# Laboratory Procedure Manual

*Analyte:* Polychlorinated dibenzo-*p*-dioxins and furans (PCDD/F) and co-planar polychlorinated biphenyls (cPCB).

*Matrix:* **Serum / Plasma**

*Method:* Solid-Phase Extraction (SPE), Purification by Acid-silica and Carbon, Analysis by Gas chromatography isotope dilution high resolution mass spectrometry (GC-IDHRMS)

Method No: **6501.04**

*Revised:* **June 27, 2016**

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

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**Important Information for Users**

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 testing the items listed in the following table:

| Lab Name | Analyte | SAS Label |
|---|---|---|
| DOXPOL_F | LBCD01 | 1,2,3,7,8-pncdd (fg/g) |
| | LBCD02 | 1,2,3,4,7,8-hxcdd (fg/g) |
| | LBCD03 | 1,2,3,6,7,8-hxcdd (fg/g) |
| | LBCD04 | 1,2,3,7,8,9-hxcdd (fg/g) |
| | LBCD05 | 1,2,3,4,6,7,8-hpcdd (fg/g) |
| | LBCD07 | 1,2,3,4,6,7,8,9-ocdd (fg/g) |
| | LBCF01 | 2,3,7,8,-Tetrachlorofuran (tcdf) (fg/g) |
| | LBCF02 | 1,2,3,7,8-Pentachlorofuran (pncdf)(fg/g) |
| | LBCF03 | 2,3,4,7,8-Pentachlorofuran (pncdf)(fg/g) |
| | LBCF04 | 1,2,3,4,7,8-Hexachlorofuran(hcxdf)(fg/g) |
| | LBCF05 | 1,2,3,6,7,8-Hexachlorofuran(hxcdf)(fg/g) |
| | LBCF06 | 1,2,3,7,8,9-Hexachlorodifuran(fg/g) |
| | LBCF07 | 2,3,4,6,7,8-Hexchlorofuran(hxcdf)(fg/g) |
| | LBCF08 | 1,2,3,4,6,7,8-Heptachlorodifuran (fg/g) |
| | LBCF09 | 1,2,3,4,7,8,9-Heptachlorodifuran (fg/g) |
| | LBCF10 | 1,2,3,4,6,7,8,9-Octachlorodifuran (fg/g) |
| | LBCHCC | 3,3',4,4',5,5'-hexachlorobiphenyl (fg/g) |
| | LBCPCB | 3,3',4,4',5-Pentachlorobiphenyl (f/g) |
| | LBCTC2 | 3,4,4',5-Tetrachlorobiphenyl (tcb)(fg/g) |
| | LBCTCD | 2,3,7,8-Tetrachloro-p-dioxin(tcdd)(fg/g) |

## Table of Contents

# Appendixes:

# 1. Clinical Relevance and Summary of Test Principle

## 1.1 Clinical Relevance

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) are two similar classes of chlorinated aromatic chemicals that are produced as contaminants or by-products. They have no known commercial or natural use. Dioxins are produced primarily during the incineration or burning of waste; the bleaching processes used in pulp and paper mills; and the chemical syntheses of trichlorophenoxyacetic acid, hexachlorophene, vinyl chloride, trichlorophenol, and pentachlorophenol. Both the synthesis and heat-related degradation of polychlorinated biphenyls (PCBs) will produce dibenzofuran byproducts. Releases from industrial sources have decreased approximately 80% since the 1980s (U.S. EPA, 2004). Today, the largest release of these chemicals occurs as a result of the open burning of household and municipal trash, landfill fires, and agricultural and forest fires. When advanced analytical techniques are used, most soil and water samples will reveal trace amounts of polychlorinated dibenzo-p-dioxins and dibenzofurans.

The coplanar and mono-ortho-substituted PCBs are chlorinated aromatic hydrocarbon chemicals that belong to the general class PCBs which were once synthesized for use as heat-exchanger, transformer, and hydraulic fluids, and also used as additives to paints, oils, window caulking, and floor tiles. Production of PCBs peaked in the early 1970s and was banned in the United States after 1979.

Together with the polychlorinated dioxins and furans, these two special classes of PCBs are often referred to as “dioxin-like” chemicals because they act in the body through a similar mechanism.

In the environment, these dioxin-like chemicals are persistent and usually occur as a mixture of congeners (i.e., compounds that differ by the numbers and positions of chlorine atoms attached to the dibenzo-p-dioxin, dibenzofuran, or biphenyl structures). The general population is exposed to low levels of polychlorinated dibenzo-p-dioxins and dibenzofurans primarily through ingestion of high-fat foods such as dairy products, eggs, and animal fats, and some fish and wildlife. Dioxin-like chemicals are measurable in U.S. meats and poultry (Hoffman et al., 2006) as a result of the accumulation of these substances in the food chain. Breast milk is a substantial source of exposure for infants (Beck et al., 1994; Lundqvist et al., 2006), though breast milk levels have been decreasing in recent years (Arisawa et al., 2005). The lesser chlorinated PCBs, including some dioxin-like PCBs, are more volatile. These PCBs can enter air of buildings containing joint sealants made with PCBs prior to 1980 and can increase background serum levels via inhalational exposure (Johansson et al., 2003; Kohler et al., 2005). Volatilization of PCBs from nearby hazardous waste sites may

also contribute to human inhalational exposure. Exposure to high levels of these chemicals has occurred in the past as a result of industrial accidents (e.g., after an explosion in a factory in Seveso, Italy); the use of accidentally contaminated cooking oils (e.g., as occurred in Yusho in Japan and Yucheng in Taiwan); the spraying of herbicides contaminated with 2,3,7,8-tetrachlorodibenzo-pdioxin (TCDD) (e.g., as Agent Orange in Vietnam); and the burning of PCBs producing polychlorinated dibenzofurans (e.g., such as from electrical transformer fires). Workplace exposures are infrequent today, but incineration plant workers and chemical synthesis workers can be exposed via inhalation and dust exposures. The dioxin-like chemicals are easily absorbed, tend to distribute into body fat, have limited metabolism, and slow elimination from the body. Serum levels may be influenced by both past (stored in body fat) and recent exposures, though the current intakes for most people are now low. Half-lives of the dioxins and furans in the body vary from three to 19 years, with the half-life of TCDD estimated at around seven years (Geyer et al., 2002).

Because exposure to these chemicals includes a mixture of varying congeners, congener-specific effects are difficult to determine (Masuda, 2001; Masuda et al., 1998). However, these four groups of chemicals (polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans, and the coplanar and mono-ortho- substituted PCBs) are considered to act through a similar mechanism to produce toxic effects. These dioxin-like effects are thought to result from interaction with the aryl hydrocarbon receptor (AhR), particularly in the induction of gene expression for cytochromes P450, CYP1A1 and CYP1A2. Dioxins and furans have a planar configuration and require four lateral chlorine atoms (2,3,7,8 positions) on the dibenzo-p-dioxin or dibenzofuran backbone to bind this receptor. The rank order of interaction with the AhR receptor by degree and position of chlorination is roughly similar for both the dioxin and furan series. The coplanar polychlorinated biphenyls (unsubstituted at ortho positions) and the mono-ortho-substituted polychlorinated biphenyls (which contain a chlorine atom at one of the ortho positions) can achieve a planar configuration and also interact with the AhR receptor. The variation in the effect on AhR among the dioxin-like chemicals is 10,000-fold, with TCDD and 1,2,3,7,8-pentachlorodibenzo-p-dioxin being the most potent. To compare potency, each of these congeners has been assigned a potency value relative to TCDD (toxic equivalency factor [TEF]). When each TEF is multiplied by the concentration of the congener, a toxic equivalency (TEQ) value is obtained. Thus, the dioxinlike toxicity contributed by each of the polychlorinated dibenzo-p-dioxins, dibenzofurans, and PCBs can then be compared. The sum of all congener TEQs in a specimen (total TEQ) can be used to compare dioxin-like activity among specimens. Many of the dioxin-like PCBs have lower potency but are found at higher concentrations than TCDD (Kang et al., 1997; Patterson et al., 1994, Van den Berg et al., 2006), so these less potent chemicals may still contribute substantially to the total TEQ.

In animal studies, TCDD and dioxin-like chemicals have demonstrated many effects including: altered transcription of genes; induction of various enzymes; wasting syndrome; hepatotoxicity; altered immune function; testicular atrophy; altered thyroid function; chloracne; porphyria; neurotoxicity; teratogenicity; and carcinogenicity (EPA, 2004). Since animal species differ dramatically in sensitivity to these chemicals, it is difficult to predict human health effects though animal studies have provided support to observations of effects in human populations. Health effects of exposure to dioxin-like chemicals in people have been observed as a result of industrial or accidental exposures involving large doses of these chemicals. Chloracne, biochemical liver test abnormalities, elevated blood lipids, fetal injury, and porphyria cutanea tarda have been reported in episodes of high exposure. Developmental effects in humans are of concern since congenital anomalies and intrauterine growth retardation were observed in offspring of Yucheng mothers exposed to cooking oil contaminated with electrical oil containing very high levels of PCB and polychlorinated dibenzofurans. Environmental serum levels of primarily non-dioxin-like PCBs, and some dioxin-like chemicals, have been associated with altered psychomotor development in newborns and children (Arisawa et al., 2005; Koopman-Esseboom et al., 1996; Longnecker et al., 2003; Lundqvist et al., 2006; U.S. EPA, 2004. Cross-sectional associations of type II diabetes or markers of insulin resistance with serum levels of TCDD, other dioxin like chemicals, non-dioxin-like PCBs and organochlorine pesticides have been reported in both highly exposed and environmentally exposed human populations, though some studies have not found an association (Calvert et al., 1999; Everett et al., 2007; Fierens et al., 2003; Fujiyoshi et al., 2006; Henriksen et al., 1997; Kang et al., 2006; Kern et al., 2004; Lee et al., 2006; Michalek et al., 1999, and 2003) and in vitro and in vivo animal studies have provided possible mechanistic plausibility. Immune effects of dioxin-like chemicals and non-dioxin-like PCBs have been reported in animal studies (Carpenter, 2006; U.S.EPA, 2004), but few or consistent effects in humans have been observed (Baccarelli et al., 2002; Halperin et al., 1998; Jung et al., 1998; IARC, 1997).

Similar to some other organochlorine-type chemicals, the dioxin-like chemicals weakly mimic or interfere with the action of estrogen; for instance, dioxin-like chemicals may decrease the effect of estrogen through induction of its metabolism. This action contrasts with the non-dioxinlike PCBs and their metabolites, which may have direct estrogenic action (Carpenter, 2006; Wang et al., 2006; Yoshida et al., 2005). Dioxin and other organochlorine chemicals have been shown to interfere with male and female reproductive development in experimental and wild animals, particularly during gestational exposure (Gao et al., 1999; Roman et al., 1998; Sonne et al., 2006; Theobald et al., 1997). In studies of women with environmental or accidental exposures, associations between dioxin-like chemical exposures and various

reproductive endpoints (Eskenazi et al., 2003; Lawson et al., 2004; Schnorr et al., 2001; Warner et al., 2004 and 2007) and endometriosis (Eskenazi et al., 2002; Fierens et al., 2003; Heilier et al., 2005; Hoffman et al., 2007) have been either absent or of unknown significance, though animal studies have demonstrated reproductive effects at high doses (Arisawa et al., 2005; U.S. EPA, 2004). In men, lowered levels of testosterone have been associated with environmental and occupational exposures to dioxin-like chemicals (Dhooge et al., 2006; Egeland et al., 1994; Gupta et al., 2006; Henriksen et al., 1996; Johnson et al., 2001; Sweeney et al., 1998) and gonadal atrophy and lowered testosterone levels have been observed in animal studies.

TCDD is classified separately by the IARC and NTP as a known human carcinogen. The U.S. EPA (2004) and IARC (1997) concluded that the aggregate evidence supports an association between high-dose TCDD exposure (e.g., encountered in contaminated occupational settings or massive unintentional releases) and increases in the all cancer category (Steenland et al., 2004). The Institute of Medicine (2005) concluded that human epidemiologic evidence is sufficient for a positive association of herbicides contaminated with TCDD and an increased risk for non-Hodgkin's lymphoma, Hodgkin's lymphoma, chronic lymphocytic leukemia, and soft tissue sarcoma. Other individual polychlorinated dibenzo-p-dioxins and dibenzofurans have not been studied sufficiently for IARC to classify their human potential for carcinogenicity, although EPA considers these other chemicals as likely human carcinogens (U.S.EPA, 2004).

## 1.2 Test Principle

This method measures the following chemical classes found in human serum by gas chromatography isotope-dilution high resolution mass spectrometry (HRGC/ID-HRMS):
7 polychlorinated dibenzo-*p*-dioxins (PCDDs)
10 polychlorinated dibenzofurans (PCDFs)
4 non-ortho-substituted (co-planar) polychlorinated biphenyls (cPCBs)

Serum specimens to be analyzed for PCDD/Fs and cPCBs are fortified with carbon-13 labeled ($^{13}C$) internal standards. The analytes of interest are extracted in hexane using a $C_{18}$ solid phase extraction (SPE) procedure followed by a Power-Prep/6 (Fluid Management Systems) automated lipid removal and enrichment procedure using multi-layered silica gel column (acidic, basic, and neutral silica) and alumina column coupled to an AX-21 carbon column. An analytical run consists of eight unknown specimens, two method blanks, and two quality control samples. The fractions containing target analytes are isolated in the reverse direction from AX-21 column with toluene.

Following toluene collection of target analyte factions, excess solvent is evaporated to 350µL using a TurboVap II (Biotage) concentration workstation and the remaining

solvent is transferred to silanized auto sampler vials containing and external recovery standard $^{13}C_6$-1234-TCDD. There after the sample is evaporated to 5uL using a TurboVap LV (Biotage) concentration workstation. PCDD/F and cPCB are measured using gas chromatography isotope dilution high resolution mass spectrometry (GC/ID-HRMS), using selected ion monitoring (SIM) at 10,000 resolving power (10% valley).

Two ion corresponding to two masses are monitored for each native ($^{13}C_{12}$) 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, relative retention time (ratio of retention time for native to isotopically labeled internal standard) compared with a standard must be within +/- 0.004, response ratios of the two $^{12}C_{12}$ and $^{13}C_{12}$ ions must be within ± 26 % of their theoretical values and analyte recovery ≥10 % and ≤ 150%. In addition, the calculated mean and range of each analyte in the quality control sample must be within their respective confidence intervals. The method detection limit (MDL) for each analyte is calculated correcting for sample weight. The total lipid content of each specimen is estimated from its total cholesterol and triglycerides values using a “summation” method (Atkins et al. 1989, Phillips et al. 1989).

Analytical results 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)]. International toxicity equivalents (I-TEQs) are also reported for PCDDs, PCDFs, cPCBs. 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

## 2.1 Biohazards

Follow Universal Precautions by wearing appropriate gloves, lab coat, and protective eye glasses while handling human serum. Serum may be contaminated with pathogens such as hepatitis or HIV; hence all safety precautions must be followed as outlined in the laboratory hazardous chemicals exposure plan. Wear gloves, lab coat and glasses at all times, and conduct all work in fume hood or biological safety cabinets (BSCs).

Place any disposable plastic, glass, and paper items (e.g., pipette tips, test tubes, and gloves) that come in contact with serum in a biohazard autoclave bag. Keep these bags in appropriate containers until they are sealed and autoclaved. When work is finished, wipe down all work surfaces where serum was handled with a 10% (v/v) sodium hypochlorite solution or an equivalent disinfectant.

After an accident the CDC/ATSDR Incident Report must be filed according to hazardous exposure control plan by supervisor.

## 2.2 Chemical hazards

### *2.2.1 Acids and Bases*

Exercise caution when handling and dispensing concentrated acids and bases such as: sulfuric acid, formic acid, hydrochloric acid and sodium hydroxide. Always remember to add acid to water. Acids and bases are capable of causing severe eye and skin damage. Wear powder-free gloves, a lab coat and safety glasses. If acids or bases come in contact with any part of the body, quickly wash the exposed area with copious quantities of water for at least 15 minutes. Use safety shower if exposed area is not limited to hands and/or arms. Use eye wash station in the event of eye exposure to acids and/or bases. In the event of an accident, lab colleagues will contact the clinic by phone or emergency medical response by dialing 9-911.

### *2.2.2 Solvents*

Solvents may penetrate skin causing long-term adverse health effects. When possible/appropriate, use the chemical fume hood when working with solvents. Exercise caution and always use gloves when handling solvents and other chemicals. In the event of spill on gloves immediately change to a new glove since solvents do penetrate many gloves with time.

## 2.3 Hazardous waste handling

### *2.3.1 Solvent waste*

Collect solvent waste in waste bottles (empty solvent bottles may be used). Clearly write **WASTE** on bottles, and the solvent(s) the waste bottle contains. If possible, always keep different solvents separated in different waste bottles, since this will make the final disposal of the different solvent wastes easier. When a bottle is filled, arrange for waste pickup according the Chemical Hygiene Plan.

### *2.3.2 Serum waste*

Dispose of serum waste originating as a waste fraction in the extraction step by completing the forms as outlined by Chemical Hygiene Plan. Also attach a Memorandum stating that the contents of the bottle are a mixture of formic acid, water, and serum that is considered to be biologically inactivated by the acid present.

### *2.3.3 Solid wastes*

Sort solid waste in three fractions and placed in metal boxes with lid according to below and Chemical Hygiene Plan:

- **Non-Biogenic Contaminated Reusable Glassware** (e.g. beakers, cylinders and other reusable glassware). As needed clean glass ware according to section 6.2.1.

- **Broken glass** includes used Pasteur pipets (including Pasteur pipets contaminated with biogenic materials), or serum bottles and vials that are not reused. When this container is filled (*i*) add approximately 1 L water to container, (*ii*) place sticker with your name, room and building number on container, (*iii*) place autoclave tape over lid and down the side of the box and (i*v*) bring the container to autoclave located in the loading dock, building 103.

- **Gloves and other plastic parts contaminated with biogenic material** - Place biohazard bag in metal container before placing any waste in container. When container is filled (*i*) add approximately 1 L water to container, (*ii*) place sticker with your name, room and building number on container, (*iii*) place autoclave tape over lid and down the side of the box, (*iv*) place autoclave sticker on container and (*v*) bring the container to DLS designated handling area.

# 3. Computerization; Data System Management

### 3.1 Data Entry and Transfer

Manual data entry has been minimized to the largest possible extent. A standard run sheet generated using an automated SAS procedure is used to capture sample/run data for each sequence of samples processed. Entries like analyst name, date of sample preparation is recorded using manual entry while sample weights are exported from the balance to the run sheet in Excel-format. Manual entry of sample weight is also an accepted procedure and used as a backup to the export function from the analytical balance.

### 3.2 Routine Computer Hard-Drive Maintenance

Defragmentation of the computer hard drive for HRMS instrument is performed monthly or on an as needed basis using Defragmentation in Windows. Other computers connected to the CDC network are maintained by ITSO (local computer support department). Data is continually backed up (mirrored) from the instrument computer to the CDC network. To prevent filling the hard drive of the instrument computer when a study is completed or as needed move instrumental data to the “Archive Network location” which also is continually backed up.

## 4. Procedures for Collecting, Storage and Handling of Specimens; Criteria for Specimen Rejection

- No special instructions for fasting or special diets are required, although, preferably the sample has been drawn in the morning before breakfast (i.e. fasting).
- The specimen type is serum or plasma.
- Required sample amount:
    - Minimum acceptable serum amount: 2.5 grams.
    - Minimum preferred serum amount: 10 grams.
    - Maximum serum amount: <40 grams
    - A similar sample amount for all samples within a study is preferable since the limit of detection (LOD) calculated is proportional to the sample size thus a consistent sample size for all samples in a study produces a consistent LOD.
- The limit of detection for the minimum acceptable serum amount 2.5 to 30 g of serum is given in Table 1.
- Preferable storage containers are thick-walled glass vials with Teflon™-lined caps. Although, other containers are acceptable but will be evaluated on a case by case basis and a background test of the material the container is made from may be conducted. Unless the container is pre-cleaned by the manufacturer, clean containers using the same procedure as for other glassware used in the current method (see section 6.1.1). Preferred container is either a 2oz or 4oz glass Qorpak bottle.

- The criteria for an unacceptable specimen are either a low volume (<2.5 mL) or suspected contamination due to improper collection procedures or collection devices. In all such cases, request a second serum specimen. Contamination of specimen could occur from contact with indoor dust from improper handling. In case a replacement specimen is not available, the recipient of the analytical data will be informed that this particular sample may have been contaminated during sample collection.
- Transport and ship frozen serum specimens on dry ice. Upon receipt, they must be kept frozen at ≤ -50 ºC until analysis. Refreeze at ≤ -50 °C any portions of the sample that remain after analytical aliquots are withdrawn. Samples thawed and refrozen several times are not compromised; however care should be taken to avoid an excessive or unnecessary number of freeze/thaw cycles.

**Table 1.** Method limit of detection (LOD, fg/gram of serum) by target analyte and used sample amount (gram). The method LOD corresponding to the minimum preferred sample amount of 10 grams are colored in blue, method LODs between the minimum preferred sample amount and the minimum acceptable sample size are colored in red. Method LODs two and four fold higher than the minimum preferred sample amount are colored in green. A sample amount greater than the minimum preferred sample amount may be used to lower the method LOD. Any sample for which the available serum amount for measurement is less than the minimum acceptable serum amount of 2.5grams will be reported as QNS (Quantify Not Sufficient) in reportable data tables.

| Class | Analyte | Serum Weight (g) | Method LOD (pg/g serum) [a] | Class | Analyte | Serum Weight (g) | Method LOD (pg/g serum) [a] |
|---|---|---|---|---|---|---|---|
| PCDD/F | 2378-TeCDD | 2.5 | 6.4 | PCDD/F | 1234678-HpCDD | 2.5 | 110 |
| | | 5 | 3.2 | | | 5 | 54 |
| | | 7.5 | 2.1 | | | 7.5 | 36 |
| | | 10 | 1.6 | | | 10 | 27 |
| | | 20 | 0.80 | | | 20 | 14 |
| | | 30 | 0.53 | | | 30 | 9.0 |
| PCDD/F | 12378-PeCDD | 2.5 | 15 | PCDD/F | OCDD | 2.5 | 400 |
| | | 5 | 7.4 | | | 5 | 200 |
| | | 7.5 | 4.9 | | | 7.5 | 130 |
| | | 10 | 3.7 | | | 10 | 100 |
| | | 20 | 1.9 | | | 20 | 50 |
| | | 30 | 1.2 | | | 30 | 33 |
| PCDD/F | 123478-HxCDD | 2.5 | 6.8 | PCDD/F | 2378-TeCDF | 2.5 | 9.2 |
| | | 5 | 3.4 | | | 5 | 4.6 |
| | | 7.5 | 2.3 | | | 7.5 | 3.1 |
| | | 10 | 1.7 | | | 10 | 2.3 |
| | | 20 | 0.85 | | | 20 | 1.2 |
| | | 30 | 0.57 | | | 30 | 0.77 |
| PCDD/F | 123678-HxCDD | 2.5 | 16 | PCDD/F | 12378-PeCDF | 2.5 | 15 |
| | | 5 | 7.8 | | | 5 | 7.6 |
| | | 7.5 | 5.2 | | | 7.5 | 5.1 |
| | | 10 | 3.9 | | | 10 | 3.8 |
| | | 20 | 2.0 | | | 20 | 1.9 |
| | | 30 | 1.3 | | | 30 | 1.3 |
| PCDD/F | 123789-HxCDD | 2.5 | 16 | PCDD/F | 23478-PeCDF | 2.5 | 11 |
| | | 5 | 8.0 | | | 5 | 5.6 |
| | | 7.5 | 5.3 | | | 7.5 | 3.7 |
| | | 10 | 4.0 | | | 10 | 2.8 |
| | | 20 | 2.0 | | | 20 | 1.4 |
| | | 30 | 1.3 | | | 30 | 0.93 |

[a] Method LOD defined the higher value of $S_0$ (Taylor, K. T. (1987) In Quality Assurance of Chemical Measurements, pp 79-82, Lewis Publishers, Washington, DC) and three times the standard deviation of blank samples. Method LOD determination based on gennerated measurements during 2015 and 1st and 2nd quarter of 2016.

**Table 1 (Continued).** Method limit of detection (LOD, fg/gram of serum) by target analyte and used sample amount (gram). The method LOD corresponding to the minimum preferred sample amount of 10 grams are colored in blue, method LODs between the minimum preferred sample amount and the minimum acceptable sample size are colored in red. Method LODs two and four fold higher than the minimum preferred sample amount are colored in green. A sample amount greater than the minimum preferred sample amount may be used to lower the method LOD. Any sample for which the available serum amount for measurement is less than the minimum acceptable serum amount of 2.5grams will be reported as QNS (Quantify Not Sufficient) in reportable data tables.

| Class | Analyte | Serum Weight (g) | Method LOD (pg/g serum) [a] | Class | Analyte | Serum Weight (g) | Method LOD (pg/g serum) [a] |
|---|---|---|---|---|---|---|---|
| PCDD/F | 123478-HxCDF | 2.5 | 13 | PCDD/F | 1234789-HpCDF | 2.5 | 8.8 |
| | | 5 | 6.6 | | | 5 | 4.4 |
| | | 7.5 | 4.4 | | | 7.5 | 2.9 |
| | | 10 | 3.3 | | | 10 | 2.2 |
| | | 20 | 1.7 | | | 20 | 1.1 |
| | | 30 | 1.1 | | | 30 | 0.73 |
| PCDD/F | 123678-HxCDF | 2.5 | 9.2 | PCDD/F | OCDF | 2.5 | 40 |
| | | 5 | 4.6 | | | 5 | 20 |
| | | 7.5 | 3.1 | | | 7.5 | 13 |
| | | 10 | 2.3 | | | 10 | 9.9 |
| | | 20 | 1.2 | | | 20 | 5 |
| | | 30 | 0.77 | | | 30 | 3.3 |
| PCDD/F | 123789-HxCDF | 2.5 | 10 | cPCB | PCB77 | 2.5 | 1700 |
| | | 5 | 5.2 | | | 5 | 860 |
| | | 7.5 | 3.5 | | | 7.5 | 570 |
| | | 10 | 2.6 | | | 10 | 430 |
| | | 20 | 1.3 | | | 20 | 220 |
| | | 30 | 0.87 | | | 30 | 140 |
| PCDD/F | 234678-HxCDF | 2.5 | 11 | cPCB | PCB81 | 2.5 | 92 |
| | | 5 | 5.6 | | | 5 | 46 |
| | | 7.5 | 3.7 | | | 7.5 | 31 |
| | | 10 | 2.8 | | | 10 | 23 |
| | | 20 | 1.4 | | | 20 | 12 |
| | | 30 | 0.93 | | | 30 | 7.7 |
| PCDD/F | 1234678-HpCDF | 2.5 | 44 | cPCB | PCB126 | 2.5 | 96 |
| | | 5 | 22 | | | 5 | 48 |
| | | 7.5 | 15 | | | 7.5 | 32 |
| | | 10 | 11 | | | 10 | 24 |
| | | 20 | 5.5 | | | 20 | 12 |
| | | 30 | 3.7 | | | 30 | 8.0 |

[a] Method LOD defined the higher value of $S_0$ (Taylor, K. T. (1987) In Quality Assurance of Chemical Measurements, pp 79-82, Lewis Publishers, Washington, DC) and three times the standard deviation of blank samples. Method LOD determination based on gennerated measurements during 2015 and 1st and 2nd quarter of 2016.

**Table 1 (Continued).** Method limit of detection (LOD, fg/gram of serum) by target analyte and used sample amount (gram). The method LOD corresponding to the minimum preferred sample amount of 10 grams are colored in blue, method LODs between the minimum preferred sample amount and the minimum acceptable sample size are colored in red. Method LODs two and four fold higher than the minimum preferred sample amount are colored in green. A sample amount greater than the minimum preferred sample amount may be used to lower the method LOD. Any sample for which the available serum amount for measurement is less than the minimum acceptable serum amount of 2.5grams will be reported as QNS (Quantify Not Sufficient) in reportable data tables.

| Class | Analyte | Serum Weight (g) | Method LOD (pg/g serum) [a] | Class | Analyte | Serum Weight (g) | Method LOD (pg/g serum) [a] |
|---|---|---|---|---|---|---|---|
| cPCB | PCB169 | 2.5 | 48 | | | | |
| | | 5 | 24 | | | | |
| | | 7.5 | 16 | | | | |
| | | 10 | 12 | | | | |
| | | 20 | 6.0 | | | | |
| | | 30 | 4.0 | | | | |

[a] Method LOD defined the higher value of $S_0$ (Taylor, K. T. (1987) In Quality Assurance of Chemical Measurements, pp 79-82, Lewis Publishers, Washington, DC) and three times the standard deviation of blank samples. Method LOD determination based on gennerated measurements during 2015 and 1st and 2nd quarter of 2016.

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

Not Applicable

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

## 6.1 Reagents and consumables

The method has been validated using the chemicals, solvents and expendables listed in Table 2 and 3. Other manufacturer's products of equivalent purity can be used after verification of chemical's and/or materials purity.

**Table 2.** Solvents and chemicals used for development of current methodology, equivalent products from other manufacturer may be used with exception to the SPE sorbent.

| Chemical/Solvent | Manufacturer | Grade/Purity |
|---|---|---|
| ***Acids*** | | |
| Formic Acid | EMD Scientific | 90% |
| | | |
| ***Solvents*** | | |
| Dichloromethane | LabSolv / TEDIA | Pesticide Grade or better |
| Dodecane | EM Science | min 99% |
| Hexane | LabSolv / TEDIA | Pesticide Grade or better |
| 50% Dichloromethane in Hexane | LabSolv / TEDIA | Pesticide Grade or better |
| Methanol | LabSolv / TEDIA | Pesticide Grade or better |
| n-Nonane | Sigma | 99% |
| Water | LabSolv / TEDIA | Pesticide Grade or better |
| Toluene | LabSolv / TEDIA | GC Grade or better |
| | | |
| ***SPE sorbents*** | | |
| XtracT® C18 SPE | UCT Chemicals | n/a |

**Table 3.** Expendables used for development of current methodology, equivalent products from other manufacturer may be used.

| **Item** | **Manufacturer/Source** |
|---|---|
| | |
| ***Glassware and caps*** | |
| Test tube (20 x 125 mm) | Fisher Scientific |
| Test tube (20 x 150 mm) | Fisher Scientific |
| Clear Polyethylene (HDPE) bottles | Fisher Scientific |
| Borosilicate Glass Pasteur pipette | Fisher Scientific |
| Boston Round (amber glass bottle) | Fisher Scientific |
| V-vial (3 or 5 mL) with septum-cap | Fisher Scientific |
| GC vials and caps | Fisher Scientific |
| | |
| ***Others*** | |
| Label printer (Brady TLS PC-Link) | Fisher Scientific |
| Plastic pipette tips | Rainin |
| Pipette dispenser | VWR |

## 6.2 Rinsing of Expendables Prior to Use

Clean all glassware including new glassware according to the following procedures to eliminate risk of sample contamination:

### *6.2.1 Culture tubes and other glassware*

First, rinse glassware in a dishwasher. Place test tubes in racks and insert them in the dishwasher. Make sure that all of the necessary cleaning chemicals (detergent and any required rinse solution) are either connected or placed in their appropriate dispensers. Start the dishwasher.

After completion of the program, transfer the glassware to an oven. After a heat cycle of at least 12 hours at >200 $^{o}$C, the glassware is ready to be used.

For satellite bottles such as glass tapered-stopper bottles intended for storing for small volume, everyday use in the BSC the normal large labels are not to be used because it would interfere with the proper procedure for re-cleaning them. Instead label by hand using a “Sharpie” pen and affix a small hazard pictogram sticker to the bottle or alternatively attach a sheet of paper on the fume hood/BSC were relevant chemicals are listed by name with appropriate pictogram.

### *6.2.2 Caps and septa*

Rinse caps and septa for test tubes prior to use to remove contaminants. This is done by Soxhalet extraction for at least 5 hours using methanol as the extraction solvent. Alternatively, if the Soxhalet apparatus cannot be used it is also acceptable to sonicate the items in methanol (20 min x 3 times). After cleaning the items, allow them to dry on aluminum foil covered with a paper towel. After the caps are completely dry, place them in a large glass beaker or in plastic re-sealable bags (not in cardboard boxes) for safe storage until used.

### *6.2.3 Gas Chromatography Vials*

Heat GC vials in an oven at >200 $^{o}$C overnight prior to use. Store vials in a closed container such as a beaker covered with aluminum foil or a Ziplock bag. The caps for GC vials are cleaned by Soxhalet extraction, using the same procedure as for caps and septa.

### *6.2.4 Pasteur Pipets*

Place glass Pasteur pipets in oven on aluminum foil and heat the oven to >200 $^{o}$C overnight. After completing the heating cycle for at least 12 hours, the pipets are ready to be used.

## 6.3 Quantitative standards

### *6.3.1 Internal standards (IS)*

The current method is validated for PCDD/Fs and cPCBs. Internal standards are typically obtained from CIL.

When opening a new ampoule transfer the standard to a Wheaton 3-mL vial. Label the vial appropriately using a computer-generated label.
Note the weight of the vial, and the date the ampoule was opened. This weight is used to detect any potential evaporation of the standard during storage (See 8.3).

### *6.3.2 Recovery standard (RS)*

The recovery standard is used for reconstitution of the extract prior to GC-ID/HRMS measurement was also purchased from Cambridge Isotopes, (CIL Woburn, MA).

The calibration standards also contain the same recovery standard at an equivalent concentration of the spiked and evaporated sample. Therefore, a comparison between the ratio of the internal standards and the recovery standard is used to calculate the absolute percent recovery of the 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.

### *6.3.3 Calibration Curve Standard (CS)*

Calibration standards are purchased from Cambridge Isotopes Laboratory (CIL, Woburn, MA) or equivalent standards manufacturing company. They were prepared in nonane, according to CDC specifications. Standards were prepared from individual stock solutions of labeled $^{13}C_{12}$-PCDDs, $^{13}C_{12}$-PCDFs, $^{13}C_{12}$-cPCBs, and native $^{12}C_{12}$-PCDDs, $^{12}C_{12}$-PCDFs, $^{12}C_{12}$-cPCBs 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, even though the concentrations in these analytical standards are very low. The calibration standard range is given in Appendix A.

When opening a new ampoule, aliquot the standard into GC vials (~5uL in each vial). Label the vials appropriately using a computer-generated label. Replace the standards used for calibration of the DFS after completion of every run.

## 6.4 Instrumentation

### *6.4.1 Power-Prep/6 Fluid Management Systems (FMS)*

The Power-Prep/6 (Fluid Management Systems, Inc., Watertown, MA) is from here on referred to as the FMS. A picture of the FMS system is given in Figure 1. Each FMS system 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 ¼” Delrin end fittings with 28 threads per inch (Rainin, Woburn, MA).

**Figure 1.** Fluid Management System (FMS) Power-Prep/6.

### *6.4.2 Gas chromatograph isotope dilution high resolution mass spectrometry (GC/ID-HRMS)*

Thermo Electron DFS, with X-caliber data systems (Thermo Electron, San Jose, CA) and Thermo TRACE 1300 GC (Thermo Electron, San Jose, CA) and a Thermo TriPlus autosampler (Thermo Electron, San Jose, CA) are used for target analyte measurements. Sample extracts are analyzed for PCDDs, PCDFs, and cPCBs by GCHRGC/ID-HRMS where 2µL are injected using an auto sampler, into a the Trace GC operated in the splitless injection mode with a flow of 1 mL/min helium through a Restek Rxi-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 45 eV, using selected ion monitoring (SIM) at 10,000 resolving power (10% valley).


**Figure 2.** Thermo Electron Double Focusing Sector (DFS) instrument.

## 6.5 Procedures for preparing quality control materials

The QC material for this assay is bovine serum fortified with target analytes that has been certified for final concentration of the target analytes. Two QC samples are analyzed in every set of 12 samples (or one in every set of 6) to ensure comparability and reliability between different sets of samples over time (according to DLS procedures manual). In addition to the QC sample, two bovine blank is analyzed in every set of 12 samples (or one in every set of 6). The method is designed to include one or two sets of 12 samples to be analyzed by GC-MS, in one batch. (See Sample preparation below).

Specific, predefined rules (DLS procedures manual) are applied in order to determine if the QC sample analyzed in one set is in agreement with previously analyzed QC samples. If the QC sample is found to be an outlier that set has to be reanalyzed or the data set to Non-Reportable.

For further details, see data handling section below and refer to the Division Policies and Procedures Manual.

### *6.5.1 Methods for preparation of quality control materials*

**Day 1** Rinse the vials (including caps in which the serum will be aliquoted) according to the procedure outlined in glassware rinsing procedures before use (see section 6.1.1. Label the vials with computer-generated labels. This label must contain a unique QC pool name, constructed from the page number in the pool note book. For example SERUM:02:03 where 02 is the notebook number and 03 is the page number. State the date of the pool preparation on the label.

Thaw the serum until the serum is completely unfrozen. Pour the serum into a large beaker (4 L) containing a heavy-duty stir bar (45-mm length). Spike with native analytes to appropriate concentration level and stir solution overnight using a magnetic stirrer.

**Day 2:** While still stirring the solution, transfer the spiked bovine serum pool to each of the vials in 6.1 mL aliquots. Cap the vials and place them in an appropriate container (e.g., a lid for Xerox paper boxes) for simple freezer shelf organization. Place one identifying label on the edge of the container and place in freezer (<-50 $^{o}$C).

## 7. Calibration and Calibration Verification

### 7.1 Calibration of Mass Spectrometer

Calibrate and tune the Thermo DFS mass spectrometer according to the instructions in the operator's manual using the appropriate calibration gas i.e., FC43 for PCDD/F and cPCB analysis. After tuning the instrument to 10,000 resolution a 100:1 signal to noise ratio is required for the injection of 0.01pg/ul of 2378-tetrachloro-p-dibenzodioxin (TCDD) with a 2ul injection (20fg on-column) and a greater than 3:1 signal to noise ratio is required for a manually injected CS1 standard for all $^{12}$C-target analytes (when the calibration standard EDF-4143 is used).

### 7.2 Creation of Calibration Curve

A linear calibration curve, consisting of at least five CS standards, is generated using the ratio of the peak area of the analyte to the labeled internal standard.

The R-squared value of the curve must be equal or greater than 0.995. Linearity of the standard curve must extend over the entire standard range on a log scale.

The highest point in the calibration curve is the highest reportable value (ie. Upper Linearity Limit). A sample exceeding this level needs to be diluted until the area counts of the $^{12}$C-target analyte is less than that of the highest concentration standard. The lowest reporting level or the method limit of detection (LOD) is given in Table 1. The method LOD is defined as the higher value of $S_0$ (Taylor, K. T. (1987) In Quality Assurance of Chemical Measurements, pp 79-82, Lewis Publishers, Washington, DC) and three times the standard deviation of blank samples. The method LOD was determined based on generated measurements during 2015 and 1st and 2nd quarter of 2016. Analytes with a determine concentration between the LOD and the lowest standard are reported as “Detected, lower than lowest standard”. The remainder of the points is evenly distributed between the two extreme concentrations (on a log scale).

Generate a new calibration curve with every new set of samples to be analyzed. Before using a new batch of standards with the current method, verify that the new standards agree within 20% of the old standard, this is accomplished by quantifying the new standard using the old standard. The certified value (pg/µl) of the new standard must be within 20% of the in-house quantified value (pg/µL). The tolerance of 20% between new and older standard is derived from the certificate of analysis giving a 10% tolerance of each standard released by CIL. Due to the fact that the response ratio between a native and $^{13}$C-labeled internal standard is measured, a maximum deviation of 20% is used. This is accomplished by quantifying the new standard using the old standard.

Note: The PCDD/F and cPCB calibration standard from CIL with Part# EDF-4143 should be diluted 1:4 with nonane prior to use.

## 7.3 Calibration Verification

Calibration verification of the test system is done by the inclusion of quality control samples with a determined concentration in every run of unknown specimens and by the analysis of Proficiency Testing (PT) samples at least twice per year. See section 10 for further information on PT procedures.

# 8. Procedure Operation Instructions; Calculations; Interpretation of Results

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

Anyone involved in sample preparation must be trained in for all sample preparation equipment, chemical handling, and have basic chemistry laboratory skills. The training may be delegated to more experienced analyst. New staff must be re-evaluated after 6 months of initial training and certification to perform the assay by the supervisor to certify that they are appropriately qualified to perform the assay. There after the re-certification is performed annually.

## 8.1 Sending aliquot of serum for lipid determination

Serum lipid concentration in serum is determined in an aliquot of the sample (100 µl) using enzymatic methods by the Clinical Chemistry Branch (CCB). Aliquot 100 µl of each sample into appropriate vials (generally, 500µL Cryovials) ***after mixing the completely thawed serum samples***; use a new pipette tip for every sample to avoid cross contamination. Label vials for lipid weight determination with Study name, Study Number and unique DLS sample ID. A lipid aliquot may have been drawn upon arrival of the samples at CDC and prior to the samples being sent to the Persistent Organic Pollutants Laboratory by the Sample Logistics Laboratory. If this is the case, no lipid aliquot needs to be drawn prior to analysis.

## 8.2 Thawing and weighing samples

Store samples in a -70° C freezer before starting analysis. Samples are taken out from the freezer to ***thaw completely***; this can be done the day before analysis and the samples placed in a refrigerator overnight. Thoroughly mix the samples by vortex. For each batch of samples, complete the run sheet. On the run sheet, enter ALL requested information.

To ensure optimum performance of the balance used for weighing serum samples, verify the balance calibration using NIST calibration weights before weighing each batch of samples. (The analytical balance is further on an annual basis recertified by external vendor). Calibration weights are placed on the balance after tarring, and the

reading is recorded on the run sheet. The difference from true value may not exceed +/- 0.01 g. If this limit is exceeded, any problems must be resolved, such as cleaning the balance tray, recalibration of balance and/or calling for service of balance.

After verifying the balance calibration, weigh serum samples into washed and burned glass 4oz bottles. Record all sample weights on the run sheet.

The target sample weight of spiked Quality Control (QC) samples (ie. SSP:01:13, etc.) is approximately 3 grams unless otherwise noted. Record the mass of the QC samples appropriately on the runsheet. The QC samples are then diluted up to 8mL with water.

Method blanks are made using 0.5 mL bovine serum unless otherwise noted. It is not necessary to record the mass of the bovine serum used. The blanks are then diluted up to 8mL with water.

## 8.3 Sample pretreatment using Gilson 215 Liquid Handler

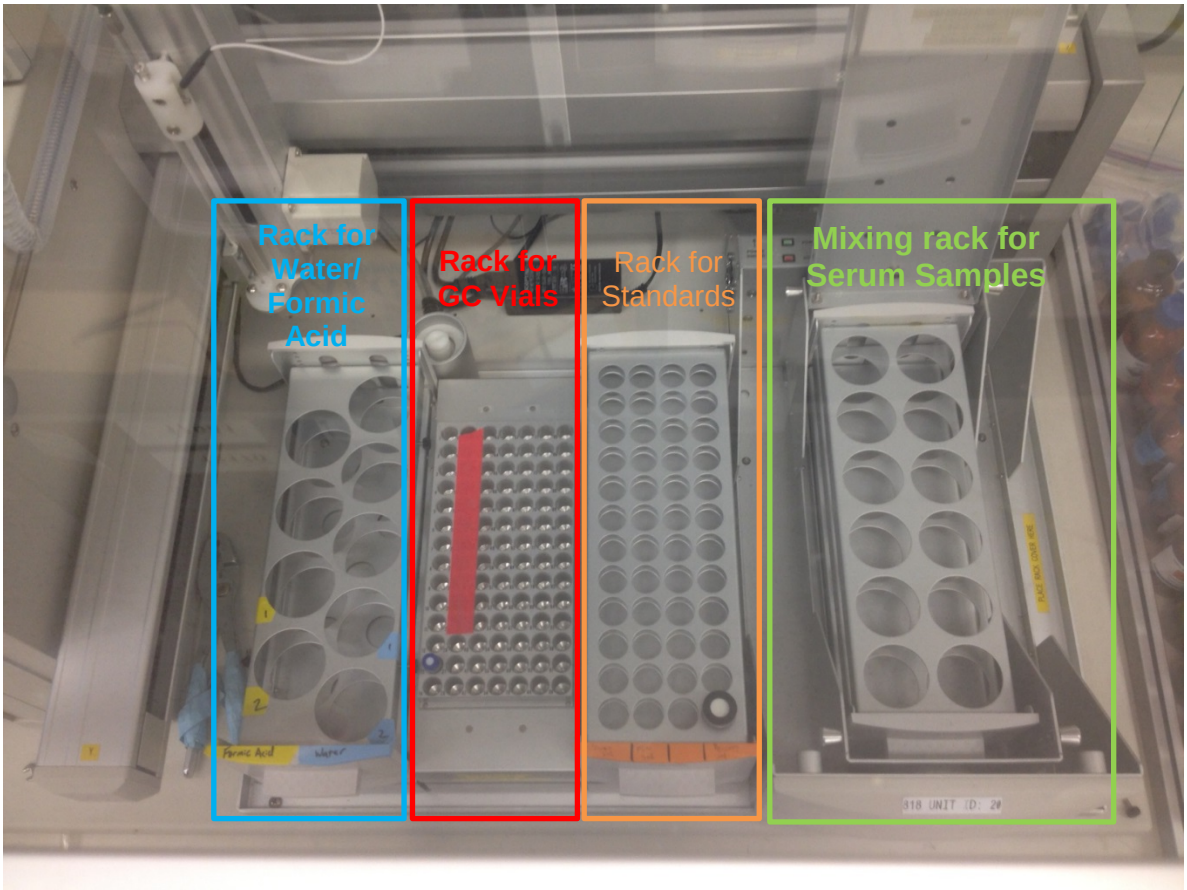

Figure 8.3: Bed Layout of Gilson 215 set up for PCDD/F and cPCB analysis.

### *8.3.1 Procedure*

A. Record the weights of the PCDDs/PCDFs and cPCBs internal standard vial before use. Place the PCDD/F and cPCB internal standard vial on the Gilson 215 in the appropriate rack (See Figure 8.3).

   **Special Note**: The PCDD/PCDF/cPCB internal standard from CIL with Part# EDF-4144B should be spiked at 25 µL to the samples using a positive displacement pipettor instead of the Gilson 215.

B. After launching the software, the main menu is displayed (Figure 8.3.B.a). For setting up the software for extraction, first click on “Applications” button in the menu. In the Application Menu (Figure 4) select the application named “New Dioxin Internal Spiking App”. Make sure that number of samples to be spiked is correct for each method in the Application window.


Figure 8.3.B.a: Detail of the Trilution Main Menu. **A:** The Application Menu button.

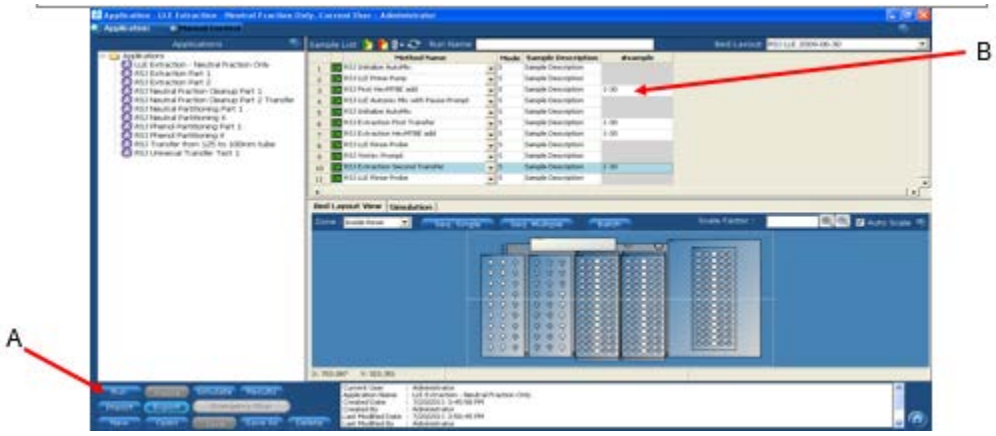


**Figure 8.3.B.b.** Detail of the Application Menu in Trilution LH. **A**: The Application Run Button. **B**: The column where the number of samples to be spiked is entered

C. The analyst has the option to use either the Gilson 215 to add water and formic acid to the samples or to add these manually. The reason for giving this choice is that both a manual addition of water and formic acid using a repeating pipettor and the Gilson 215 provide adequate precision. If the analyst chooses to use the Gilson to add water and formic acid then place 125mL Boston Round bottles with water and formic acid in the appropriate rack and enter the volume of each to be added to each sample in the Application window.

D. Place serum samples in the rack on the Gilson bed shown in Figure 8.3. Make sure that the sample bottles have caps tightened sufficiently and that each sample is in the correct position in the rack. Close and secure the lid on the Gilson mixing rack, the 818 AutoMix.

E. Briefly re-inspect the Gilson bed and the entries in the Trilution application. If everything is correct then click the RUN button in the Trilution Application window to start the run.

F. When the Gilson method has finished, record the weight of the internal standard vials used. Calculate the accuracy of the internal standard spike and if outside the acceptable range (95-105%, applicable for automated spiking on the Gilson 215 only) consult laboratory supervisor for how to proceed.

G. If the analyst chooses to add the formic acid and water to the samples manually, a graduated cylinder or adjustable dispenser may be used. Measure a volume of formic acid equal to the weight of the serum and add it to the serum. Mix the

serum/formic acid mixture using a rocker or vortexer at a slow speed that does not result in a vortex greater than 1½". Then measure a volume of high purity water equal to the volume of formic acid and add it to the serum formic acid mixture. Mix the sample by vortex or shaker.

## 8.4 Solid-Phase Extraction by $C_{18}$ Cartridge

### *8.4.1 Activation of the $C_{18}$ SPE cartridges.*

A. Attach one 10g $C_{18}$ SPE cartridge per sample to the clamp stand. Connect via Luer lock fittings to the Viton tubing which lead into the peristaltic pump and out to a waste beaker. To activate the $C_{18}$ column prior to extraction elute the following volumes through the SPE cartridge:
    - 2 void volumes of methanol, followed by
    - 2 void volumes of dichloromethane, followed by
    - 1 void volume of methanol and, lastly, followed by
    - 2 void volumes of high purity water.
B. Do not let $C_{18}$ go dry during activation by adding more solvent when the solvent layer reaches the frit above the $C_{18}$ sorbent or stop the pump. Discard this pre-rinse solution in an appropriate waste container.
C. As the water level from the second volume approaches the frit above the $C_{18}$, the peristaltic pump should be turned off to allow the analyst adequate time to apply sample to the cartridges.

### *8.4.2 $C_{18}$ Extraction of PCDD/Fs and cPCBs*

A. Pour sample mixture into the SPE cartridge. Rinse the sample container with 10 mL high purity water and add water to SPE cartridge. Allow this sample mixture to completely drain through the $C_{18}$ column. Use a slow rate (1-3mL per minute) to allow serum a long contact time with $C_{18}$ SPE cartridge for primary and secondary interaction with reverse phase material.
B. Rinse the SPE cartridge with 10 mL high purity water to elute serum residues to waste.
C. Move cartridges to the vacuum manifold to dry under vacuum (15 psi) for >60 minutes until dry.
D. Using the peristaltic pump, elute PCDD/Fs and cPCBs into labeled solvent rinsed glass test tubes. Measure hexane elution solvent with a dispenser, apply to SPE cartridge and collect eluate as follows:
    - 12 mL hexane (1-3mL per minute)
    - 12 mL hexane (1-3mL per minute)
    - 6 mL hexane (5mL per minute)

E. Remove any water extracted from cartridge with Pasteur pipette and cap tubes with screw caps until ready to start FMS Power-Prep/6 System.

## 8.5 Cleanup and Isolation of PCDD/Fs and cPCBs

### *8.5.1 Principle of Power-Prep Sample Cleanup Procedure:*

The Power-Prep/6 system is used to remove lipids and other biogenic materials present in the serum eluate 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 followed by 2% DCM/hexane. The PCDD/Fs and cPCBs are eluted on to the carbon columns with 50% dichloromethane/hexane. The PCDD/Fs and cPCBs are retained by the carbon. The PCDD/Fs and cPCBs fractions are removed from the carbon columns with toluene in the reverse direction. Table 4 describes the Power Prep/6 program:

**Table 4.** Program for Clean-up of a Sample for PCDD/Fs and cPCBs through the 6-Carbon Column POWER-Prep/6 System.

| Step | Flow Rate (mL/min) | Volume (mL) | Valve Positions | Procedure |
|---|---|---|---|---|
| 1 | 9 | 120 | 01122006 | Wet silica column with hexane |
| 2 | 15 | 15 | 01222006 | Bypass hexane to waste |
| 3 | 9 | 50 | 01212006 | Wet alumina column with hexane |
| 4 | 15 | 15 | 05222006 | Change to toluene |
| 5 | 6 | 50 | 05221226 | Elute carbon column (Forward direction) |
| 6 | 15 | 15 | 03222006 | Change to 50% DCM in hexane |
| 7 | 6 | 10 | 03221226 | Elute carbon column with 50% DCM in hexane (Forward direction) |
| 8 | 15 | 15 | 01222006 | Change to hexane |
| 9 | 6 | 25 | 01221226 | Elute carbon column with hexane (Forward direction) |
| 10 | 5 | 35 | 06112006 | Add sample to silica/alumina column |
| 11 | 5 | 90 | 01112006 | Elute silica/alumina column with hexane to waste |
| 12 | 15 | 15 | 02222006 | Change to 2% DCM in hexane |
| 13 | 9 | 60 | 02212006 | Elute alumina column with 2% DCM in hexane to waste |
| 14 | 14 | 15 | 03222006 | Change to 50% DCM in hexane |
| 15 | 5 | 120 | 03211226 | Elute alumina/carbon columns with 50% DCM in hexane to waste |
| 16 | 15 | 15 | 05222006 | Change to toluene |
| 17 | 5 | 45 | 0522111X | Elute carbon column with toluene to collected fraction 1 through 6 (Reverse direction) |
| 18 | 6 | 2 | 01221111 | Purge carbon column with hexane |
| 19 | 1 | 0.1 | 00000000 | All valves off |

### *8.5.2 Daily function checks*

Daily function checks are performed by purging the FMS system with 50% dichloromethane in hexane (file = RINSE1) and hexane (file = RINSE2). Place collection lines in round deep dish for waste and initiate procedure.

Check valves, fittings, and all columns for leaks by filling the solvent reservoirs and flushing the lines. Run the RUNSE function check program. Note in notebook that the

entire sample volume is drawn from the sample tube and that the backpressure does not exceed 30pst. If applicable (e.g., change valve), also record in Equipment Maintenance Log.

### *8.5.3 Sample Cleanup Procedure*

A. Install one acid/neutral/base silica, one alumina, and one carbon column per sample on each FMS module.

B. Insert manifold solvent lines into their appropriate solvent bottles: hexane, 50% dichloromethane in hexane, 2% dichloromethane in hexane, and toluene.

C. Attach sample test tube to the manifold in their proper position according to notebook number and insert intake tubing into sample. Place correct lines in 200 mL TurboVap collection tubes.

D. Start a run (file = 14DIOXIN.stp). Check all fittings for possible leaks as the program wets the columns. To stop the program to tighten any leaks press the "Halt" switch.

E. Collect 45 mL of tolune fraction in the 200 mL TurboVap tube. This is the target analyte fraction containing PCDD/F and cPCBs.

### *8.5.4 Evaporation and transfer to final GC-vial*

A. Samples from the FMS cleanup step are evaporated to approximately 0.5 mL using the Caliper TurboVap evaporator. Start the evaporation with the following settings as a general guide: 50 $^{o}$C water bath temperature and ~5psi line pressure. ***It is imperative that the samples are not evaporated to dryness at this step, since all volatile analytes would be lost.***

B. Transfer the sample to the GC vial spiked with 100uL of Dioxin Recovery Standard. The Recovery Standard is added to the GC vails using the Gilson 215 running the Application named, “New Dioxin Recovery Std Spiking App”. **MAKE CERTAIN THAT THE SAMPLES ARE TRANSFERRED TO THE CORRECT VIAL !!!** Rinse the sample TurboVap tube with ~0.5mL of dichloromethane and transfer to the corresponding GC-vial.

C. Evaporate samples until <1uL remains using the Caliper TurboVap LV evaporator. The TurboVap LV for GC vials should not contain water. Start the evaporation with the following settings as a guide: ~5-10psi line pressure and no heat.

D. Then reconstitute the samples with 5uL of nonane using either a GC syringe or an electric pipettor.

E. Cap the GC vials with cleaned PFTE-lined caps.

F. Complete any lab notes, and log samples into HRMS freezer and computer.

## 8.6 Gas chromatography isotope dilution high resolution mass spectrometry (GC/ID-HRMS) analysis

### *8.6.1 GC-MS Setup for PCDD/Fs and cPCBs*

GC/IDHRMS analysis of PCDDs/PCDFs/cPCBs only is performed on a DFS (Thermo Fisher, Bremen, Germany) instrument. The chromatographic separations are carried out on a Thermo 1300 gas chromatograph (GC) (Thermo Scientific) fitted with an Rxi-5MS capillary GC column (30-m length, 0.25 mm I.D. and 0.25-µm film thickness) made by Restek (Bellefonte, PA). The carrier gas is helium with a flow rate of 1.0ml/minute. The injection liner is a Restek splitless liner (4mm x 6.5 mm x 78.5 mm) with deactivated glass wool.

**The GC program used for PCDD/Fs and cPCBs are:**

- Start at 140$^{o}$C with a hold of 1 minute
- Then ramp to 230$^{o}$C (50 $^{o}$C/minute) and hold for 11 minutes
- Then ramp to 241$^{o}$C (1.5 $^{o}$C/minute)
- Then ramp to 320 (20$^{o}$C/minute) and hold for 2 minutes

Mass Spectrometer settings are given in Table 4 and MID settings are summarized in Appendix A for PCDD/F and cPCB analysis.

**Table 5.** GC/ID-HRMS settings used for PCDD/F and cPCB analysis.

| Parameter | Measure |
|---|---|
| Emission Current | 1 mA |
| Ionizing electron energy | 45 eV |
| Accelerating Voltage | 4800 V |
| Trap Current | 1 A |
| Source temperature | 275 oC |
| Transfer line temperature | 275 oC |
| Mass Resolution | 10,000 |

### *8.6.2 Mass Spectrometer Selected Ion Monitoring (SIM) Setup*

Calibrate and tune the mass spectrometer to 10,000 resolving power (RP) at 10% peak height. Multi-group analyses for all 2,3,7,8- substituted PCDD/Fs on the DFS mass spectrometers consist of six MID groups. The analyses are conducted in selected ion monitoring mode. The accurate masses and selected ion monitoring (SIM) window is given in Appendix A.

After installation of a new GC column, inject a calibration standard and determine the retention time windows for all the congeners. For each congener, update the retention times relative to the $^{13}C_{12}$-labeled isomer present for each congener group in the quantification-file containing integration parameters.

### *8.6.3 Daily Signal-to-noise (S/N) ratio Function Check*

Inject 2µL of a 0.010 pg/µL 2,3,7,8-TCDD Signal-to-noise (S/N) ratio check standard using the following GC program:

**The GC program used for S/N function check are:**

- Start at 140 ºC with a hold of 2 minute
- Then ramp to 220ºC (30ºC/minute) and hold for 2 minutes
- Then ramp to 240ºC (15ºC/minute) and hold for 5 minutes

Check the sensitivity of the instrument by verifying that the S/N ratio for the unlabeled 2,3,7,8-TCDD (m/z 319.8965) is greater than 100:1 in order to begin analyzing study samples. 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.

### *8.6.4 Daily Slope Function Check*

A calibration curve (n≥5 calibration points) with every batch of samples containing up to 36 samples, QC and blanks. The $r^2$ of the $\log_{10}$-transformed calibration curve is confirmed to be ≥0.995 by processing the data in a SAS program. Typical output from the SAS program which is stored at the network location ‘Y:’ is given in Figure 3. First order linear regression in used after $\log_{10}$ transformation.

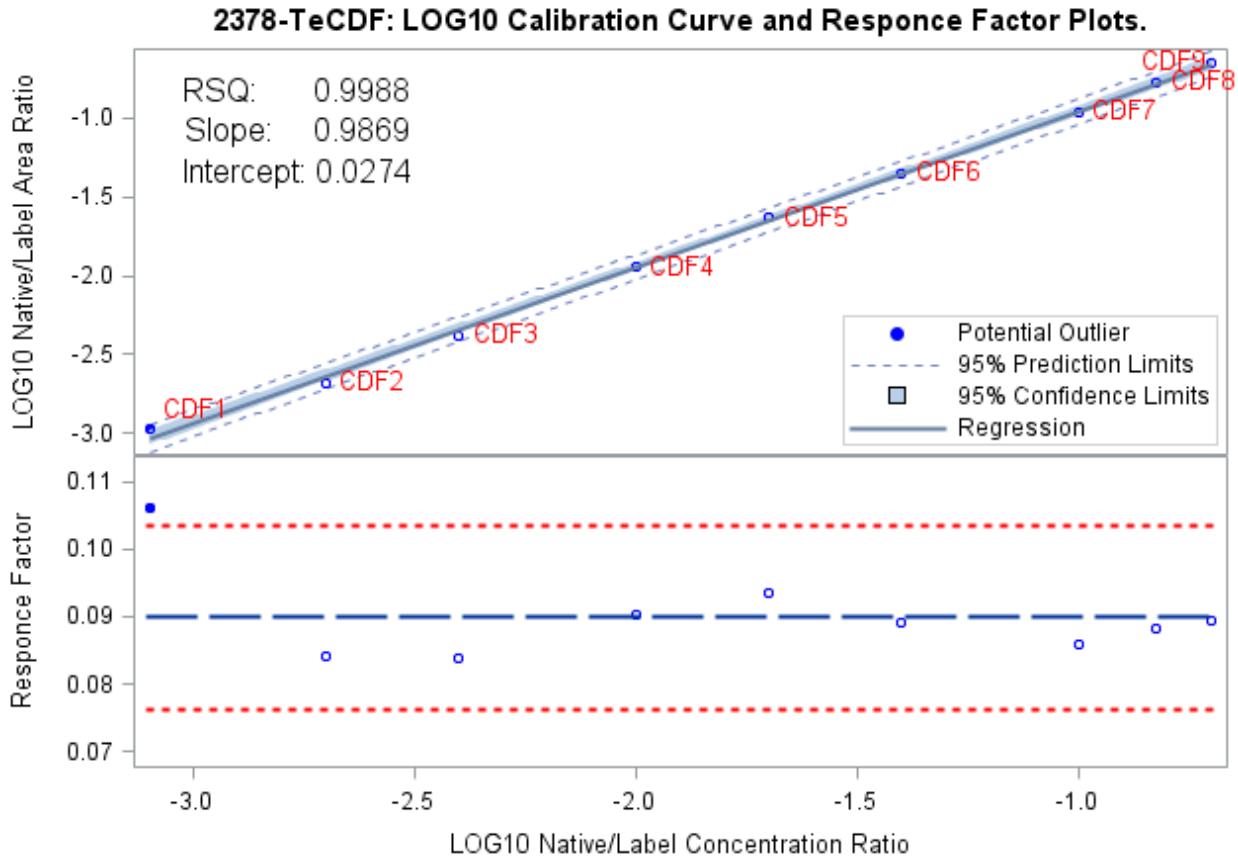


**Figure 3.** Typical output from SAS program used for confirmation of linearity of calibration curve. The top graph gives the calibration curve with the calibration points (CDF1 through CDF9 labeled). The slope, intercept and $r^2$ are given in the top graph. Lower part of the graph presents the average response factor (blue dashed line) with +/-3 standard deviations (red dashed line). Response factor of individual data points within +/- 3SD of the response factor are given as unfilled blue circles while data points outside +/-3 STD are given with filled circles. An acceptable calibration curve must have an $r^2$ >0.995.

### *8.6.5 Replacement and periodic maintenance of key components*

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

Daily, check the sensitivity of the instrument by verifying that the S/N ratio for the unlabeled 2,3,7,8-TCDD (m/z 319.8965) is greater than 100:1. If the S/N ratio is unsatisfactory, check the tuning (retune if necessary), cut 1-2 inches from the GC end of the GC 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.

Check the pressure in the nitrogen and helium tanks before beginning a run. Replace the nitrogen and helium gas cylinders when the tank pressure approaches 500 psi. Do not let the carrier gas (helium) cylinder pressure go below 500 psi since heavier contaminant gases will start to be released if the pressure of the cylinder is too low.

Clean the auto sampler solvent bottles prior to beginning a run. Pour out the old solvent and rinse well with fresh solvent and then refill the solvent bottles with fresh solvent. Do not top off the solvent in the bottles. Also, clean out the waste collection bottles. Replace the solvent bottles periodically with new, clean bottles.

Instrument Preventive Maintenance (PM) is performed by service technician once or more per year. The ion volume is cleaned and replaced monthly or as needed. The multiplier is changed once the setting is greater than 2400, or as needed. The outer source is replaced at the time of a PM or as needed. The GC column is replaced as needed, usually every month or after about 500 samples have been analyzed. Reference inlet septum and liner and auto sampler syringe are replaced after every 1-2 analytical runs. Magnetic calibration (MCAL) is performed at the time of a PM or as needed. Electric calibration (ECALIB) is performed at the time of a PM or as needed. Multiplier gain check is performed when a new multiplier is installed or as needed.

### *8.6.6 Mass Spectral Analysis of Processed Specimen*

Prior to injecting a sample into the GC, the auto sampler syringe is rinsed more than 5 times with nonane. After injecting a sample, the auto sampler syringe is rinsed 10 times with toluene, followed by 10 nonane rinses or more.

The 12 sample extracts from a cleanup run are considered one analytical run. One to three analytical runs may be batched as one sequence for the GC/ID-HRMS analysis for PCDD/F and cPCBs. The GC-HRMS analyst should always refer to the run sheet for information regarding individual sample type and/or comments from the sample preparation lab.

Area counts, retention times for each analyte and its corresponding internal standard is measured and saved and an Excel-file. The analytical data in processed using a SAS program to calculate individual sample concentration and set report codes based on

sample QC criteria, e.g. recovery, relative retention time, and isotopic ratio. Tolerances for sample QC criteria is given in Appendix A. Further details on batch quality control criteria are given in the Division of Laboratory Sciences Policy Manual.

Accurate masses and SIM window is given in Appendix A.

## 9. Reportable Range of Results

The linear range of each standard calibration curve determines the highest and lowest analytical values of an analyte that are reportable. However, samples with a concentration exceeding the highest reportable limit may be re-extracted using a smaller volume and re-analyzed, so that the result is in the reportable range. Alternatively, if no more sample is available the sample may be diluted with solvent so that the area count of the high concentration analyte is less than that of the highest calibration standard included in the run.

### 9.1 Linearity Limits

Calibration standards are linear for all analytes through the range of concentrations evaluated. Samples exceeding the calibration curve must be diluted or analyzed using a smaller volume of serum.

Certificate of analysis for all standards used are stated in the certificate of analysis as provided by the manufacturer, Cambridge Isotope Laboratory (CIL).

### 9.2 Limit of detection

The lowest reporting level or the method limit of detection (LOD) is given in Table 2. The method LOD is defined as the higher value of $S_0$ (Taylor, K. T. (1987) In Quality Assurance of Chemical Measurements, pp 79-82, Lewis Publishers, Washington, DC) and three times the standard deviation of blank samples. The method LOD was determined based on generated measurements during 2015 and 1st and 2nd quarter of 2016. Analytes with a determine concentration between the LOD and the lowest standard are reported as “Detected, lower than lowest standard”.

## 9.3 Precision

The precision of the method is reflected in the variance of quality control samples analyzed over time. Typical coefficients of variance (CV) of the method are listed in Table 6.

**Table 6.** Typical coefficients of variance (CV) of the procedure.

| Analyte | Mean (fg/g serum) | CV (%) | Analyte | Mean (fg/g serum) | CV (%) |
|---|---|---|---|---|---|
| 2378-TeCDD | 85 | 12 | 1234678-HpCDF | 291 | 11 |
| 12378-PeCDD | 93 | 12 | 1234789-HpCDF | 78 | 13 |
| 123478-HxCDD | 81 | 11 | OCDF | 91 | 14 |
| 123678-HxCDD | 264 | 11 | PCB77 | 375 | 11 |
| 123789-HxCDD | 82 | 11 | PCB81 | 360 | 9 |
| 1234678-HpCDD | 461 | 11 | PCB126 | 694 | 10 |
| OCDD | 2132 | 11 | PCB169 | 692 | 9 |
| 2378-TeCDF | 96 | 10 | | | |
| 12378-PeCDF | 94 | 10 | | | |
| 23478-PeCDF | 188 | 10 | | | |
| 123478-HxCDF | 79 | 10 | | | |
| 123678-HxCDF | 84 | 10 | | | |
| 123789-HxCDF | 87 | 10 | | | |
| 234678-HxCDF | 88 | 11 | | | |

## 9.4 Analytical specificity

GC/ID-HRMS is used for sample analysis. This instrumentation offers a high mass resolution (10,000 resolution) measurement which provides excellent specificity. In addition, two ions are monitored for each native analyte and $^{13}$C-labeled internal standard. For each measurement, the ratio between these two ions is verified to be with +/- 26% from the theoretical isotope ratio. This provides additional confirmation of the identity and specificity of the target analyte measurement.

In addition, the relative retention time of native compound divided with its $^{13}$C-internal standard is verified for each measurement to eliminate the risk of mistakes during integration.

# 10. Quality Assessment and Proficiency Testing

## 10.1 Quality Assessment

In this method, a set of samples is generally defined as 8 unknown samples, prepared and analyzed together with 2 analytical blanks and 2 QC sample. Quality control limits are established by characterizing assay precision with repeated analyses of the QC pool.

For QA/QC purposes measurement of a target analyte in a set of samples is considered valid only after the QA/QC sample have fulfilled the following criteria further detail is given in the DLS Procedure Manual.:

- If all of the QC samples are within 2σ limits, then accept the run
- If one or more QC results is outside the 2σ limits, then apply the rules below and reject the run if any conditions are met.
    - **Extreme outliner:** the result is outside the characterization mean by more than 4σ.
    - **$1_{3\sigma}$**, Average of three QCs is outside of the 3σ limit.
    - **$2_{2\sigma}$**, QC results from two consecutive runs are outside of 2σ limit on the same side of the mean.
    - **$R_{4\sigma}$ sequential**, QC results from two consecutive runs are outside of 2σ limit on opposite sides of mean.
    - **$10_x$ sequential**, QC results from ten consecutive runs are on the same side of the mean.

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

## 10.2 Proficiency testing (PT)

Currently the only established PT program for this assay is the Arctic Monitoring and Assessment program (AMAP) in which our lab participates. In this program, 2 serum samples are received twice per year and analyzed with respect to PCDD/Fs, and cPCBs. The program provides a report after each set of PT samples has been reported.

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

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

## 12. Limitations of Method, Interfering Substances and Conditions

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

## 13. Reference Ranges (Normal Values)

Reference ranges have been reported for PCDD/Fs and cPCBs in the NHANES survey and are available at www.cdc.gov/exposurereport

## 14. Critical Call Results ("Panic Values")

The health effects resulting from exposure to PCDD/Fs and cPCBs are currently unclear. Therefore, no "panic values" have been established. Test results in this laboratory are reported in support of epidemiological studies, not clinical assessments.

## 15. Specimen Storage and Handling During Testing

Store serum samples in -70 $^{o}$C freezer before and after analysis. Keep extracts at room temperature covered with aluminum foil for storage, due to documented UV-sensitivity of target analytes.

After analysis, keep GC vials in Styrofoam boxes for storage at room temperature until the final analytical data have been reported.

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

If the analytical system fails, refrigerate the samples (at 4 - 8 $^{o}$C) until the analytical system is restored to functionality. If long-term interruption (greater that one day) is anticipated, then store serum specimens at -70 $\pm$ 10 $^{o}$C.

The method is designed to run on a GC/ID-HRMS instrument, and is not generally transferable to other instrumentation. If the system fails, store sample extracts at room temperature covered with aluminum foil until the analytical system is restored to functionality.

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

Study subject data is reported in two concentration units (fg/mL serum) and adjusted based on serum lipids (pg/g lipid).

Once the validity of the data is established by the QC/QA system outlined above, these results are verified by a DLS statistician, and the data are reported in both hard copy and electronic copy. These data and a cover letter will be routed through the appropriate channels for approval (i.e. supervisor, QA/QC officer branch chief, division director) as outlined in the DLS Policy and Procedure Manual. After approval at the division level, the report will be sent to the contact person or principal investigator who requested the analyses.

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

If greater than 0.2 mL of sample remains following successful completion of analysis, this material must be returned to storage at -70 $\pm$ 5 °C in case reanalysis is required. These samples shall be retained until valid results have been obtained and reported and sufficient time has passed for review of the results.

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

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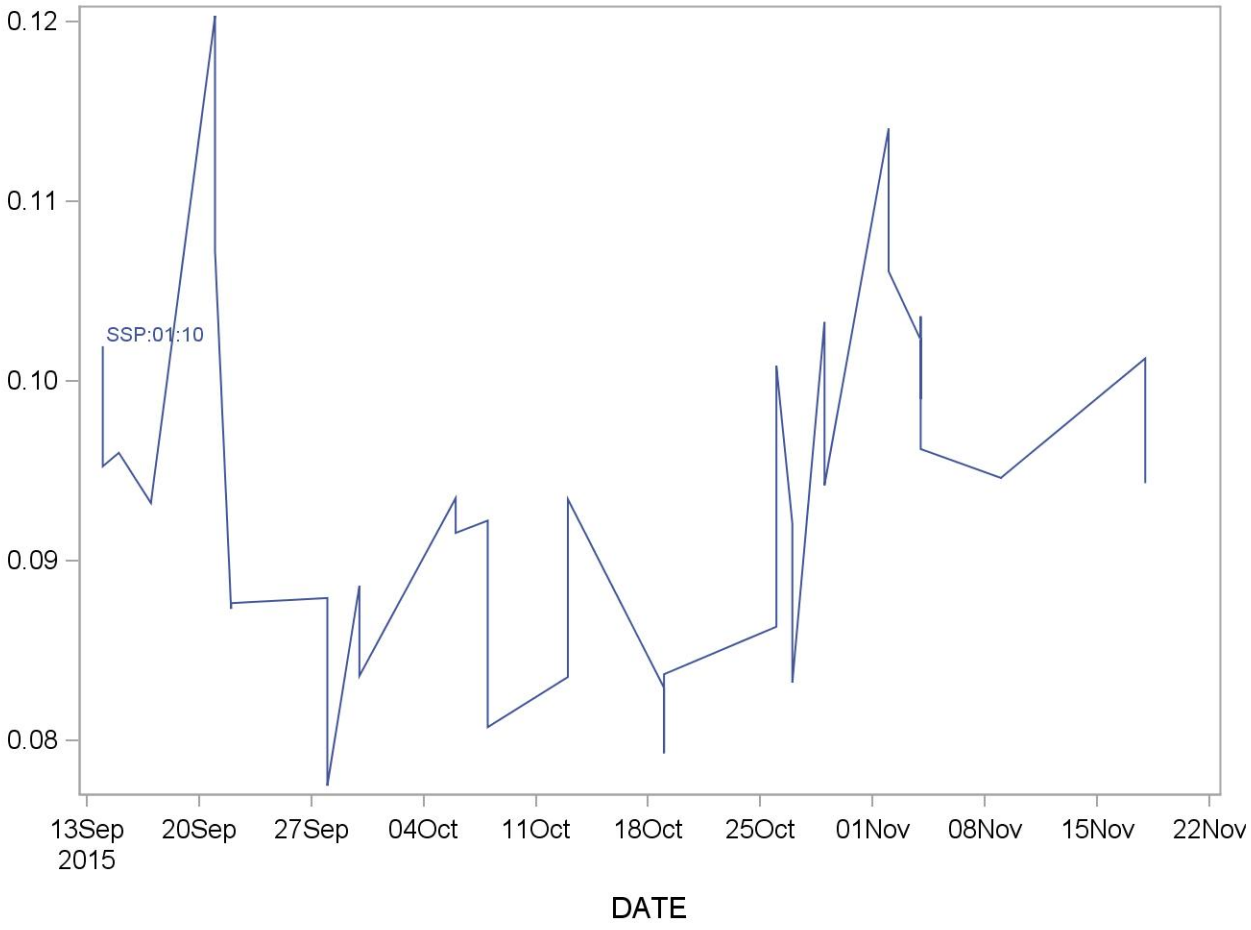
# 20. SUMMARY STATISTICS AND QC GRAPHS

See next pages.

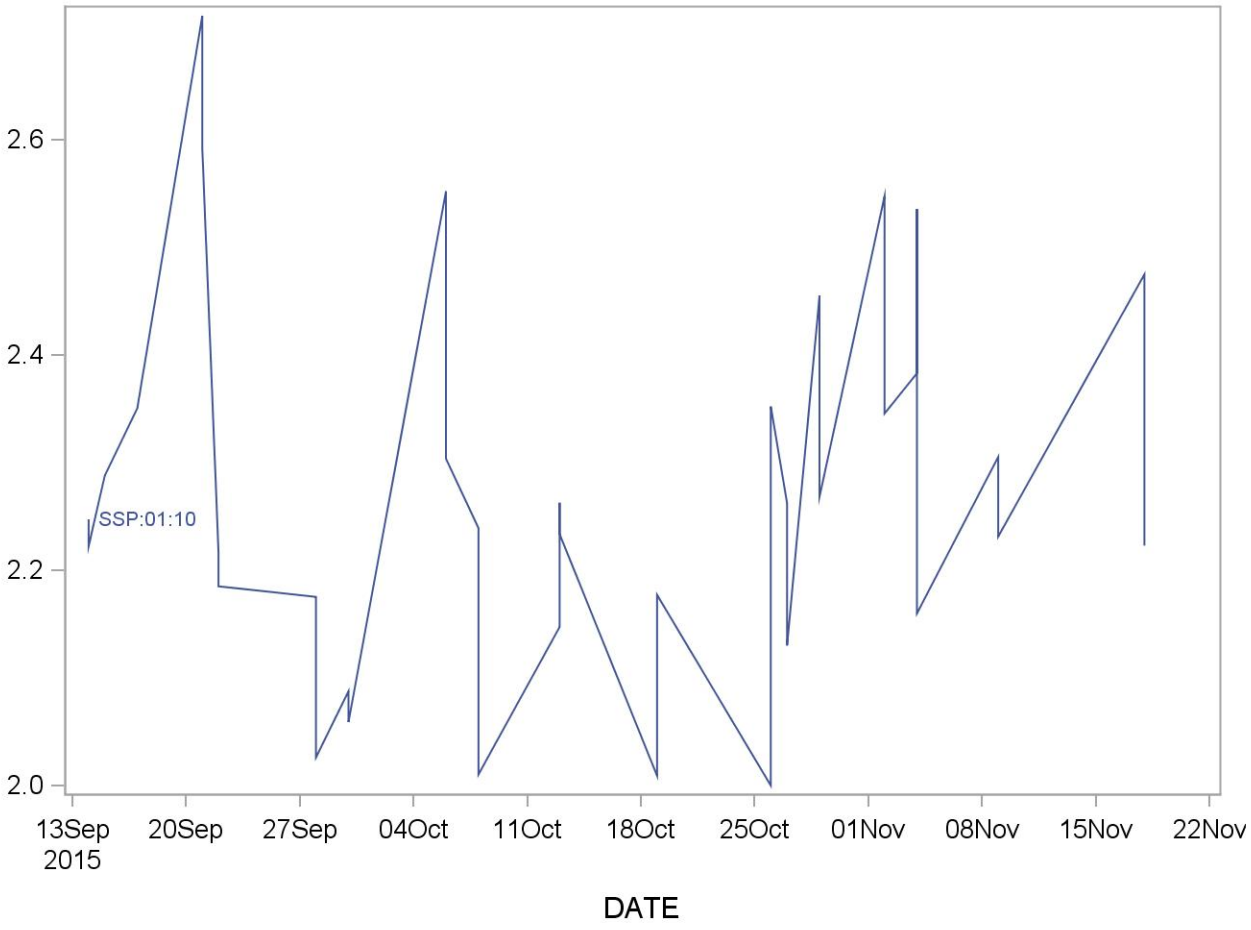
## 2009-2010 Summary Statistics and QC Chart for 1,2,3,4,6,7,8,9-Octachlorodifuran (fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 39 | 14SEP15 | 18NOV15 | 0.094033 | 0.009436 | 10.0 |



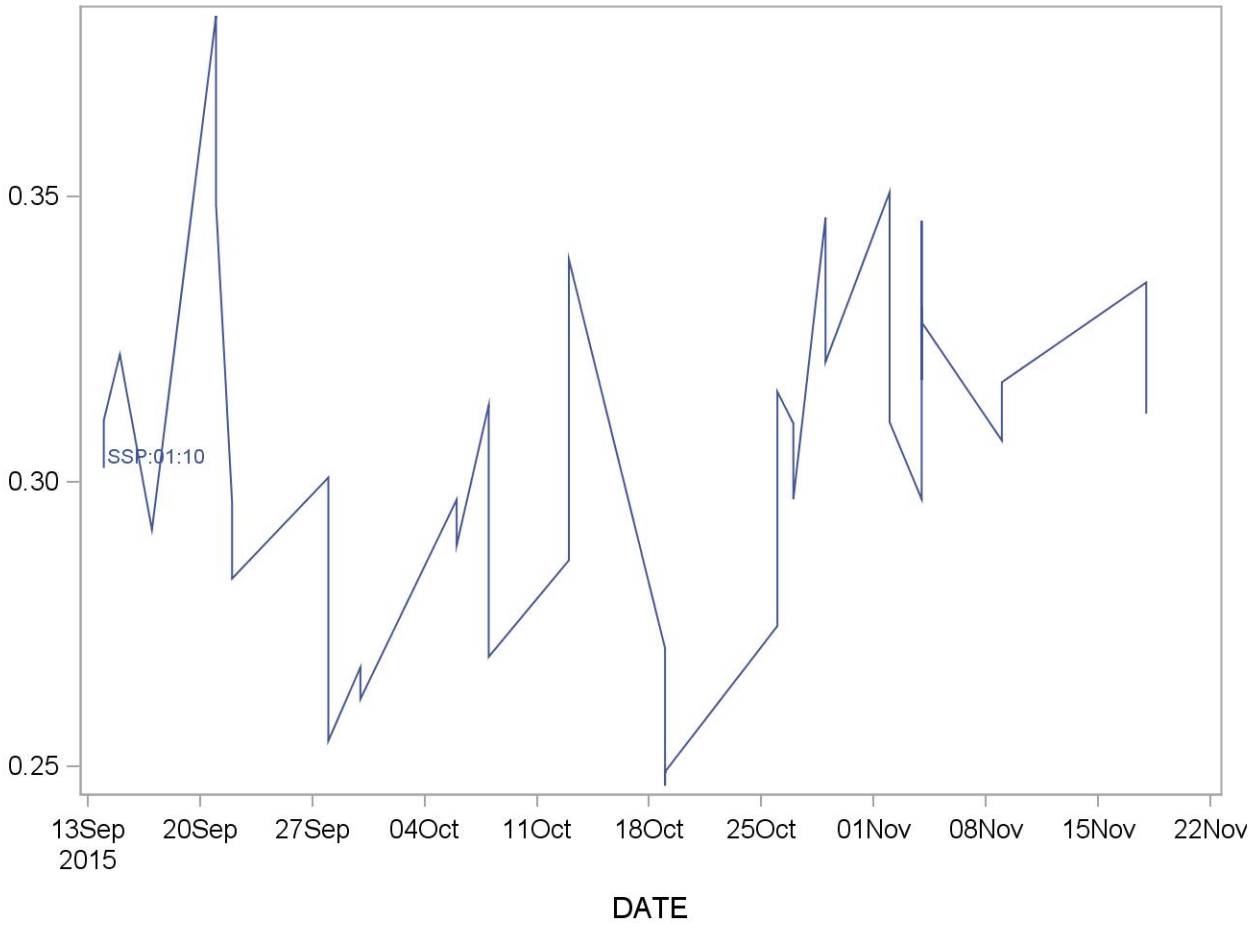
## 2009-2010 Summary Statistics and QC Chart for 1,2,3,4,6,7,8,9-ocdd (fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 39 | 14SEP15 | 18NOV15 | 2.268844 | 0.170374 | 7.5 |



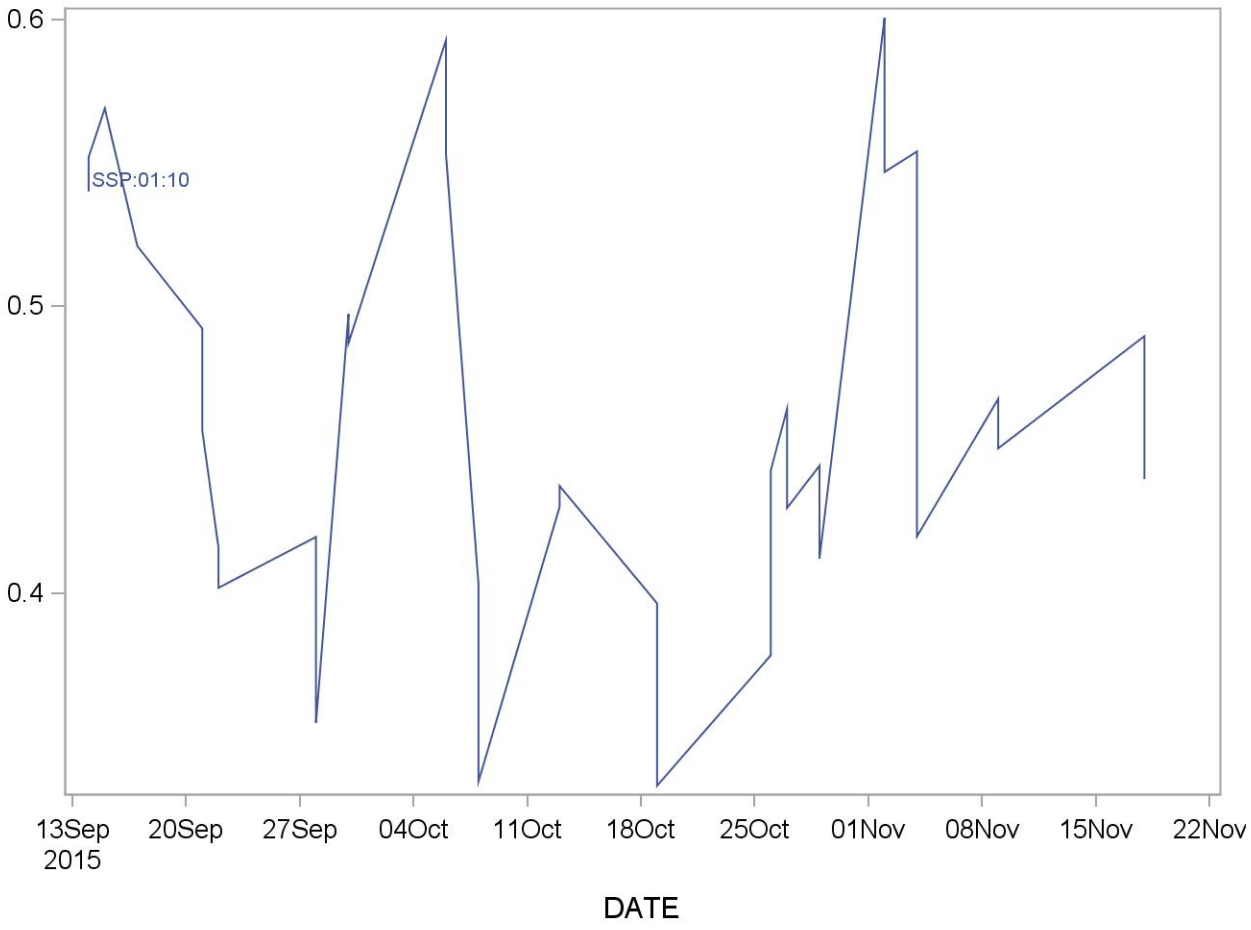
## 2009-2010 Summary Statistics and QC Chart for 1,2,3,4,6,7,8-Heptachlorodifuran (fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 39 | 14SEP15 | 18NOV15 | 0.304932 | 0.030396 | 10.0 |



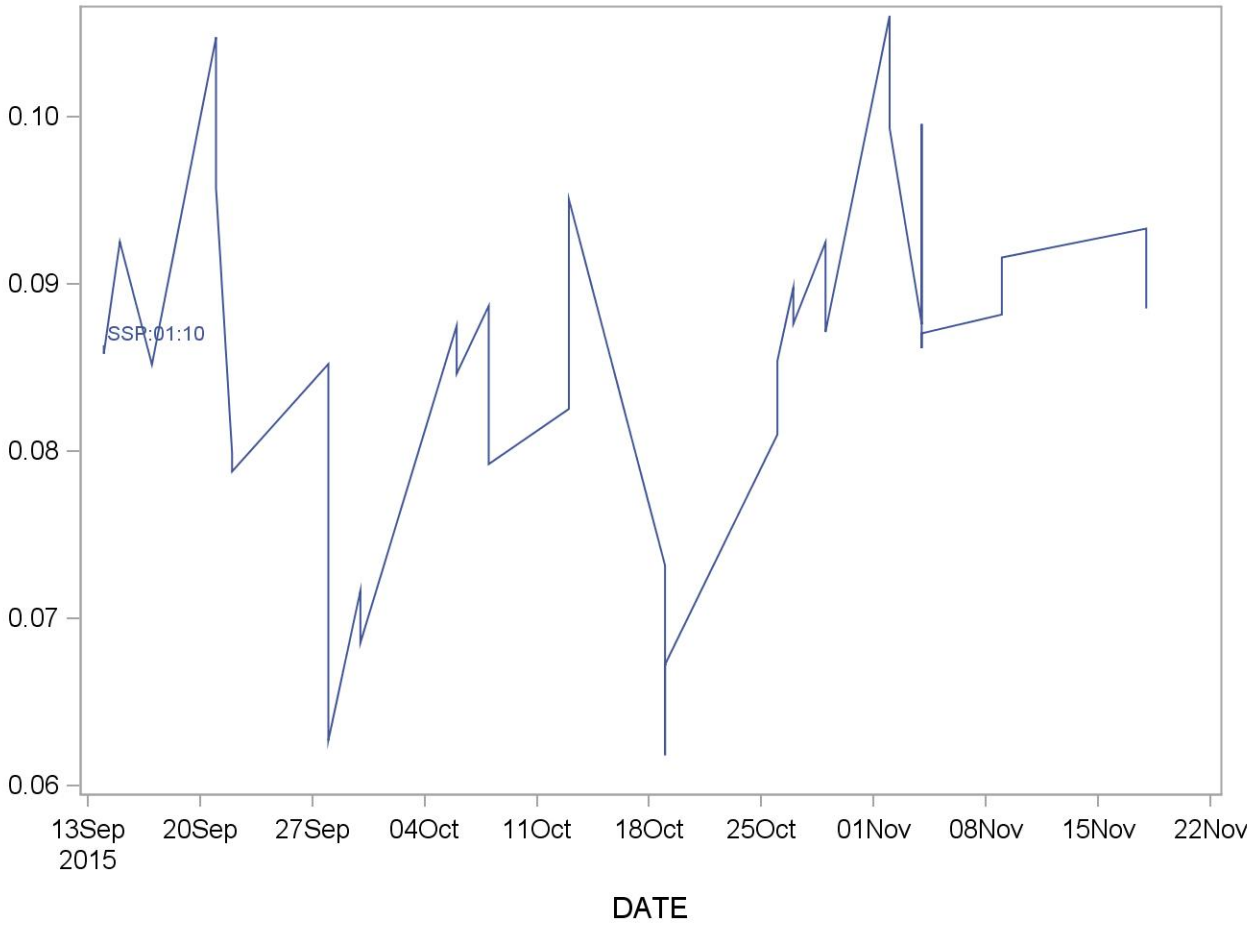
## 2009-2010 Summary Statistics and QC Chart for 1,2,3,4,6,7,8-hpcdd (fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 37 | 14SEP15 | 18NOV15 | 0.463279 | 0.069548 | 15.0 |



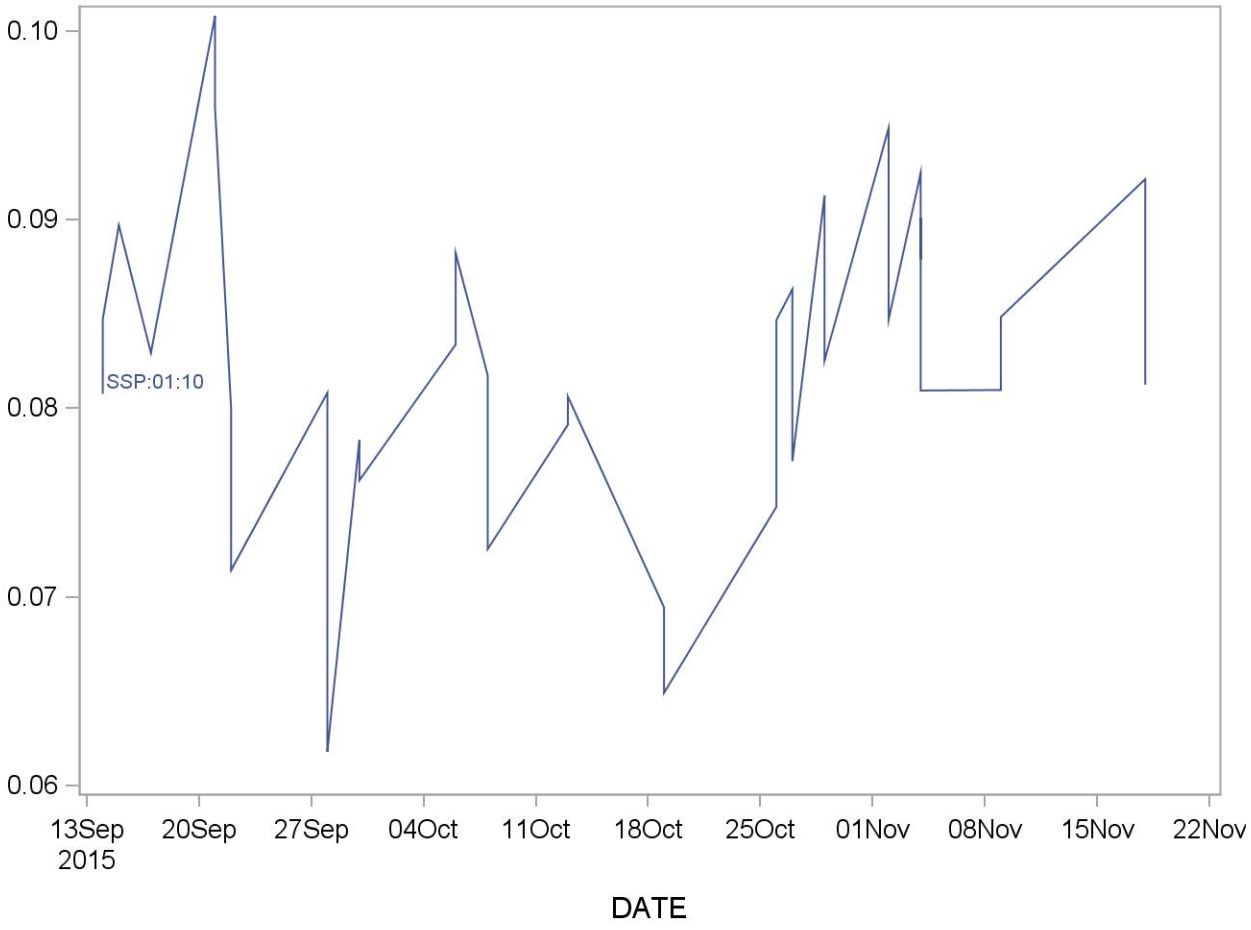
## 2009-2010 Summary Statistics and QC Chart for 1,2,3,4,7,8,9-Heptachlorodifuran (fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 39 | 14SEP15 | 18NOV15 | 0.085643 | 0.010032 | 11.7 |



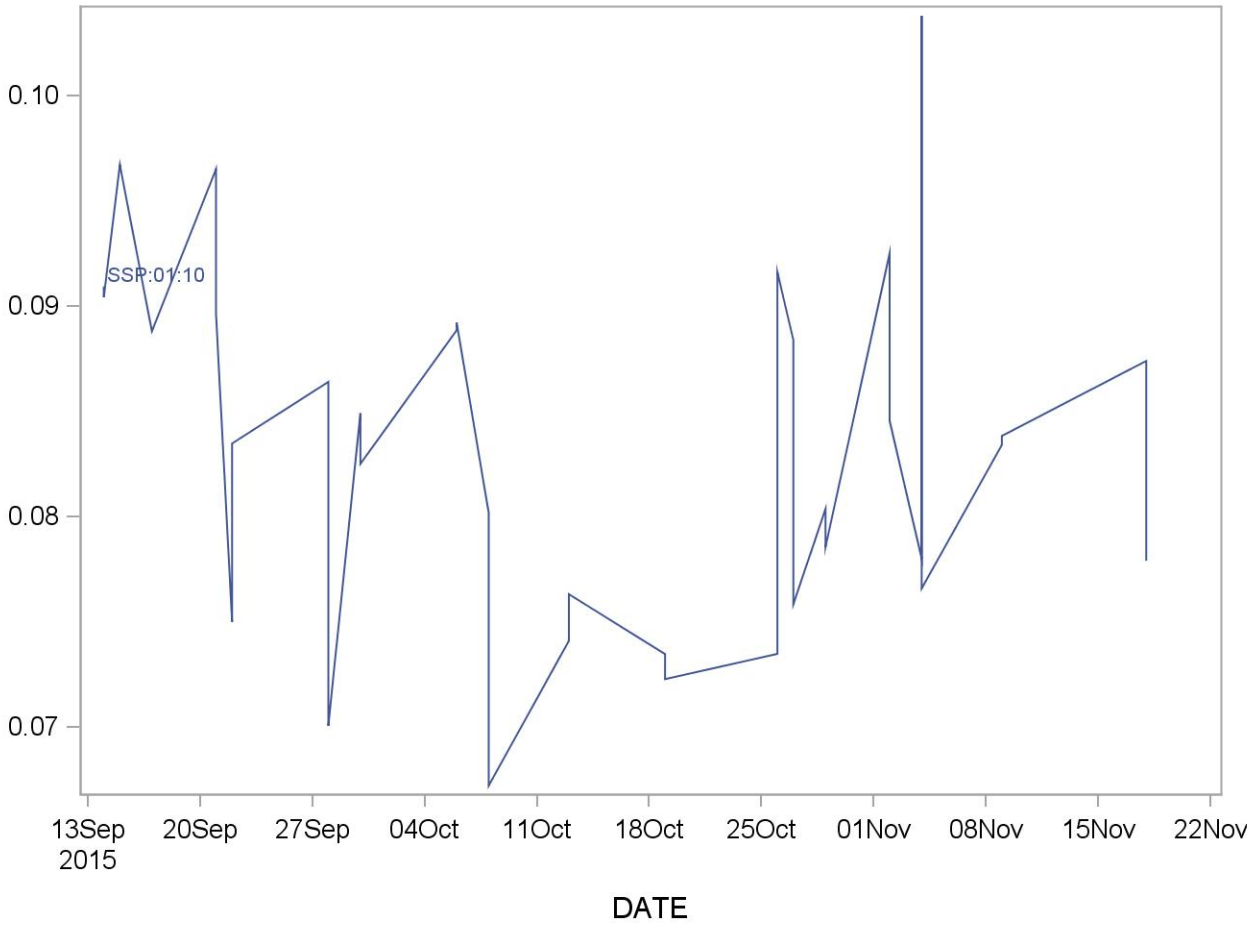
## 2009-2010 Summary Statistics and QC Chart for 1,2,3,4,7,8-Hexachlorofuran(hcxdf)(fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 37 | 14SEP15 | 18NOV15 | 0.082447 | 0.008266 | 10.0 |



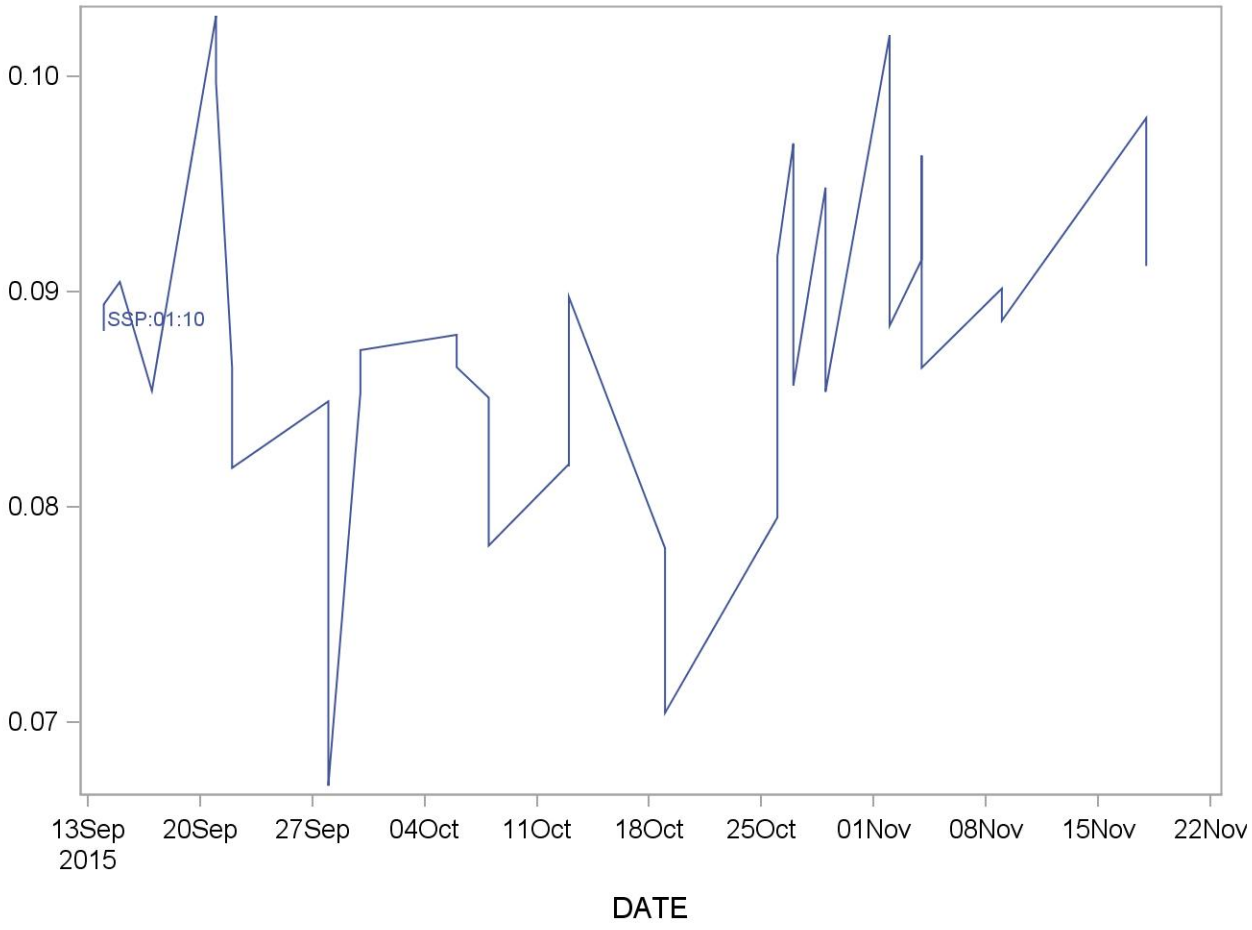
## 2009-2010 Summary Statistics and QC Chart for 1,2,3,4,7,8-hxcdd (fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 39 | 14SEP15 | 18NOV15 | 0.082770 | 0.008310 | 10.0 |



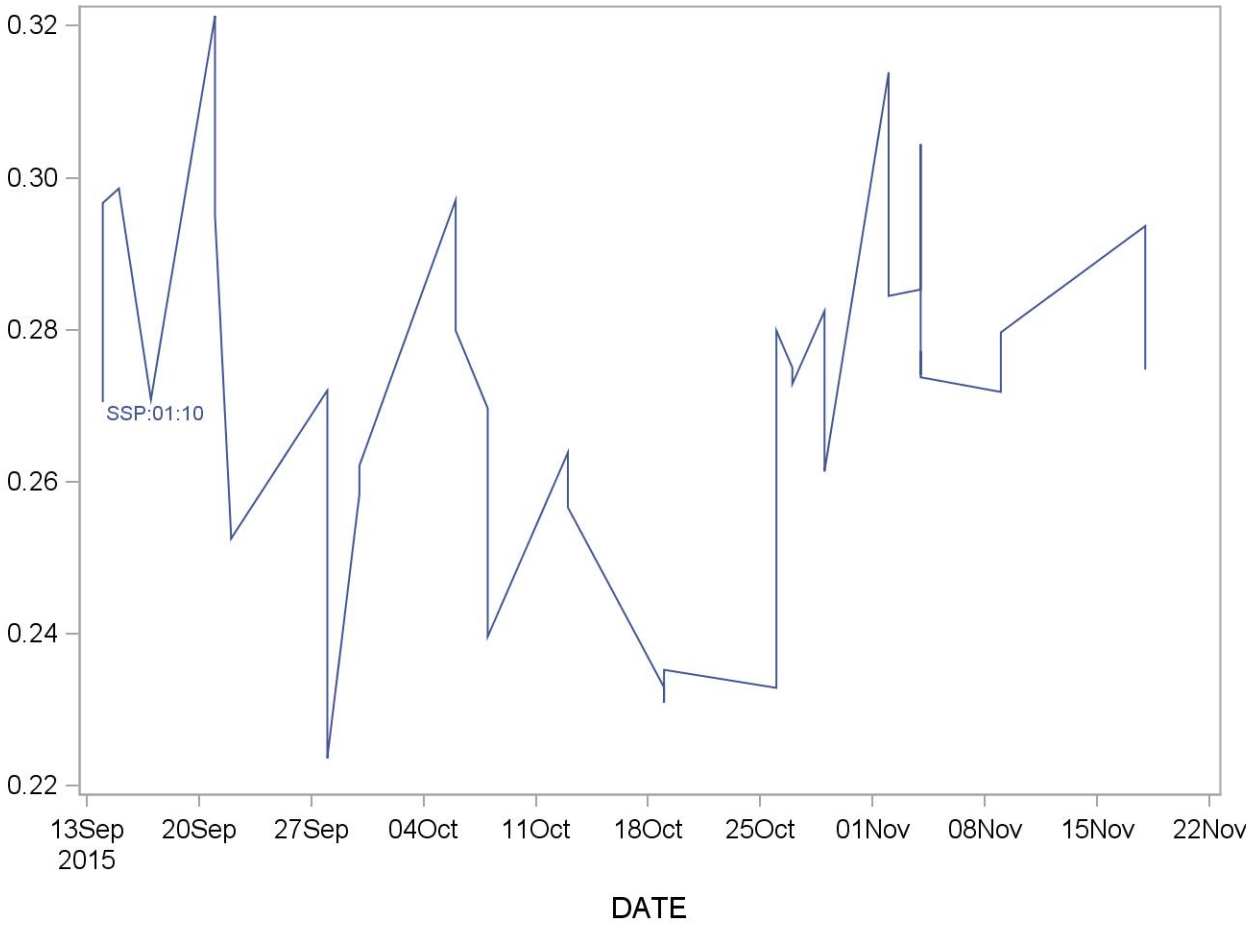
## 2009-2010 Summary Statistics and QC Chart for 1,2,3,6,7,8-Hexachlorofuran(hxcdf)(fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 39 | 14SEP15 | 18NOV15 | 0.087463 | 0.007783 | 8.9 |



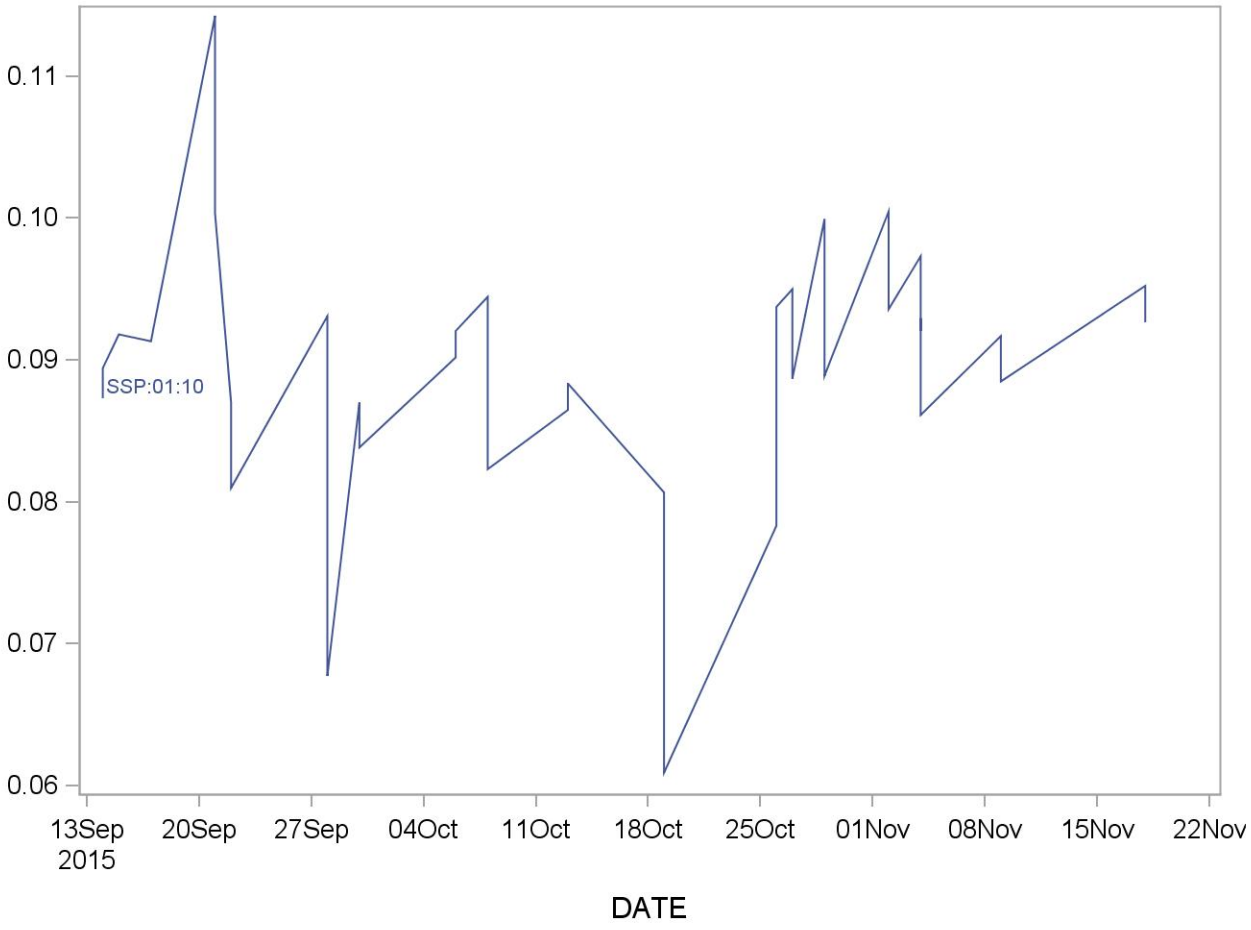
## 2009-2010 Summary Statistics and QC Chart for 1,2,3,6,7,8-hxcdd (fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 39 | 14SEP15 | 18NOV15 | 0.271177 | 0.022907 | 8.4 |



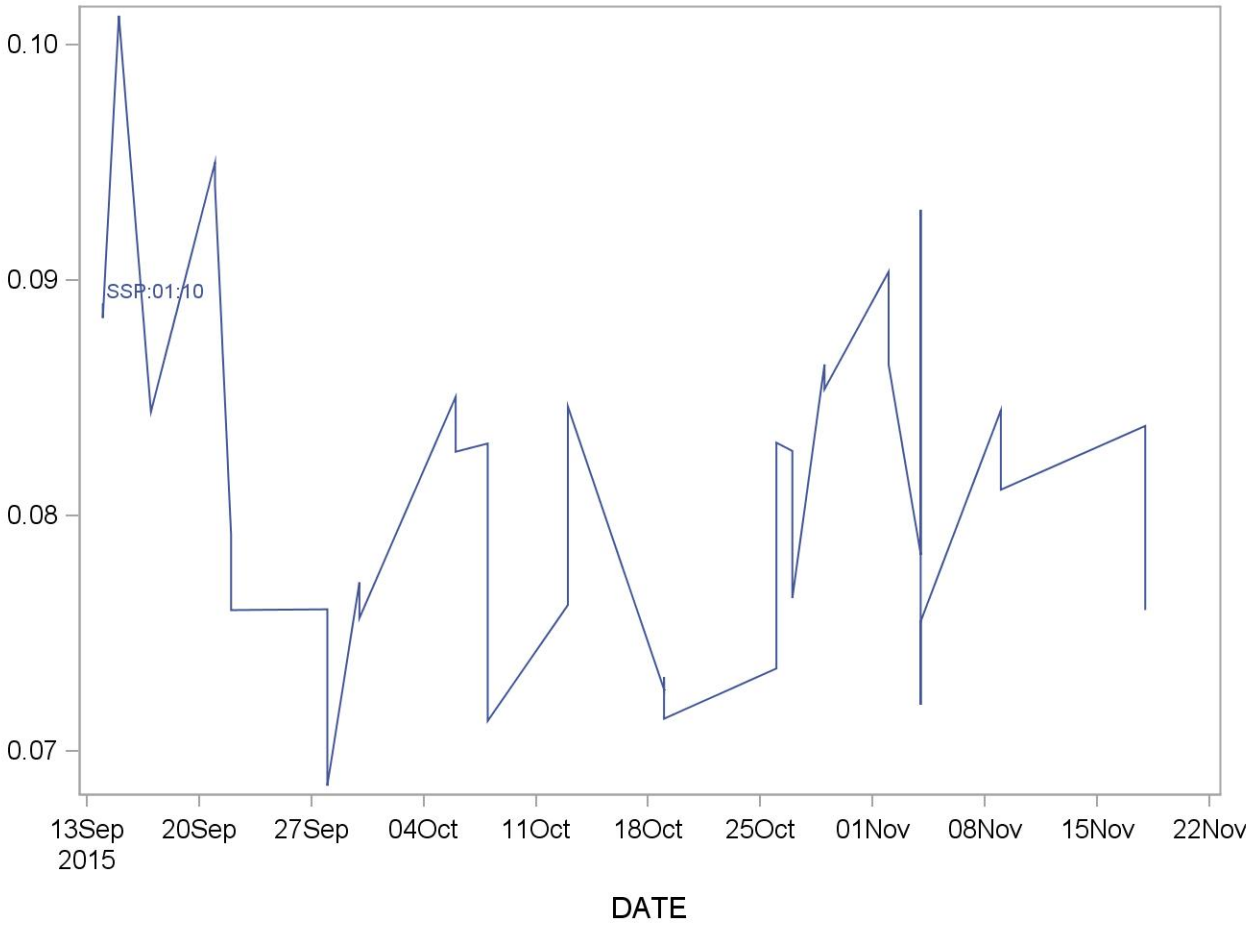
## 2009-2010 Summary Statistics and QC Chart for 1,2,3,7,8,9-Hexachlorodifuran(fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 37 | 14SEP15 | 18NOV15 | 0.089532 | 0.008969 | 10.0 |



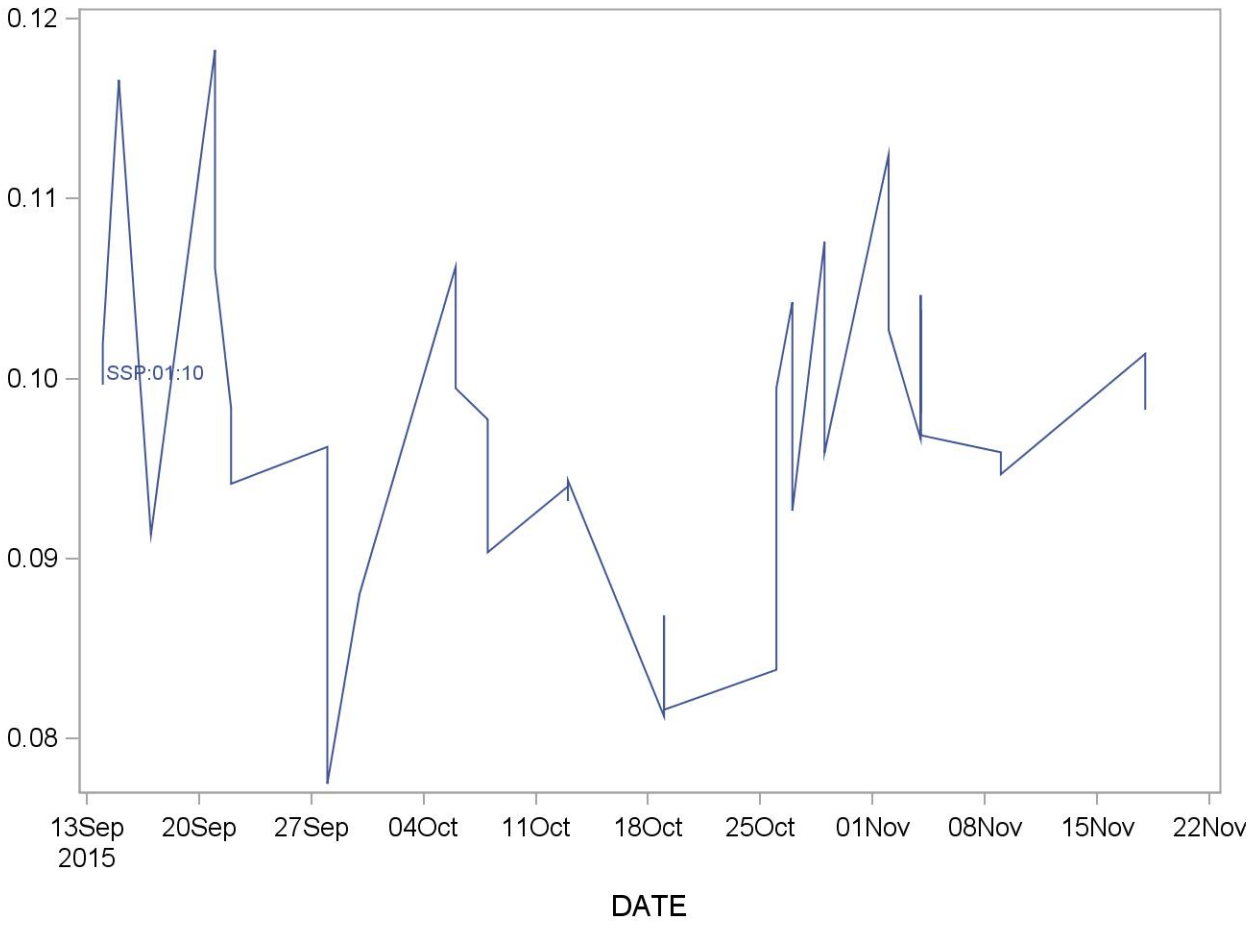
## 2009-2010 Summary Statistics and QC Chart for 1,2,3,7,8,9-hxcdd (fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 39 | 14SEP15 | 18NOV15 | 0.081516 | 0.007489 | 9.2 |



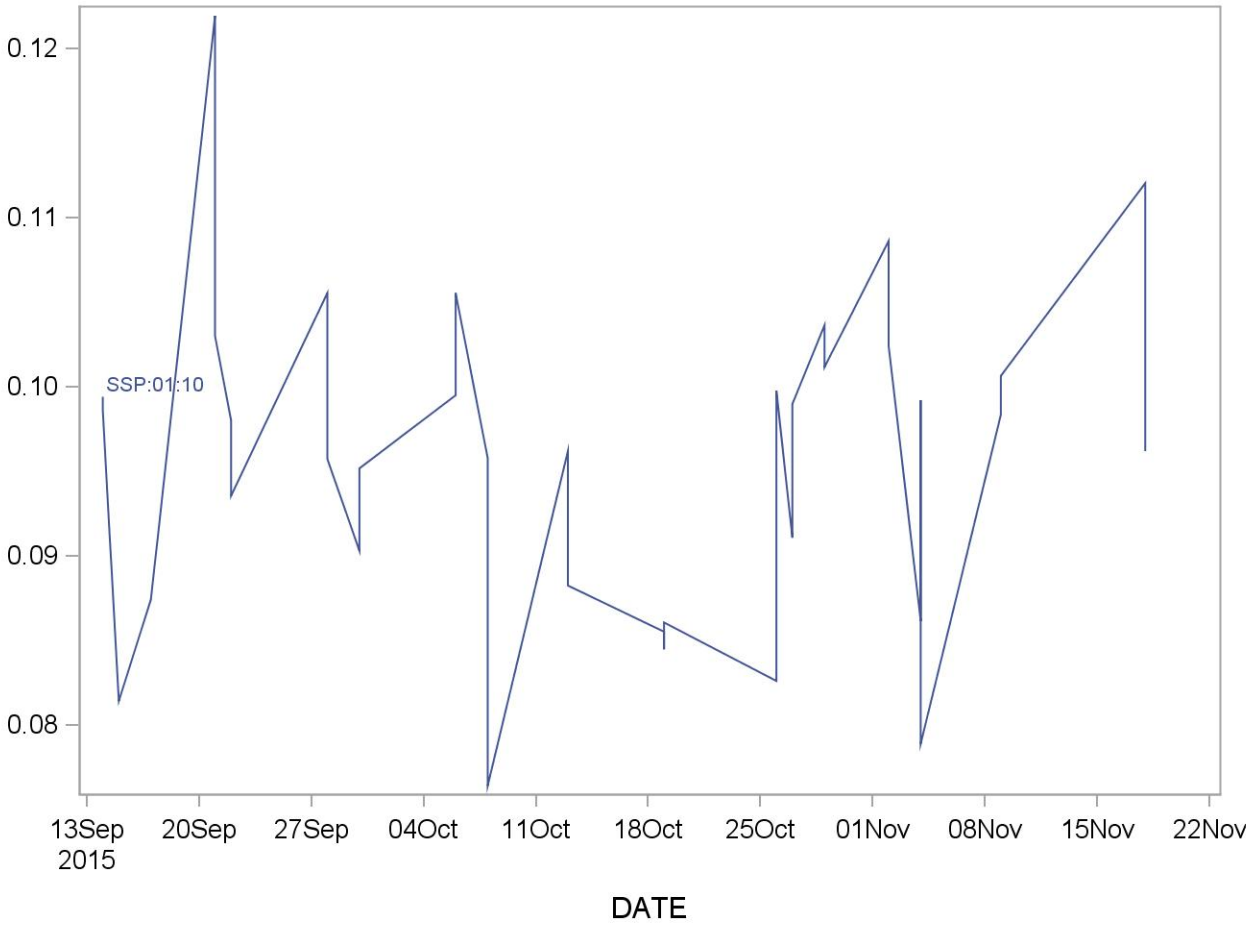
## 2009-2010 Summary Statistics and QC Chart for 1,2,3,7,8-Pentachlorofuran (pncdf)(fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 39 | 14SEP15 | 18NOV15 | 0.097187 | 0.008969 | 9.2 |



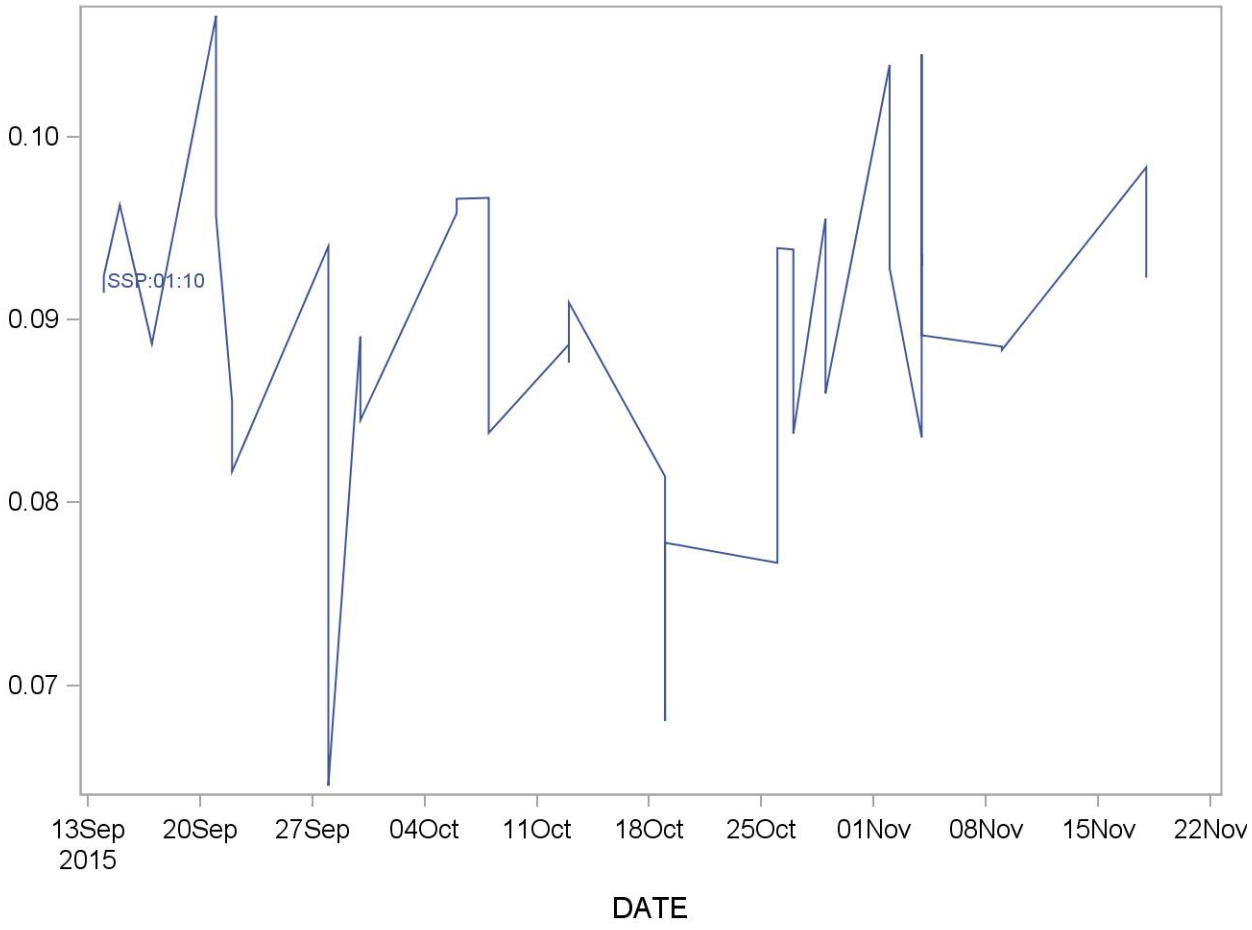
## 2009-2010 Summary Statistics and QC Chart for 1,2,3,7,8-pncdd (fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 39 | 14SEP15 | 18NOV15 | 0.095691 | 0.009307 | 9.7 |



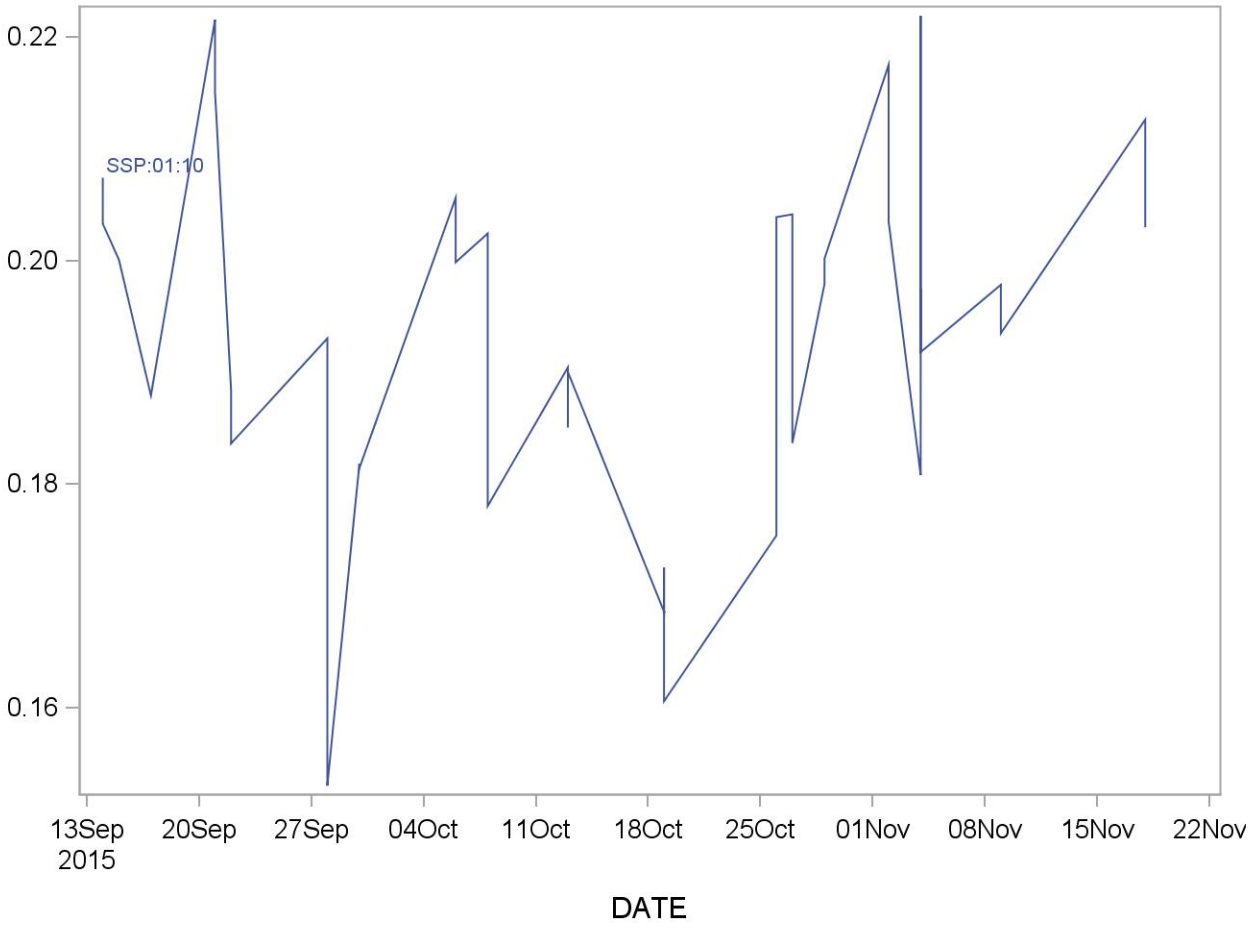
## 2009-2010 Summary Statistics and QC Chart for 2,3,4,6,7,8-Hexchlorofuran(hxcdf)(fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 39 | 14SEP15 | 18NOV15 | 0.089616 | 0.008667 | 9.7 |



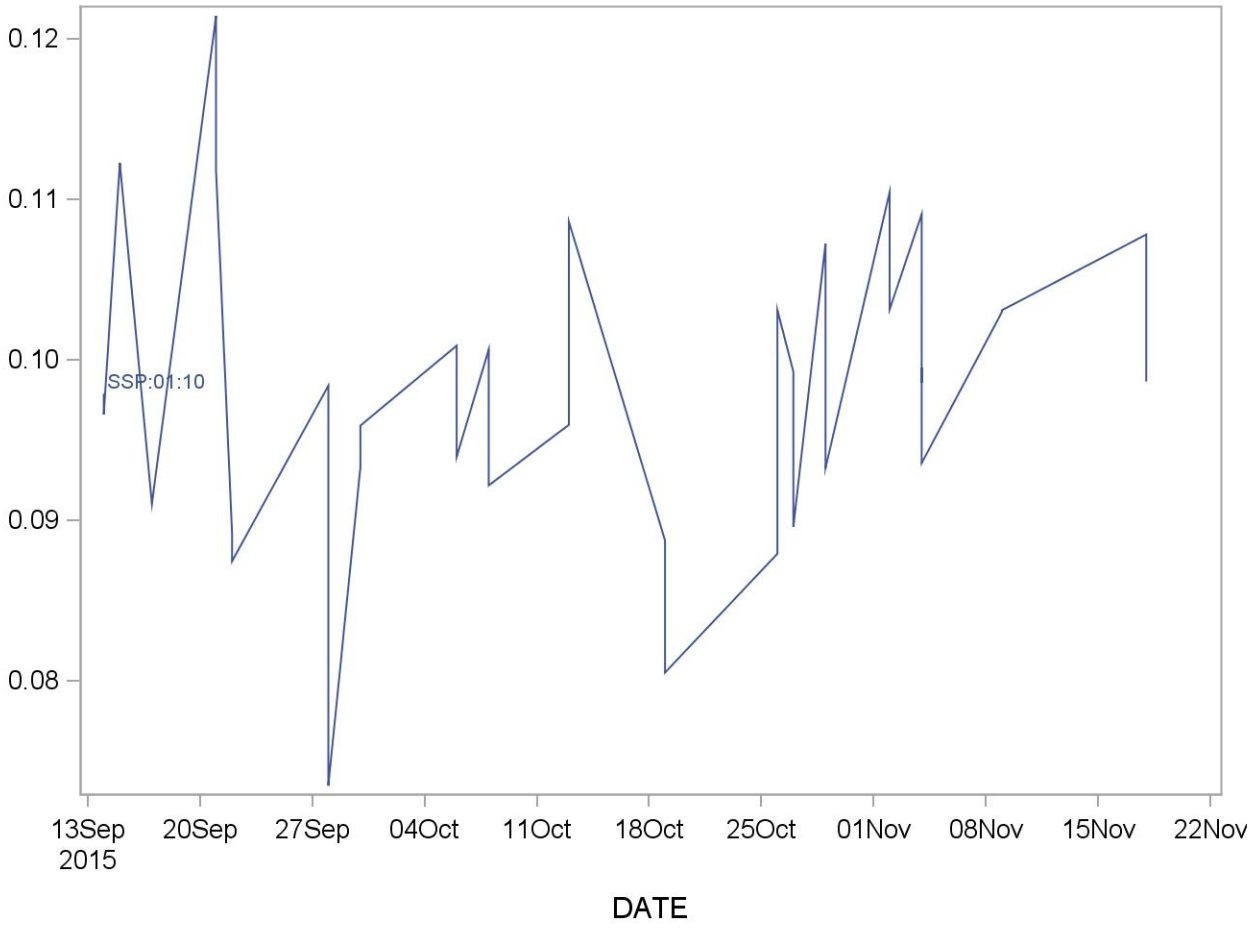
## 2009-2010 Summary Statistics and QC Chart for 2,3,4,7,8-Pentachlorofuran (pncdf)(fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 39 | 14SEP15 | 18NOV15 | 0.193504 | 0.015601 | 8.1 |



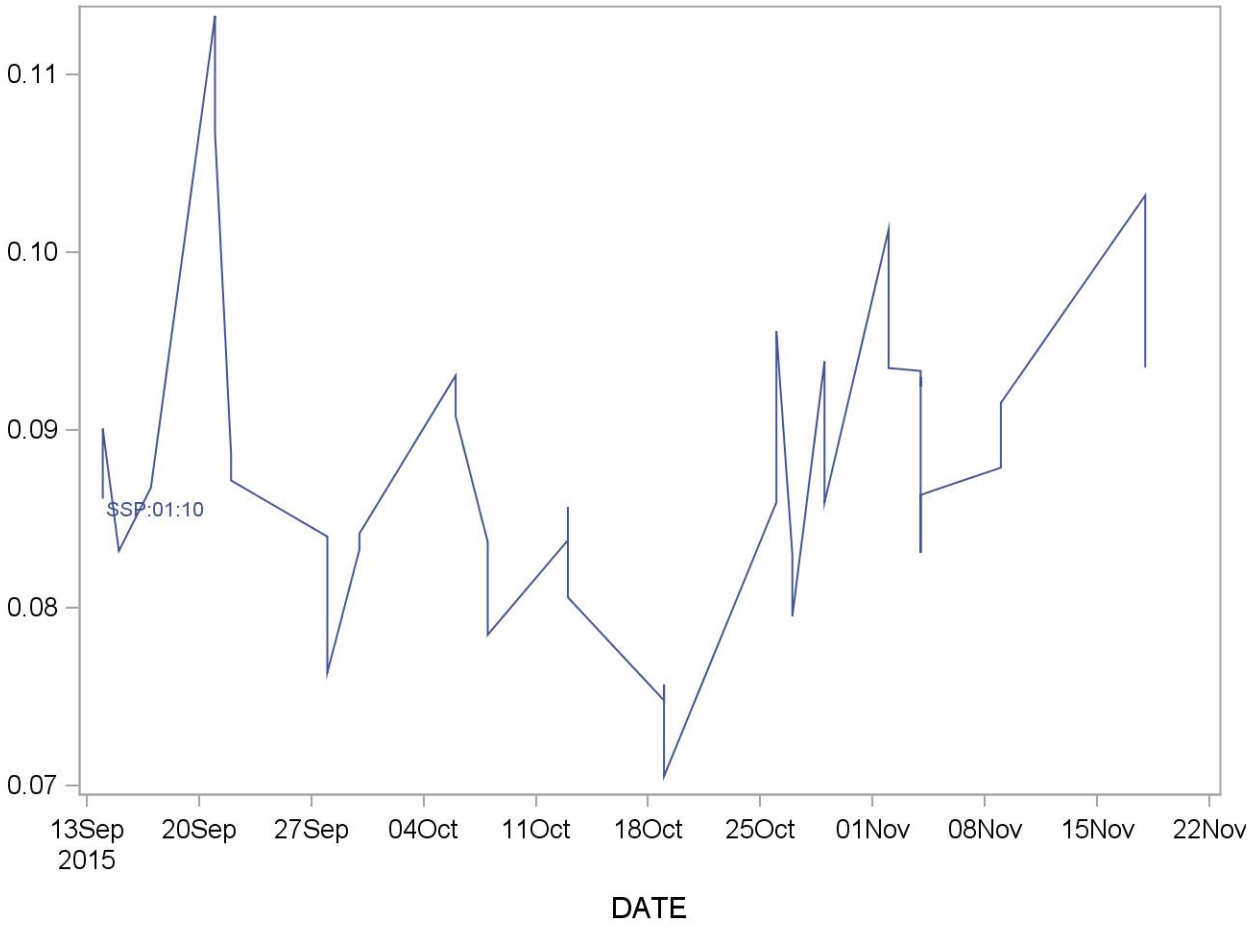
## 2009-2010 Summary Statistics and QC Chart for 2,3,7,8-Tetrachlorofuran (tcdf) (fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 37 | 14SEP15 | 18NOV15 | 0.098240 | 0.009386 | 9.6 |



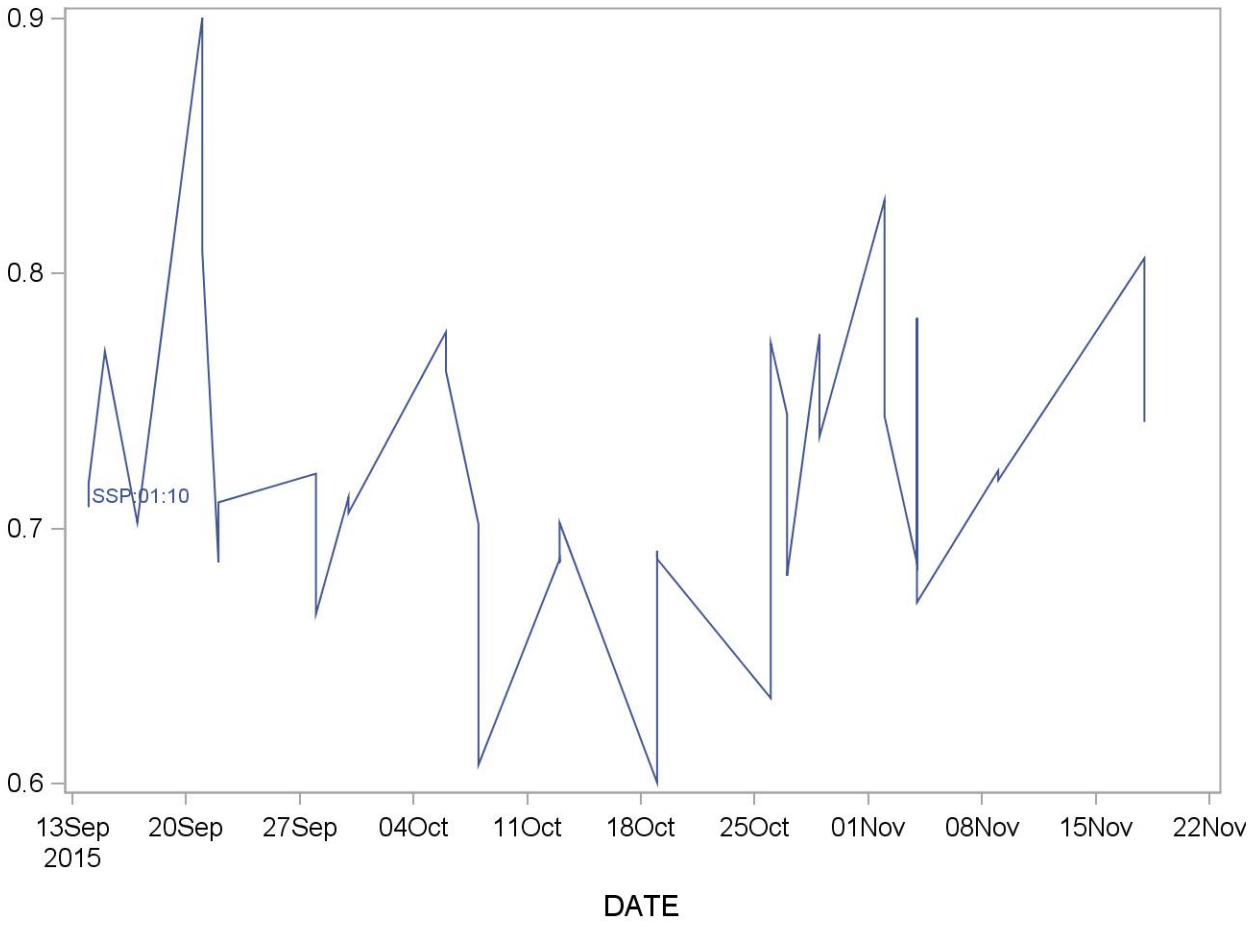
## 2009-2010 Summary Statistics and QC Chart for 2,3,7,8-Tetrachloro-p-dioxin(tcdd)(fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 39 | 14SEP15 | 18NOV15 | 0.087939 | 0.008643 | 9.8 |



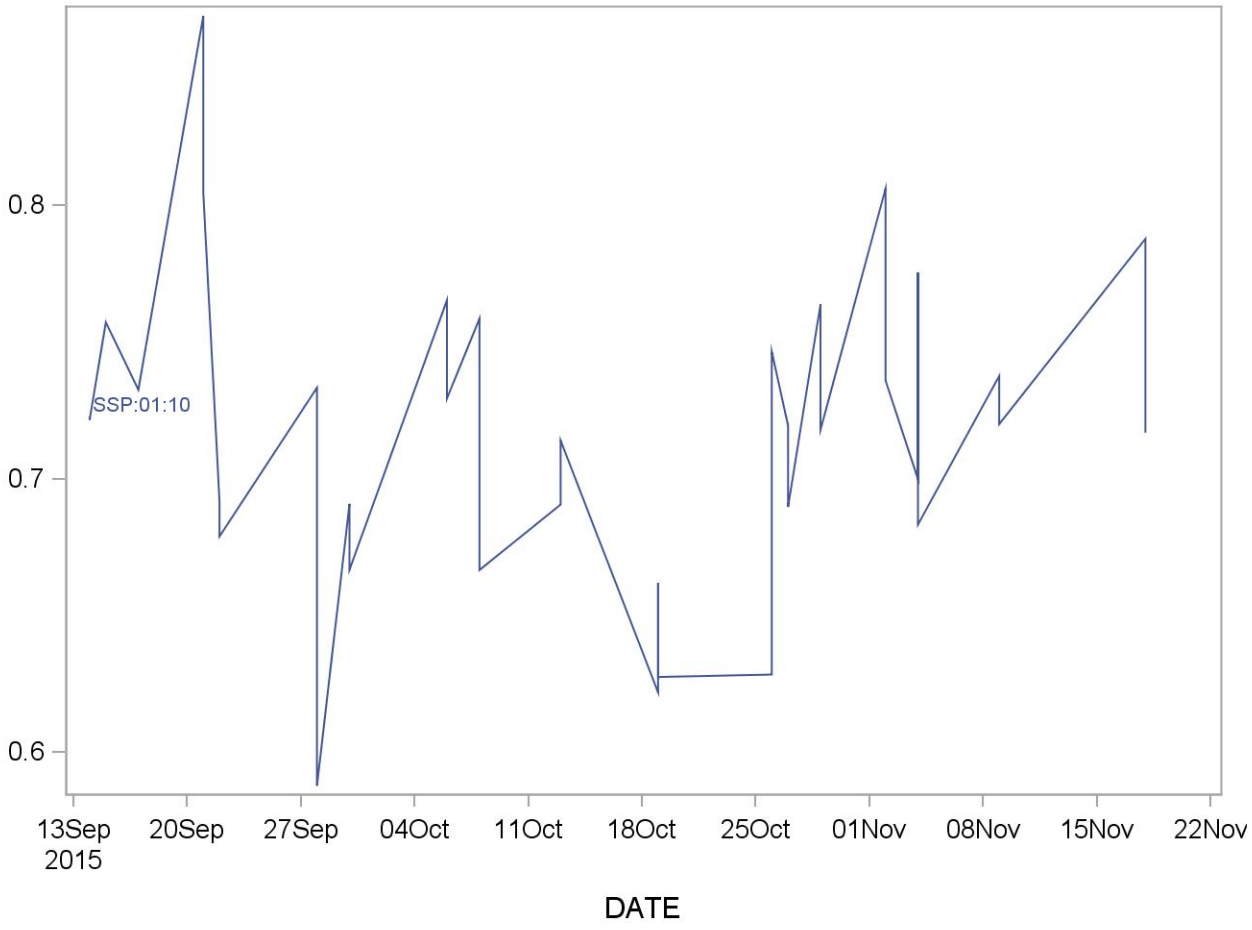
## 2009-2010 Summary Statistics and QC Chart for 3,3',4,4',5,5'-hexachlorobiphenyl (fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 39 | 14SEP15 | 18NOV15 | 0.722899 | 0.057906 | 8.0 |



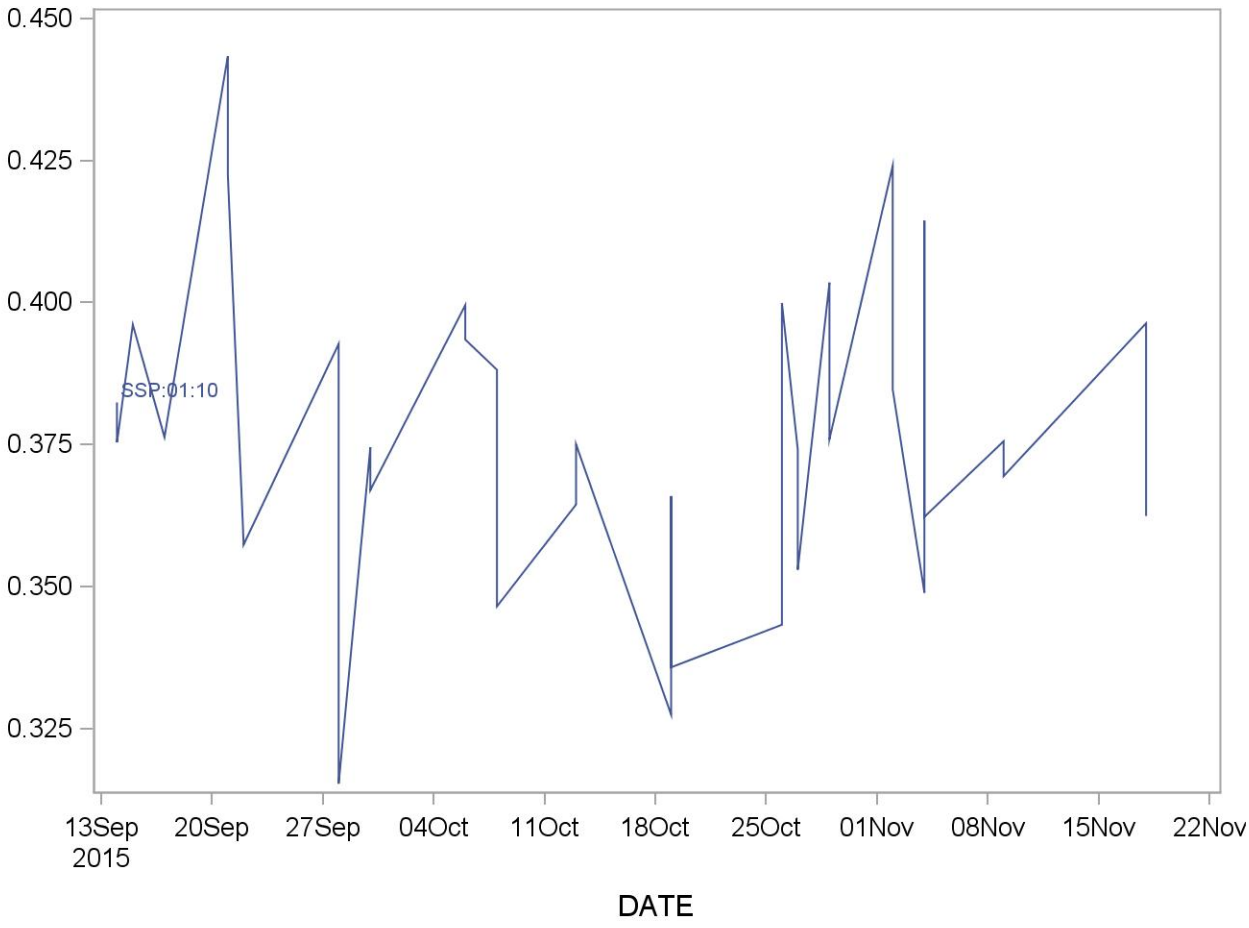
## 2009-2010 Summary Statistics and QC Chart for 3,3',4,4',5-Pentachlorobiphenyl (fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 39 | 14SEP15 | 18NOV15 | 0.717352 | 0.054601 | 7.6 |



## 2009-2010 Summary Statistics and QC Chart for 3,4,4',5-Tetrachlorobiphenyl (tcb)(fg/g)

| Lot | N | Start Date | End Date | Mean | Standard Deviation | Coefficient of Variation |
|---|---|---|---|---|---|---|
| SSP:01:10 | 39 | 14SEP15 | 18NOV15 | 0.376245 | 0.026384 | 7.0 |



**Appendix A.** Typical accurate masses, target isotopic ratios, $^{13}C$ label standard used, selected ion monitoring window (SIM) and lock and calibration masses used for high resolution isotope dilution measurements of polychlorinated (PCDD/F) and coplanar polychlorinated biphenyls (cPCBs). Also given are sample quality control (QC) criteria, i.e, relative retention time and recovery. Rare changes to these parameters may occur due to observed interferences, currently used and historical settings are stored at the network location Y:\LOOKUP_TABLES.

| Analyte | Accurate Masses | | | | Actual Label used | SIM[1] | Lock / Cali Mass | Sample QC criteria | | | | Calibration standard |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $^{12}C$-masses | | $^{13}C$-masses | | | | | $^{12}C$ Isotope | $^{13}C$ Isotope | RRT Limit[2] | Recovery Limits | range (low / high) |
| | Quan mass | Ratio Mass | Quan mass | Ratio Mass | | | | Ratio Limits | Ratio Limits | | (%) | (pg/uL)[3] |
| ***Polychlorinated dibenzo-p-dioxins (PCDDs)*** | | | | | | | | | | | | |
| 2378-TeCDD | 319.8965 | 321.8936 | 331.9368 | 333.9338 | 2378-TeCDD | 3 | 313.9839 / 351.9802 | 0.95 - 1.61 | 0.95 - 1.61 | +/- 0.004 | 10 - 150 | 0.001 - 10 |
| 12378-PeCDD | 353.8576 | 355.8546 | 365.8978 | 367.8949 | 12378-PeCDD | 4 | 313.9839 / 375.9807 | 1.18 - 2.02 | 1.18 - 2.02 | +/- 0.004 | 10 - 150 | 0.001 - 10 |
| 123478-HxCDD | 389.8156 | 391.8127 | 401.8559 | 403.8530 | 123478-HxCDD | 5 | 351.9802 / 413.977 | 0.59 - 1.01 | 0.59 - 1.01 | +/- 0.004 | 10 - 150 | 0.001 - 10 |
| 123678-HxCDD | 389.8156 | 391.8127 | 401.8559 | 403.8530 | 123678-HxCDD | 5 | 351.9802 / 413.977 | 0.59 - 1.01 | 0.59 - 1.01 | +/- 0.004 | 10 - 150 | 0.01 - 100 |
| 123789-HxCDD | 389.8156 | 391.8127 | 401.8559 | 403.8530 | 123789-HxCDD | 5 | 351.9802 / 413.977 | 0.59 - 1.01 | 0.59 - 1.01 | +/- 0.004 | 10 - 150 | 0.001 - 10 |
| 1234678-HpCDD | 423.7767 | 425.7737 | 435.8169 | 437.8140 | 1234678-HpCDD | 6 | 413.977 / 463.9743 | 0.71 - 1.21 | 0.71 - 1.21 | +/- 0.004 | 10 - 150 | 0.01 - 100 |
| 1234679-HpCDD | 423.7767 | 425.7737 | 435.8169 | 437.8140 | 1234678-HpCDD | 5 | 351.9802 / 413.977 | 0.71 - 1.21 | 0.71 - 1.21 | +/- 0.004 | 10 - 150 | 0.001 - 10 |
| OCDD | 457.7377 | 459.7348 | 469.7780 | 471.7750 | OCDD | 7 | 413.977 / 463.9743 | 0.83 - 1.41 | 0.83 - 1.41 | +/- 0.004 | 10 - 150 | 0.1 - 1000 |
| ***Polychlorinated dibenzo-p-furans (PCDDs)*** | | | | | | | | | | | | |
| 2378-TeCDF | 303.9016 | 305.8987 | 315.9419 | 317.9389 | 2378-TeCDF | 3 | 313.9839 - 351.9802 | 0.95 - 1.61 | 0.95 - 1.61 | +/- 0.004 | 10 - 150 | 0.001 - 10 |
| 12378-PeCDF | 337.8627 | 339.8597 | 349.9029 | 351.9000 | 12378-PeCDF | 4 | 313.9839 - 375.9807 | 1.18 - 2.02 | 1.18 - 2.02 | +/- 0.004 | 10 - 150 | 0.001 - 10 |
| 23478-PeCDF | 337.8627 | 339.8597 | 349.9029 | 351.9000 | 23478-PeCDF | 4 | 313.9839 - 375.9807 | 1.18 - 2.02 | 1.18 - 2.02 | +/- 0.004 | 10 - 150 | 0.001 - 10 |
| 123478-HxCDF | 373.8207 | 375.8178 | 385.8610 | 387.8580 | 123478-HxCDF | 5 | 351.9802 - 413.977 | 0.59 - 1.01 | 0.59 - 1.01 | +/- 0.004 | 10 - 150 | 0.001 - 10 |
| 123678-HxCDF | 373.8207 | 375.8178 | 385.8610 | 387.8580 | 123678-HxCDF | 5 | 351.9802 - 413.977 | 0.59 - 1.01 | 0.59 - 1.01 | +/- 0.004 | 10 - 150 | 0.001 - 10 |
| 123789-HxCDF | 373.8207 | 375.8178 | 385.8610 | 387.8580 | 123789-HxCDF | 5 | 351.9802 - 413.977 | 0.59 - 1.01 | 0.59 - 1.01 | +/- 0.004 | 10 - 150 | 0.001 - 10 |
| 234678-HxCDF | 373.8207 | 375.8178 | 385.8610 | 387.8580 | 234678-HxCDF | 5 | 351.9802 - 413.977 | 0.59 - 1.01 | 0.59 - 1.01 | +/- 0.004 | 10 - 150 | 0.001 - 10 |
| 1234678-HpCDF | 407.7818 | 409.7788 | 419.8220 | 421.8191 | 1234678-HpCDF | 6 | 413.977 - 463.9743 | 0.71 - 1.21 | 0.71 - 1.21 | +/- 0.004 | 10 - 150 | 0.01 - 100 |
| 1234789-HpCDF | 407.7818 | 409.7788 | 419.8220 | 421.8191 | 1234678-HpCDF | 6 | 413.977 - 463.9743 | 0.71 - 1.21 | 0.71 - 1.21 | +/- 0.004 | 10 - 150 | 0.001 - 10 |
| OCDF | 441.7428 | 443.7398 | 453.7830 | 455.7801 | OCDF | 7 | 413.977 - 463.9743 | 0.83 - 1.41 | 0.83 - 1.41 | +/- 0.004 | 10 - 150 | 0.001 - 10 |
| ***coplanar PCBs (cPCBs)*** | | | | | | | | | | | | |
| PCB77 | 289.9224 | 291.9194 | 301.9626 | 303.9597 | PCB77 | 3 | 313.9839 - 351.9802 | 0.95 - 1.61 | 0.95 - 1.61 | +/- 0.004 | 10 - 150 | 0.01 - 100 |
| PCB81 | 289.9224 | 291.9194 | 301.9626 | 303.9597 | PCB81 | 3 | 313.9839 - 351.9802 | 0.95 - 1.61 | 0.95 - 1.61 | +/- 0.004 | 10 - 150 | 0.01 - 100 |
| PCB126 | 325.8804 | 327.8775 | 337.9207 | 339.9177 | PCB126 | 3 | 313.9839 - 351.9802 | 0.47 - 0.81 | 0.47 - 0.81 | +/- 0.004 | 10 - 150 | 0.01 - 100 |
| PCB169 | 359.8415 | 361.8385 | 371.8817 | 373.8788 | PCB169 | 4 | 313.9839 - 375.9807 | 0.59 - 1.01 | 0.59 - 1.01 | +/- 0.004 | 10 - 150 | 0.01 - 100 |

[1] Selected Ion Monitoring Window; [2] Relative retention time deviation limit. Calculated against $^{13}C$-labled standard; [3] Standard part number EDF-5524 obtained from Cambride Isotope Laboratories (www.isotope.com)