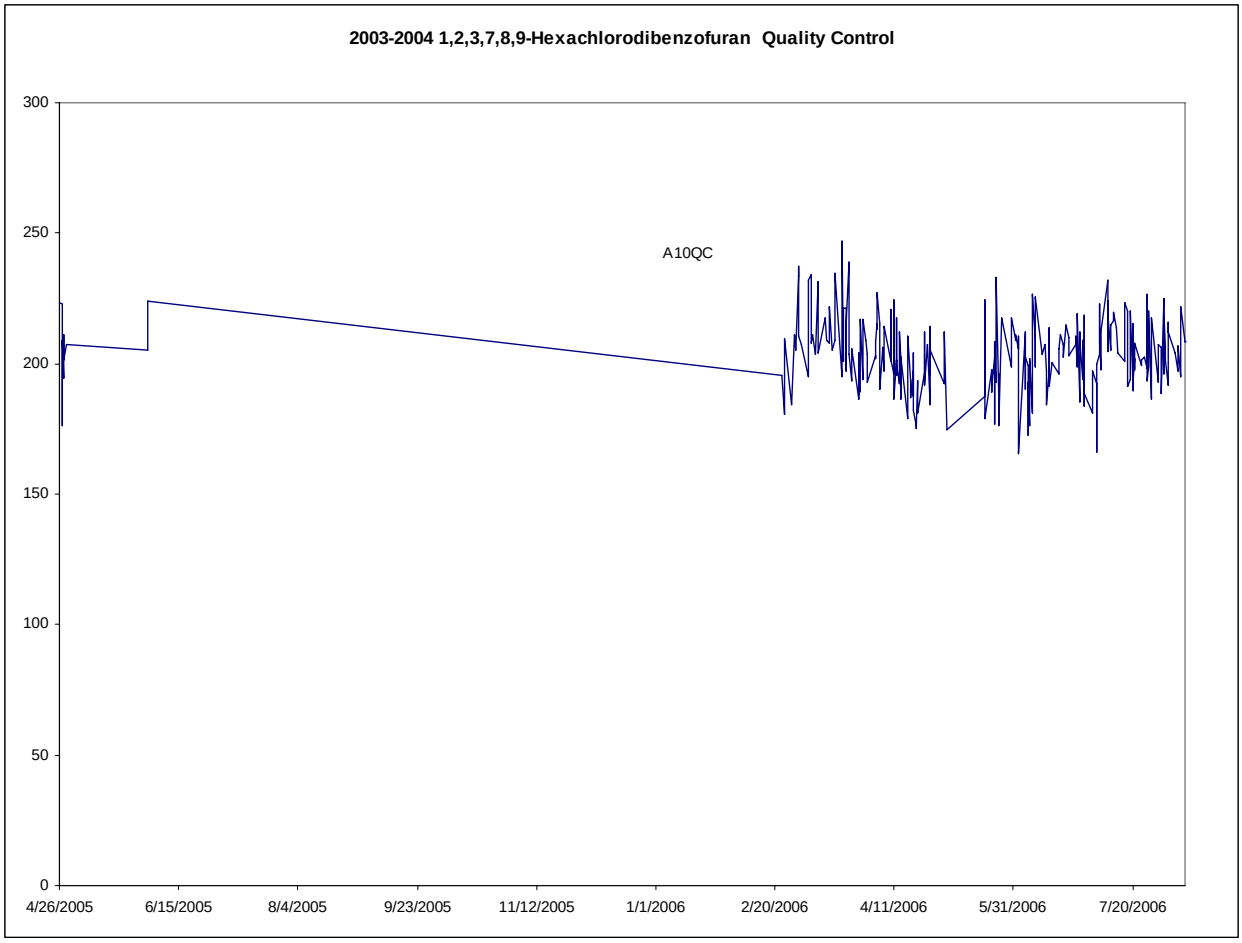
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	256	4/26/2005	8/11/2006	208.9242	13.5093	6.5



L. 1,2,3,7,8,9-Hexachlorodibenzofuran

Summary Statistics for 1,2,3,7,8,9-Hexachlorodibenzofuran by Lot

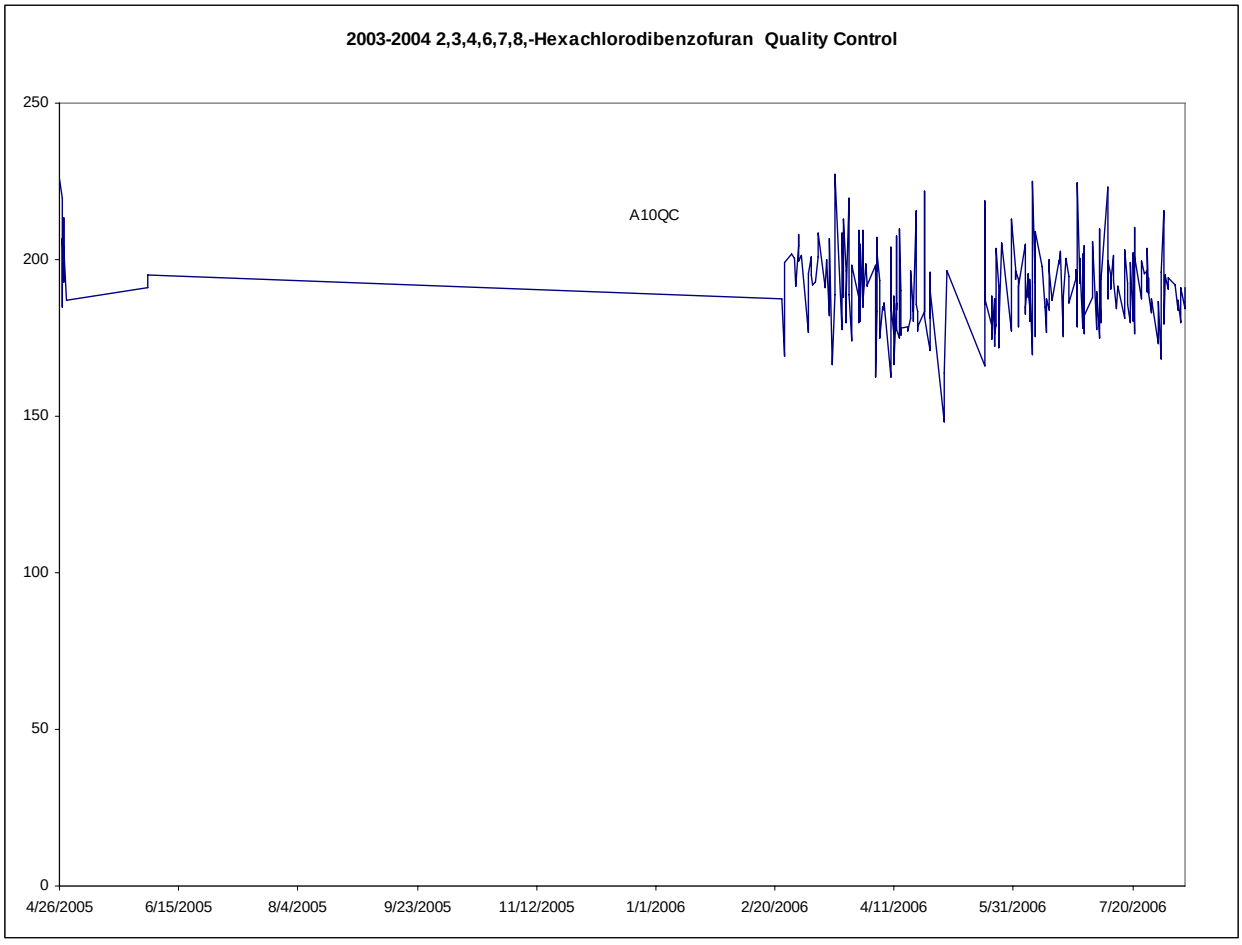
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	256	4/26/2005	8/11/2006	203.8666	13.892	6.8



M. 2,3,4,6,7,8,-Hexachlorodibenzofuran

Summary Statistics for 2,3,4,6,7,8,-Hexachlorodibenzofuran by Lot

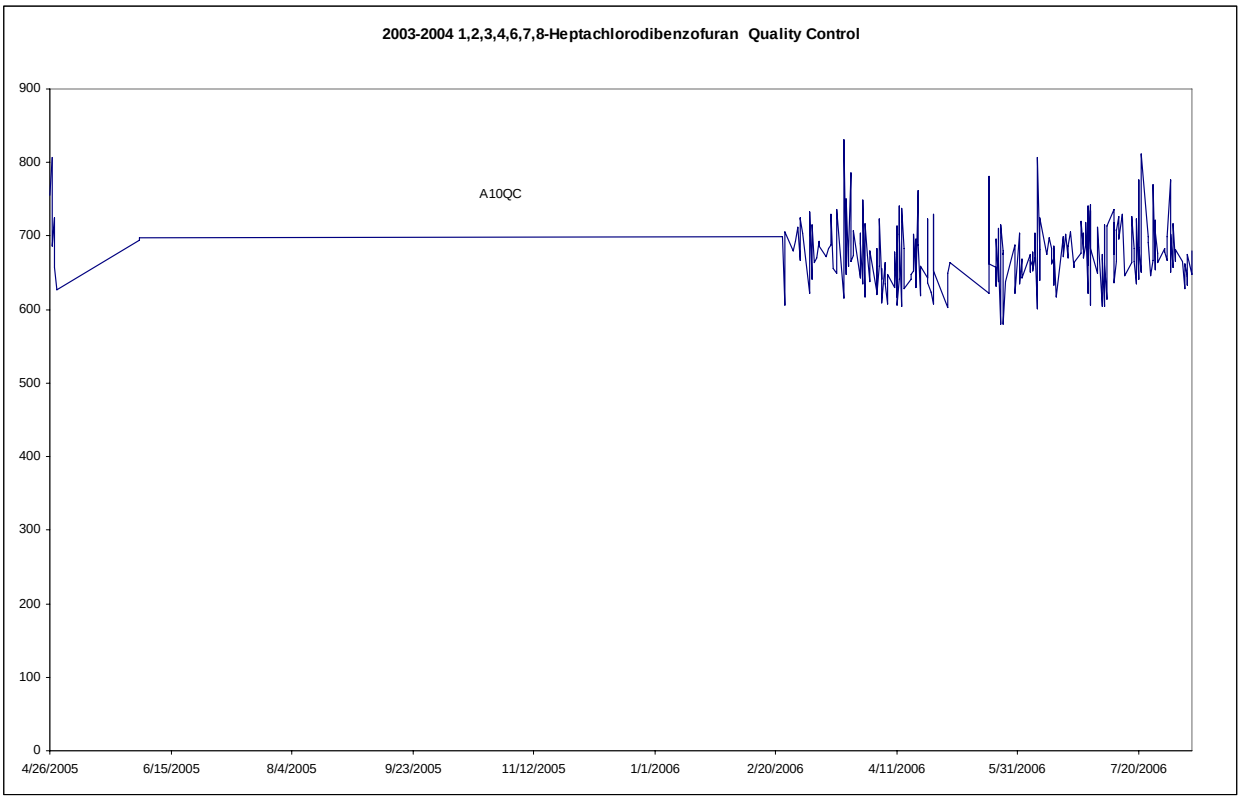
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	256	4/26/2005	8/11/2006	190.6865	12.5924	6.6



N. 1,2,3,4,6,7,8-Heptachlorodibenzofuran

Summary Statistics for 1,2,3,4,6,7,8-Heptachlorodibenzofuran by Lot

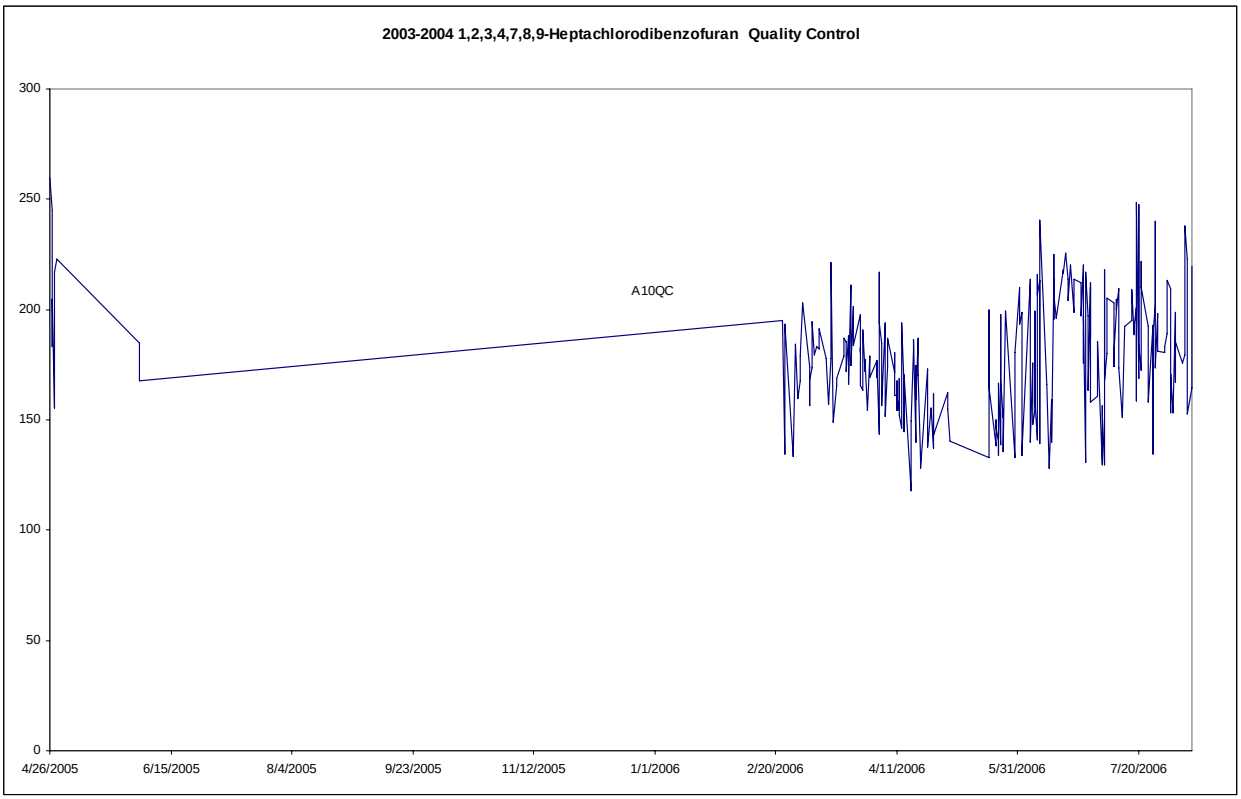
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	255	4/26/2005	8/11/2006	677.6476	42.2159	6.2



0. 1,2,3,4,7,8,9-Heptachlorodibenzofuran

Summary Statistics for 1,2,3,4,7,8,9-Heptachlorodibenzofuran by Lot

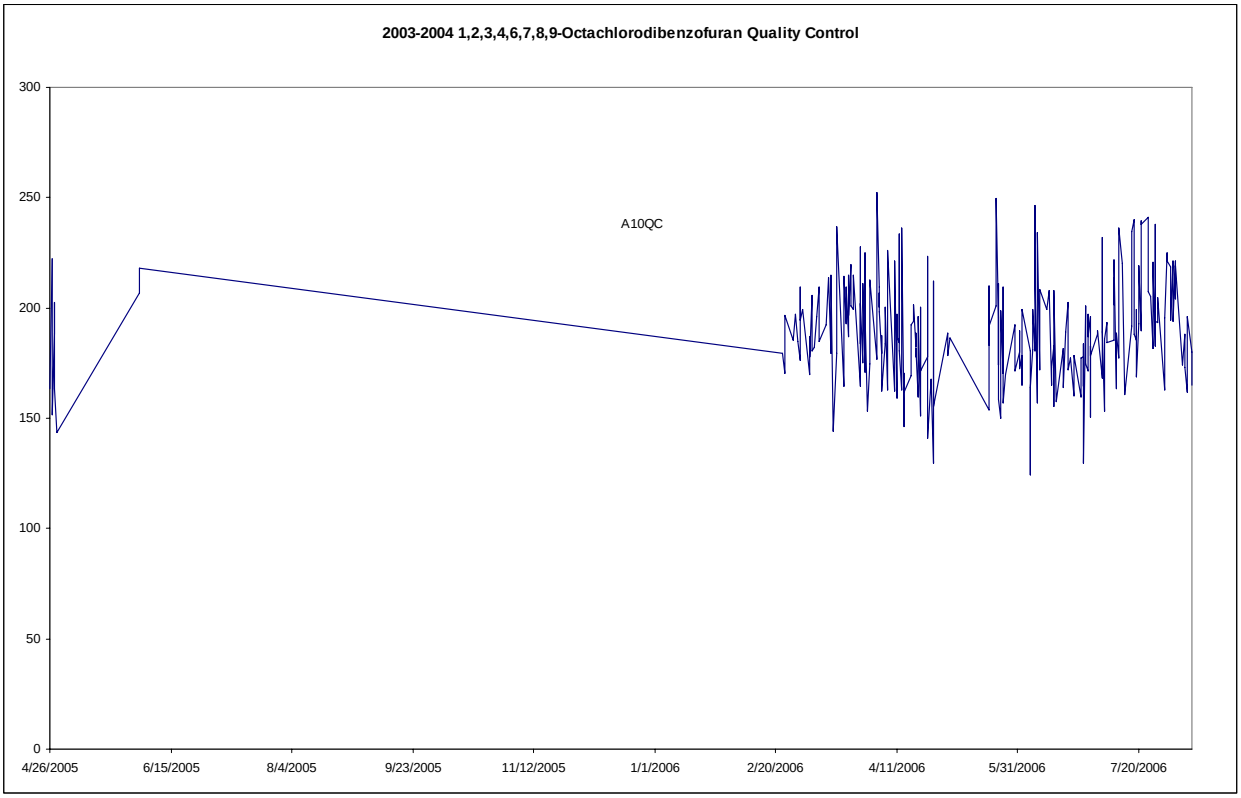
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	254	4/26/2005	8/11/2006	178.1154	27.2255	15.3



P. 1,2,3,4,6,7,8,9-Octachlorodibenzofuran

Summary Statistics for 1,2,3,4,6,7,8,9-Octachlorodibenzofuran by Lot

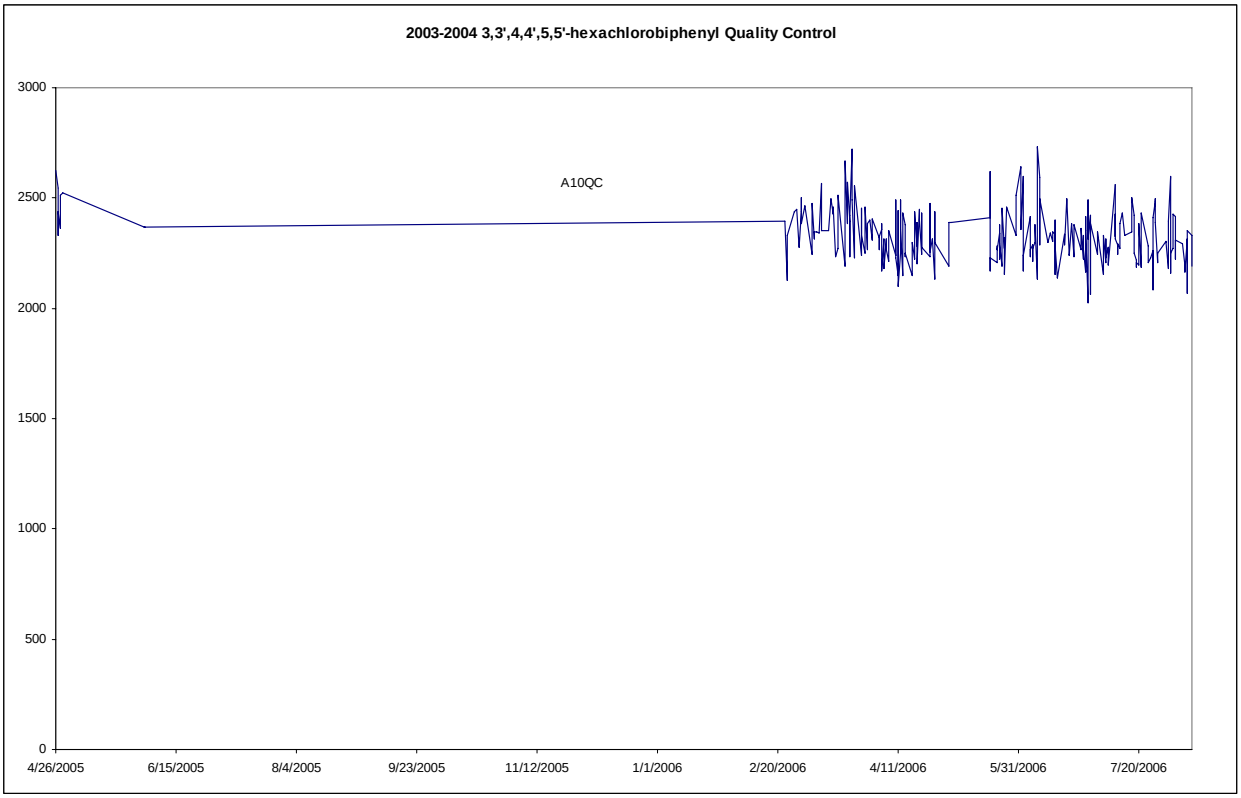
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	254	4/26/2005	8/11/2006	189.552	23.6198	12.5



Q. 3,3',4,4',5,5'-hexachlorobiphenyl

Summary Statistics for 3,3',4,4',5,5'-hexachlorobiphenyl by Lot

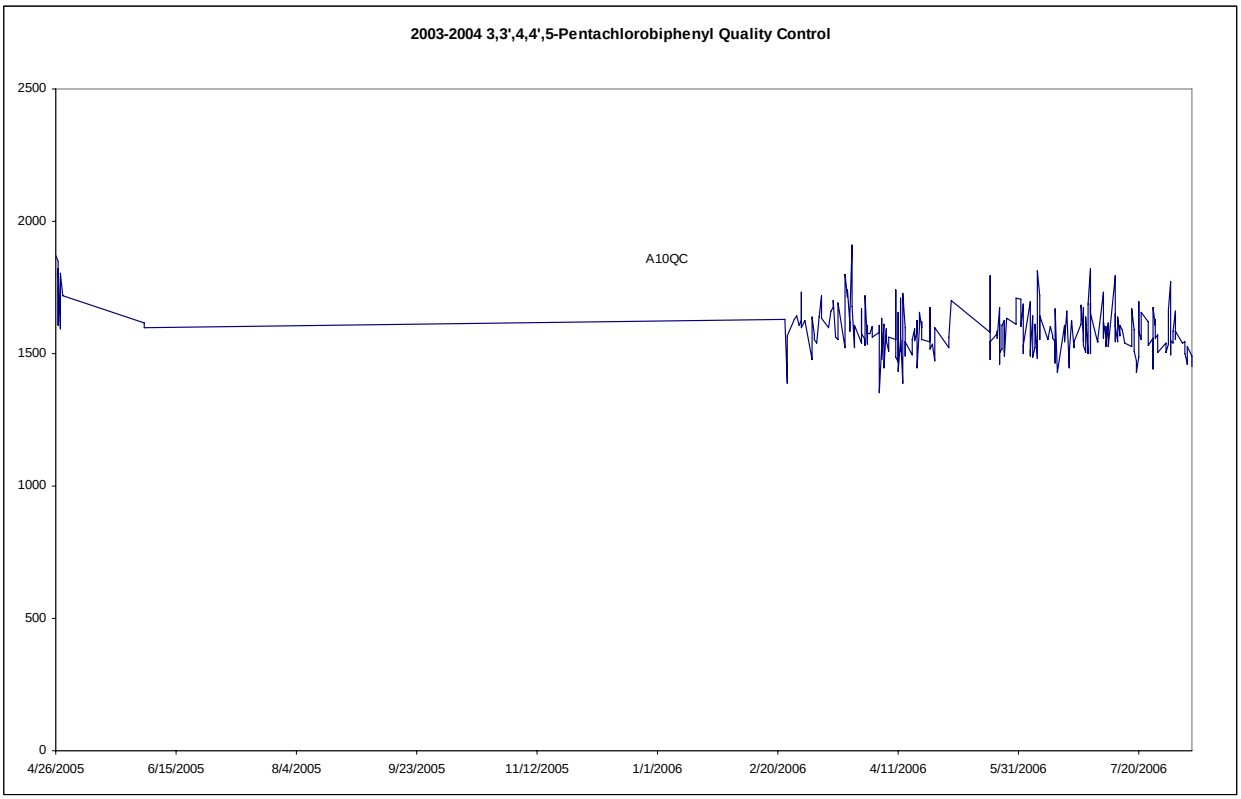
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	256	4/26/2005	8/11/2006	2332.8801	123.0475	5.3



R. 3,3',4,4',5-Pentachlorobiphenyl

Summary Statistics for 3,3',4,4',5-Pentachlorobiphenyl by Lot

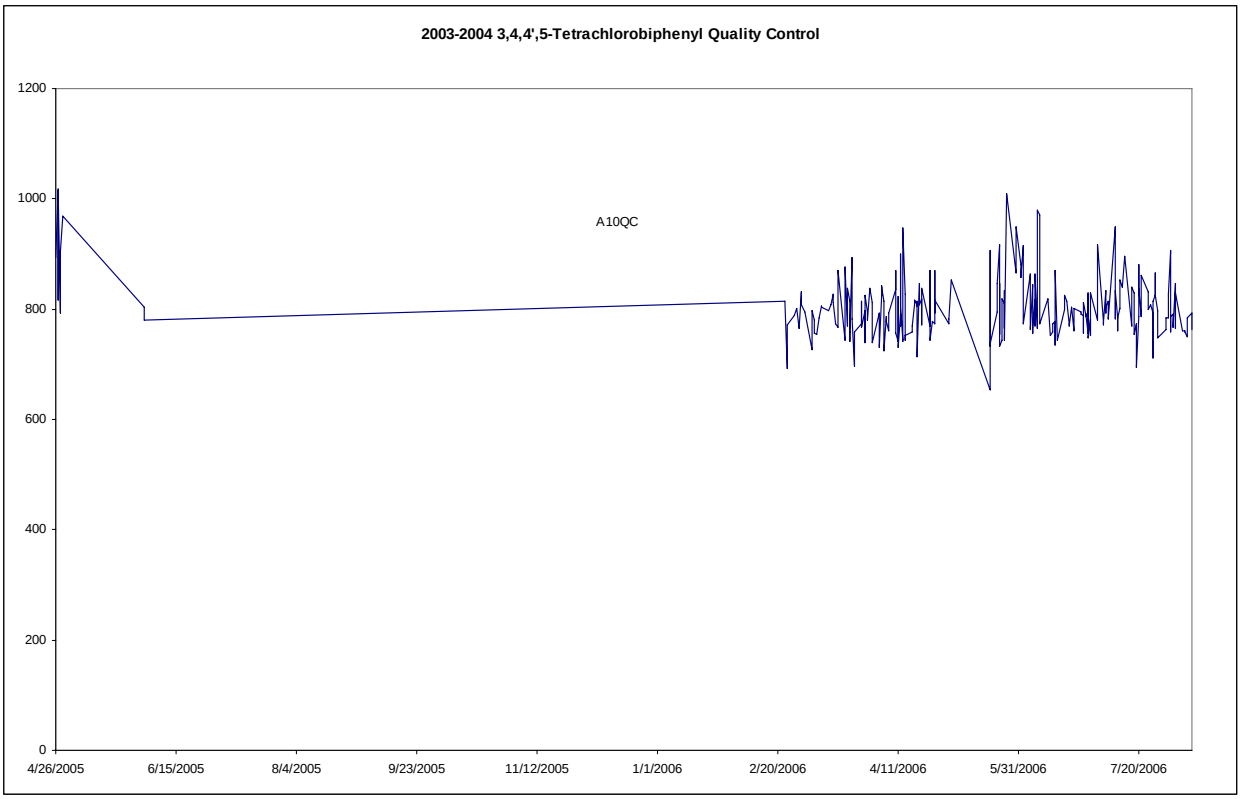
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	255	4/26/2005	8/11/2006	1587.6076	88.6036	5.6



S. 3,4,4',5-Tetrachlorobiphenyl

Summary Statistics for 3,4,4',5-Tetrachlorobiphenyl by Lot

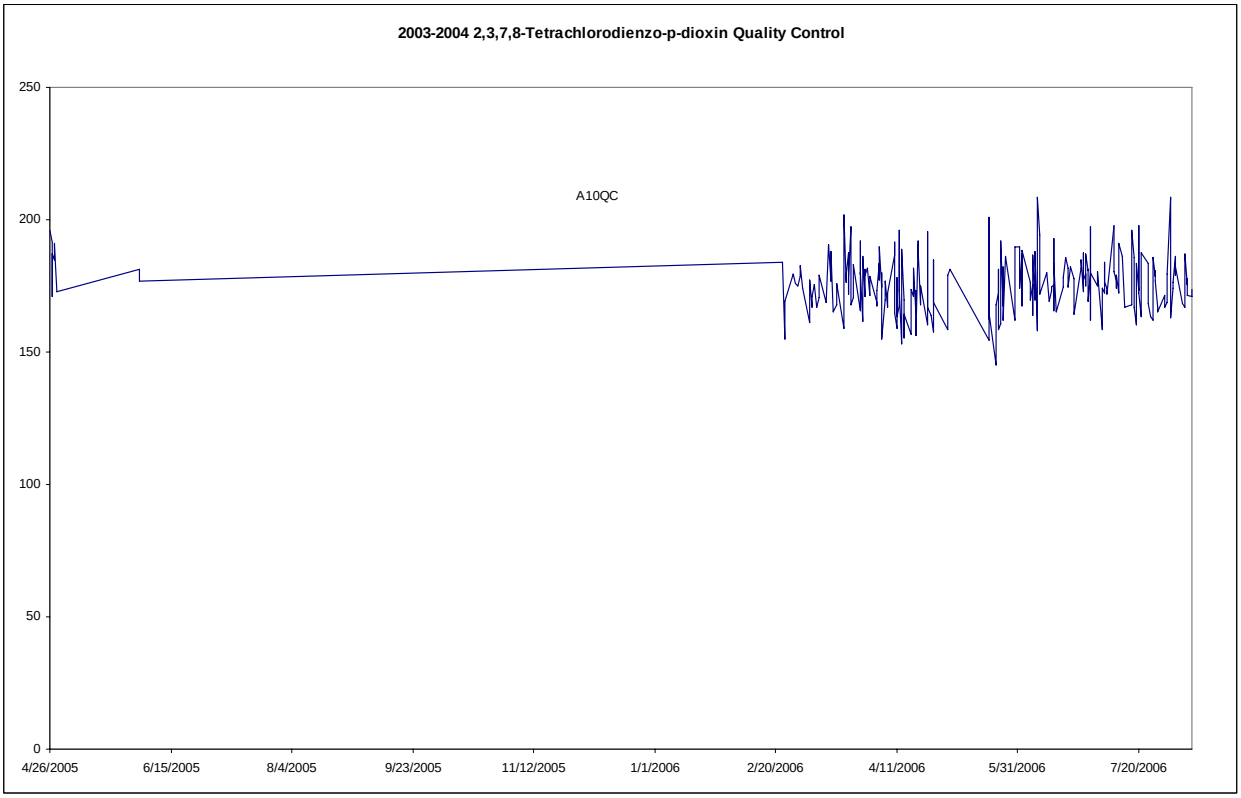
Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	255	4/26/2005	8/11/2006	802.7084	56.1601	7.0



T. 2,3,7,8-Tetrachlorodienzo-p-dioxin

Summary Statistics for 2,3,7,8-Tetrachlorodienzo-p-dioxin by Lot

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
A10QC	256	4/26/2005	8/11/2006	175.7262	10.6538	6.1



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