Laboratory Procedure Manual

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

Matrix: **Serum / Plasma**

Method: Liquid-Liquid Extraction (LLE), Purification by Acid-silica and Carbon, Analysis by High Resolution Gas Chromatography-High Resolution Mass Spectrometry (HRGC-HRMS)

Method No: **6501.08**

Revised: **January 10, 2023**

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:

Data File name	Variable Name	SAS Label
DOXPOL_I	SAMPLEID	Pool identification number
	RIAGENDR	Gender
	RIDRETH3	Ethnicity - Recode
	RIDAGGRP	Age at Screening Adjudicated - Recode
	RIANSMP	Number of samples in a pool
	WTSMSMPA	Sum of adjusted subsample weights
	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-hcxdf(fg/g)
	LBCF05	1,2,3,6,7,8-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)
	LBCHXC	3,3',4,4',5,5'-hexachlorobiphenyl(fg/g)
	LBCPCB	3,3',4,4',5-Pentachlorobiphenyl (fg/g)
	LBCTC2	3,4,4',5-Tetrachlorobiphenyl (tcb)(fg/g)
	LBCTCD	2,3,7,8-Tetrachloro-p-dioxn(tcdd)(fg/g)
	LBCD01LA	1,2,3,7,8-pncdd lipid adjusted (pg/g)
	LBCD02LA	1,2,3,4,7,8-hxcdd lipid adjusted (pg/g)
	LBCD03LA	1,2,3,6,7,8-hxcdd lipid adjusted (pg/g)
	LBCD04LA	1,2,3,7,8,9-hxcdd lipid adjusted (pg/g)
	LBCD05LA	1,2,3,4,6,7,8-hpcdd lipid adjusted(pg/g)
	LBCD01LA	1,2,3,7,8-pncdd lipid adjusted (pg/g)
	LBCD07LA	1,2,3,4,6,7,8,9-ocdd Lipid Adjust (pg/g)
	LBCF01LA	2,3,7,8-tcdf lipid adjusted (pg/g)
	LBCF02LA	1,2,3,7,8-pncdf lipid adjusted (pg/g)
	LBCF03LA	2,3,4,7,8-pncdf lipid adjust (pg/g)

DOXPOL_I	LBCF04LA	1,2,3,4,7,8-hcxdf lipid adjusted (pg/g)
	LBCF05LA	1,2,3,6,7,8-hxcdf lipid adjusted (pg/g)
	LBCF06LA	1,2,3,7,8,9-hxcdf lipid adjusted (pg/g)
	LBCF07LA	2,3,4,6,7,8-hxcdf lipid adjusted (pg/g)
	LBCF08LA	1,2,3,4,6,7,8-hpcdf lipid adjust (pg/g)
	LBCF09LA	1,2,3,4,7,8,9-hpcdf lipid adjust (pg/g)
	LBCF10LA	1,2,3,4,6,7,8,9-ocdf lipid adjust(pg/g)
	LBCHXCLA	3,3',4,4',5,5'-hxcb Lipid Adjust(pg/g)
	LBCPCBLA	3,3',4,4',5-pncb lipid adjusted (pg/g)
	LBCTC2LA	3,4,4',5-tcb lipid adjusted (pg/g)
	LBCTCDLA	2,3,7,8-tcdd lipid adjusted (pg/g)
	LBDD01LC	1,2,3,7,8-pncdd comment code
	LBDD02LC	1,2,3,4,7,8-hxcdd comment code
	LBDD03LC	1,2,3,6,7,8-hxcdd comment code
	LBDD04LC	1,2,3,7,8,9-hxcdd comment code
	LBDD05LC	1,2,3,4,6,7,8-hpcdd comment code
	LBDD07LC	1,2,3,4,6,7,8,9-ocdd comment code
	LBDF01LC	2,3,7,8-tcdf comment code
	LBDF02LC	1,2,3,7,8-pncdf comment code
	LBDF03LC	2,3,4,7,8-pncdf comment code
	LBDF04LC	1,2,3,4,7,8-hcxdf comment code
	LBDF05LC	1,2,3,6,7,8-hxcdf comment code
	LBDF06LC	1,2,3,7,8,9-hxcdf comment code
	LBDF07LC	2,3,4,6,7,8-hxcdf comment code
	LBDF08LC	1,2,3,4,6,7,8-hpcdf comment code
	LBDF09LC	1,2,3,4,7,8,9-hpcdf comment code
	LBDF10LC	1,2,3,4,6,7,8,9-ocdf comment code
	LBDHXCLC	3,3',4,4',5,5'-hxcb comment code
	LBDPCBLC	3,3',4,4',5-pncb comment code
	LBDTC2LC	3,4,4',5-tcb comment code
	LBDTCDLC	2,3,7,8-tcdd comment code

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 high resolution gas chromatography-high resolution mass spectrometry (HRGC/HRMS):

- 7 Polychlorinated dibenzo-*p*-dioxins (PCDDs) [n=7]
- Polychlorinated dibenzofurans (PCDFs) [n=10]
- Co-planar polychlorinated biphenyls (cPCBs) [n=4]

Serum specimens are fortified with carbon-13 labeled (^{13}C) internal standards. The analytes of interest are extracted in hexane using an automated liquid-liquid extraction method 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 auto sampler vials. The samples are there after fortified with recovery standard and evaporated to 5uL using a TurboVap LV (Biotage) concentration workstation. PCDD/F and cPCB are injected using a TriPlus auto sampler (Thermo Scientific), into a TRACE 1300 gas chromatograph (Thermo Scientific) equipped with a Pressure Temperature Vaporization (PTV) programmable injector. A DB5-MS UI capillary column (60m x 0.25 mm x 0.25 µm film thickness) or equivalent are used for chromatographic separation before entering a Thermo Scientific DFS high resolution magnetic sector mass spectrometer operated in Electron Ionization mode at 45 eV, using selected ion monitoring (SIM) at 10,000 resolving power (5% peak height).

Two ions corresponding to two masses are monitored for each native ($^{13}C_{12}$) compound and its corresponding $^{13}C_{12}$-internal standard. The instrumental response 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 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 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 control limits.

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. Wear 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 Chemical Hygiene and Exposure control Plans. Wear gloves, lab coat and glasses at all times, and conduct all work in fume hood or biological safety cabinets (BSCs).

Place disposable plastic, glass, and paper (e.g., pipette tips, autosampler tubes, and gloves) that come in contact with serum in the appropriate waste container, as described in the Chemical Hygiene Plan. Keep autoclaveable 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 equivalent.

After an accident, the CDC/ATSDR Incident Report must be filed according to Chemical Hygiene and Exposure control Plans.

2.2 Chemical hazards

Consult the laboratory’s Chemical Hygiene Plan and Exposure Control Plan for specific information regarding the chemicals used in the laboratory.

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 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.

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

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.

2.3.3 Solid wastes

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

- **Non-Biogenic Contaminated Reusable Glassware** (e.g. beakers, cylinders and other reusable glassware). If appropriate, these glassware may be cleaned / re-cleaned.

- **Broken glass** includes used glassware contaminated with biogenic materials, or serum bottles and vials that are not reused. When this container is filled: (*i*) place CDC Autoclave label on the container, (*ii*) place autoclave tape over lid and down the side of the box, and (*iii*) bring the container to autoclave located in the loading dock of Building 103.

  Used Pasteur pipettes are placed in a separate container, as described in the Chemical Hygiene Plan. When filled, (*i*) place CDC Autoclave label on the container, (*ii*) place autoclave tape over lid and down the side of the box, and (*iii*) bring the container to autoclave located in the loading dock of Building 103.

- **Gloves and other plastic parts contaminated with biogenic material** - Place biohazard bag in metal container before placing any waste in container. When filled, (*i*) place CDC Autoclave label on the container, (*ii*) place autoclave tape over lid and down the side of the box, and (*iii*) bring the container to the DLS designated handling area.

3. Computerization; Data System Management

3.1 Data Entry and Transfer

Sample analysis results generated by this method are stored in SAS and/or Microsoft Excel™ software. The analytical results should include at least the analysis date; analytical run number, quality-control (QC) results for the run, results of specimen analysis by specimen identification (ID), and method identifier.

3.2 Routine Computer Hard-Drive Maintenance

Defragment the computer hard drive regularly by using software such as Norton Utilities™ to maximize computer performance and maintain data integrity for files on the hard drive.

3.3 Data Backup and Schedule of Back-ups

Instrument raw data files are mirrored through a local network connection with each HRMS instrument computer to a local share drive Network Path: \\192.168.210.3\volume_1) which is mirrored to a network share drive (Network Path: DLSNAS-E78DD7). Between the 10th and the 14th of each month all generated instrument raw data is copied into the folder X:\LONG_TERM_BACKUP_001 on the network share for compression into a monthly compressed ZIP-file. The creation of the ZIP-file is an automatic process that runs at mid-night on the 15th of each month. The created ZIP-file is called POPLab_YYYY-MM-DD where YYYY-MM-DD is a date time stamp. After completion of the monthly backup all instrument operators will be informed over email that the backup has been completed and any raw-files from the preceding month should be transferred to a local archive folder on the instrument computer. After completion of the monthly backup the compressed ZIP-file will be made available on the lab share in the folder Z:__Shared_Folders_01_BACKUP_GOING_BACK_3_MONTHS which is a synchronized folder between the lab and network share drives. The monthly backup ZIP-file will be made available on the Lab Share for at a minimum 3 months after which older backups are accessible on the Network Share drive in the folder X:\LONG_TERM_BACKUP_001\ZipFiles.

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 for analysis: 20 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 PTFE-lined caps. The preferred container is either a 2oz or 4oz glass Qorpak bottle. Although, other containers may be acceptable, they will be evaluated on a case by case basis and a background test of the materials may be conducted. Clean the containers using the same procedure as for other glassware and closers used in the current method.
- 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 and in accordance with safety protocols outlined in the CDC Chemical Hygiene Plan and the CDC Intrafacility Specimen and Sample Transfer Policy. Upon receipt, they must be kept frozen at ≤ -60 ^{o}C until time for analysis. Refreeze at ≤ -60 °C any portions of the sample that remain after analytical aliquots are withdrawn.

Table 1. Method limit of detection (LOD) and Taylor LOD [a] and blank LOD (defined as three times the standard deviation of blank samples analyzed in paralell with study samples). LOD calculation based on calibration curves and method blanks measued between February, 2021 and August, 2022. The method LOD is expressed categorized based on the available sample size. The method LOD corresponding to the minimum prefered sample amount of 10 grams are collored in blue, method LODs between the minimum prefered sample amount and the minimum acceptable sample size are collored in red. Method LODs two and three fold higher than the minimum prefered sample amount are collored in green. A sample amount greather than the minimum prefered sample amount may be used to lower the method LOD. Any sample for which the available serum amount for measurement is less then the minimum acceptable serum amount of 2.5grams will be reported as QNS (Quantify Not Sufficent) in reportable data tabels. The concentration of the lowest available calibration standard is given as pg/uL and pg/5uL where the concentration expressed as per five microliters is directly comparable to the Taylor LOD and blank LOD.

Class	Analyte	Serum Weight (g)	Taylor LOD (pg)		Blank LOD (pg)		Lowest standard		Method LOD [b]
			N	LOD	N	LOD	pg/uL	pg/5uL	(pg/g serum)
PCDD/F	2378-TeCDD	2.5	65	0.012	45	0.041	0.003	0.015	0.016
		5							0.008
		7.5							0.005
		10							0.004
		20							0.002
		30							0.001
PCDD/F	12378-PeCDD	2.5	65	0.026	46	0.099	0.003	0.015	0.040
		5							0.020
		7.5							0.013
		10							0.010
		20							0.005
		30							0.003
PCDD/F	123478-HxCDD	2.5	65	0.013	47	0.055	0.003	0.015	0.022
		5							0.011
		7.5							0.007
		10							0.005
		20							0.003
		30							0.002
PCDD/F	123678-HxCDD	2.5	65	0.025	46	0.10	0.03	0.15	0.041
		5							0.020
		7.5							0.014
		10							0.010
		20							0.005
		30							0.003

[a] Taylor, K. T. (1987) In Quality Assurance of Chemical Measurements, pp 79-82, Lewis Publishers, Washington, DC
[b] Method LOD defined as the higher value of Taylor LOD and blank LOD.

Table 1 (continued). Method limit of detection (LOD) and Taylor LOD [a] and blank LOD (defined as three times the standard deviation of blank samples analyzed in paralell with study samples). LOD calculation based on calibration curves and method blanks measued between February, 2021 and August, 2022. The method LOD is expressed categorized based on the available sample size. The method LOD corresponding to the minimum prefered sample amount of 10 grams are collored in blue, method LODs between the minimum prefered sample amount and the minimum acceptable sample size are collored in red. Method LODs two and three fold higher than the minimum prefered sample amount are collored in green. A sample amount greather than the minimum prefered sample amount may be used to lower the method LOD. Any sample for which the available serum amount for measurement is less then the minimum acceptable serum amount of 2.5grams will be reported as QNS (Quantify Not Sufficent) in reportable data tabels. The concentration of the lowest available calibration standard is given as pg/uL and pg/5uL where the concentration expressed as per five microliters is directly comparable to the Taylor LOD and blank LOD.

Class	Analyte	Serum	Taylor LOD (pg)		Blank LOD (pg)		Lowest standard		Method LOD [b]
		Weight (g)	N	LOD	N	LOD	pg/uL	pg/5uL	(pg/g serum)
PCDD/F	123789-HxCDD	2.5	65	0.019	47	0.073	0.003	0.015	0.029
		5							0.015
		7.5							0.010
		10							0.007
		20							0.004
		30							0.002
PCDD/F	1234678-HpCDD	2.5	65	0.024	44	0.26	0.03	0.15	0.104
		5							0.052
		7.5							0.035
		10							0.026
		20							0.013
		30							0.009
PCDD/F	OcCDD	2.5	65	0.23	43	0.55	0.3	1.5	0.221
		5							0.110
		7.5							0.074
		10							0.055
		20							0.028
		30							0.018
PCDD/F	2378-TeCDF	2.5	65	0.018	47	0.038	0.003	0.015	0.015
		5							0.008
		7.5							0.005
		10							0.004
		20							0.002
		30							0.001

[a] Taylor, K. T. (1987) In Quality Assurance of Chemical Measurements, pp 79-82, Lewis Publishers, Washington, DC

[b] Method LOD defined as the higher value of Taylor LOD and blank LOD.

Table 1 (continued). Method limit of detection (LOD) and Taylor LOD [a] and blank LOD (defined as three times the standard deviation of blank samples analyzed in paralell with study samples). LOD calculation based on calibration curves and method blanks measued between February, 2021 and August, 2022. The method LOD is expressed categorized based on the available sample size. The method LOD corresponding to the minimum prefered sample amount of 10 grams are collored in blue, method LODs between the minimum prefered sample amount and the minimum acceptable sample size are collored in red. Method LODs two and three fold higher than the minimum prefered sample amount are collored in green. A sample amount greather than the minimum prefered sample amount may be used to lower the method LOD. Any sample for which the available serum amount for measurement is less then the minimum acceptable serum amount of 2.5grams will be reported as QNS (Quantify Not Sufficent) in reportable data tabels. The concentration of the lowest available calibration standard is given as pg/uL and pg/5uL where the concentration expressed as per five microliters is directly comparable to the Taylor LOD and blank LOD.

Class	Analyte	Serum	Taylor LOD (pg)		Blank LOD (pg)		Lowest standard		Method LOD [b]
		Weight (g)	N	LOD	N	LOD	pg/uL	pg/5uL	(pg/g serum)
PCDD/F	12378-PeCDF	2.5	65	0.011	46	0.030	0.003	0.015	0.012
		5							0.006
		7.5							0.004
		10							0.003
		20							0.001
		30							0.001
PCDD/F	23478-PeCDF	2.5	65	0.031	47	0.041	0.003	0.015	0.016
		5							0.008
		7.5							0.005
		10							0.004
		20							0.002
		30							0.001
PCDD/F	123478-HxCDF	2.5	65	0.003	46	0.057	0.003	0.015	0.023
		5							0.011
		7.5							0.008
		10							0.006
		20							0.003
		30							0.002
PCDD/F	123678-HxCDF	2.5	65	0.014	47	0.071	0.003	0.015	0.029
		5							0.014
		7.5							0.010
		10							0.007
		20							0.004
		30							0.002

[a] Taylor, K. T. (1987) In Quality Assurance of Chemical Measurements, pp 79-82, Lewis Publishers, Washington, DC
[b] Method LOD defined as the higher value of Taylor LOD and blank LOD.

Table 1 (continued). Method limit of detection (LOD) and Taylor LOD [a] and blank LOD (defined as three times the standard deviation of blank samples analyzed in paralell with study samples). LOD calculation based on calibration curves and method blanks measued between February, 2021 and August, 2022. The method LOD is expressed categorized based on the available sample size. The method LOD corresponding to the minimum prefered sample amount of 10 grams are collored in blue, method LODs between the minimum prefered sample amount and the minimum acceptable sample size are collored in red. Method LODs two and three fold higher than the minimum prefered sample amount are collored in green. A sample amount greather than the minimum prefered sample amount may be used to lower the method LOD. Any sample for which the available serum amount for measurement is less then the minimum acceptable serum amount of 2.5grams will be reported as QNS (Quantify Not Sufficent) in reportable data tabels. The concentration of the lowest available calibration standard is given as pg/uL and pg/5uL where the concentration expressed as per five microliters is directly comparable to the Taylor LOD and blank LOD.

Class	Analyte	Serum	Taylor LOD (pg)		Blank LOD (pg)		Lowest standard		Method LOD [b]
		Weight (g)	N	LOD	N	LOD	pg/uL	pg/5uL	(pg/g serum)
PCDD/F	123789-HxCDF	2.5	65	0.013	46	0.049	0.003	0.015	0.019
		5							0.010
		7.5							0.006
		10							0.005
		20							0.002
		30							0.002
PCDD/F	234678-HxCDF	2.5	65	0.02	46	0.44	0.003	0.015	0.175
		5							0.088
		7.5							0.058
		10							0.044
		20							0.022
		30							0.015
PCDD/F	1234678-HpCDF	2.5	65	0.032	46	0.10	0.03	0.15	0.041
		5							0.020
		7.5							0.014
		10							0.010
		20							0.005
		30							0.003
PCDD/F	1234789-HpCDF	2.5	65	0.020	46	0.021	0.003	0.015	0.009
		5							0.004
		7.5							0.003
		10							0.002
		20							0.001
		30							0.001

[a] Taylor, K. T. (1987) In Quality Assurance of Chemical Measurements, pp 79-82, Lewis Publishers, Washington, DC

[b] Method LOD defined as the higher value of Taylor LOD and blank LOD.

Table 1 (continued). Method limit of detection (LOD) and Taylor LOD [a] and blank LOD (defined as three times the standard deviation of blank samples analyzed in paralell with study samples). LOD calculation based on calibration curves and method blanks measued between February, 2021 and August, 2022. The method LOD is expressed categorized based on the available sample size. The method LOD corresponding to the minimum prefered sample amount of 10 grams are collored in blue, method LODs between the minimum prefered sample amount and the minimum acceptable sample size are collored in red. Method LODs two and three fold higher than the minimum prefered sample amount are collored in green. A sample amount greather than the minimum prefered sample amount may be used to lower the method LOD. Any sample for which the available serum amount for measurement is less then the minimum acceptable serum amount of 2.5grams will be reported as QNS (Quantify Not Sufficent) in reportable data tabels. The concentration of the lowest available calibration standard is given as pg/uL and pg/5uL where the concentration expressed as per five microliters is directly comparable to the Taylor LOD and blank LOD.

Class	Analyte	Serum	Taylor LOD (pg)		Blank LOD (pg)		Lowest standard		Method LOD [b]
		Weight (g)	N	LOD	N	LOD	pg/uL	pg/5uL	(pg/g serum)
PCDD/F	OcCDF	2.5	65	0.028	47	0.063	0.003	0.015	0.025
		5							0.013
		7.5							0.008
		10							0.006
		20							0.003
		30							0.002
cPCB	PCB81	2.5	65	0.015	47	0.10	0.03	0.15	0.042
		5							0.021
		7.5							0.014
		10							0.010
		20							0.005
		30							0.003
cPCB	PCB126	2.5	65	0.033	45	0.11	0.03	0.15	0.043
		5							0.021
		7.5							0.014
		10							0.011
		20							0.005
		30							0.004
cPCB	PCB169	2.5	65	0.028	45	0.095	0.03	0.15	0.038
		5							0.019
		7.5							0.013
		10							0.010
		20							0.005
		30							0.003

[a] Taylor, K. T. (1987) In Quality Assurance of Chemical Measurements, pp 79-82, Lewis Publishers, Washington, DC

[b] Method LOD defined as the higher value of Taylor LOD and blank LOD.

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.

Chemical/Solvent	Manufacturer	Grade/Purity
Acids		
Sulfuric Acid	Sigma-Aldrich	99.999%
Hydrochloric Acid	Sigma-Aldrich	37% (w/w) in H_2O
Solvents		
Dichloromethane	Fisher Scientific	Optima Grade or better
Hexane	Fisher Scientific	Optima Grade or better
Methanol	LabSolv / TEDIA	GC Grade or better
n-Nonane	Sigma-Aldrich	In-house Distilled
Water	LabSolv / TEDIA	HPLC Grade or better
Toluene	Fisher Scientific	Optima Grade or better
Diethyl Ether	Fisher Scientific	Pesticide Grade or better
Ethanol	Fisher Scientific	Molecular Biology Grade or better
SPE Sorbents		
Neutral Silica Gel	UCT Chemicals	100-200 Mesh, Heat Treated

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

Item	Manufacturer/Source
Glassware and caps	
20 x 150mm Test tubes	Fisher Scientific
PTFE-lined cap for 20x150 test tube	Fisher Scientific
2oz (60mL) Round bottle	Qorpak
PTFE-Lined open-top cap for 2oz Bottle	Lab Depot
Borosilicate Glass Pasteur pipette	Fisher Scientific/VWR
V-vial (3 or 5 mL) with septum-cap	Fisher Scientific/Wheaton
GC vials and caps	Waters
TurboVap Evaporation Vials	Biotage
Others	
Label printer (Brady TLS PC-Link)	Fisher Scientific
Plastic pipette tips	Rainin/ Metler Toledo

6.2 Rinsing of Expendables Prior to Use

Clean all glassware including new glassware and materials to eliminate risk of sample contamination. In brief, a general description is given below:

6.2.1 Culture tubes and other glassware

First, rinse glassware in a dishwasher (Labconco, Steam Scrubber or equivalent dish washer). Place test tubes in racks and insert them in the dishwasher. Place detergent in reservoir in the door, and start the dishwasher using a program **appropriate for glassware.** After completion of the program, transfer the glassware to an oven. After a heat cycle of at least 12 hours at 250 ^{o}C, the glassware is ready to be used.

For satellite bottles such as glass tapered-stopper bottles intended for storing small volumes for everyday use, adhesive labels are not to be used, because the glue from such labels may interfere with the proper procedure for re-cleaning them. Instead, label by hand using a “Sharpie” pen and attach a sheet of paper on the fume hood/BSC - where relevant chemicals are listed by name with any appropriate hazard pictograms.

6.2.2 Caps and septa

Rinse caps and septa for test tubes prior to use to remove contaminants. This is done by Soxhlet extraction for at least 24 hours using methanol as the extraction solvent. Alternatively, if the Soxhlet 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. After the materials are completely dry, place them in an appropriate, clean glass container 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 250 °C for at least 12 hours prior to use. Store vials in an appropriate, clean glass container or in plastic re-sealable bags for safe storage until used. The caps for GC vials are cleaned by Soxhlet extraction, using the same procedure as for other caps and septa described above.

6.2.4 Pasteur Pipets

Place glass Pasteur pipets in an oven on aluminum foil and heat the oven to 250 °C for at least 12 hours. After completing the heating cycle, the pipets are ready to be used.

6.2.5 Plastic Pipette Tips

Pipette tips are cleaned by Soxhlet extraction using the same procedure as for caps and septa described above.

6.3 Quantitative standards

6.3.1 Internal standards (IS)

The current method is validated for PCDD/Fs and cPCBs. Purchase these standards from Cambridge Isotope Laboratories, Inc. (CIL).

When opening a new ampoule transfer the standard to clean Wheaton 3-mL V-vials. Label the vial appropriately using a computer-generated label. Record 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. One vial of each standard is consumed in each analytical run on the automated liquid handler.

6.3.2 Recovery standard (RS)

The recovery standard is used for reconstitution of the extract prior to GC-ID/HRMS measurement and was also purchased from Cambridge Isotope Laboratories, Inc. Nonane and dodecane are present in the standard to act as a “keeper” (a solvent that will not evaporate or evaporate to a lesser degree during evaporation steps) to reduce evaporation losses during the final evaporation step

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. In addition, a mass is monitored with an offset to the accurate mass of the recovery standard equivalent to a theoretical peak that can be resolved at 10,000 resolution. Confirming that the area ratio of this offset mass to the area of the recovery standard is less than 0.05 allows researchers to show that the mass spectrometer remained at 10,000 mass-resolving power during the analysis of each sample.

When opening a new ampoule the standard is transferred to a clean Wheaton 3-mL V-vial and the vial is labeled using a computer-generated label. Record the weight of the vial and the date the ampoule was opened on the label. The weight is used to detect any potential evaporation of the standard during storage. One vial of each standard is consumed in each analytical run on the automated liquid handler.

6.3.3 Calibration Curve Standard (CS)

Calibration standards are purchased from Cambridge Isotope Laboratories, Inc. The calibration standards includes several individual calibration levels denoted CSX, where X=1 through 10.

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 Fluid Management Systems (FMS) Power-Prep NG

The Power-Prep (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 6.4.1. Each FMS system consists of a 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.

Figure 6.4.1. Fluid Management System (FMS) Power-Prep NG.

6.4.2 Gilson Liquid handler Robots

Liquid handling is automated using Gilson liquid handler robots, cf. Figure 6.4.2.2. A Gilson 215 Liquid Handler is used for Internal and Recovery standard fortification. Place the samples in the rack to the far right as shown in in Figure 6.4.2.1. The probe (moving arm) picks up and dispenses reagents (internal standards, methanol, water, etc.) to the samples according to a predefined sequence.

Recovery of the internal standards, as a percentage, is an important quality measurement of the analytical run. In order to enable recovery measurements, in this automated procedure, recovery standard will be added to empty GC vials located in a rack at the far left in Figure 6.4.2.1. These GC vials will be stored capped until the last step of the sample preparation method in which the purified extract will be transferred to the GC vials and mixed with the recovery standard.

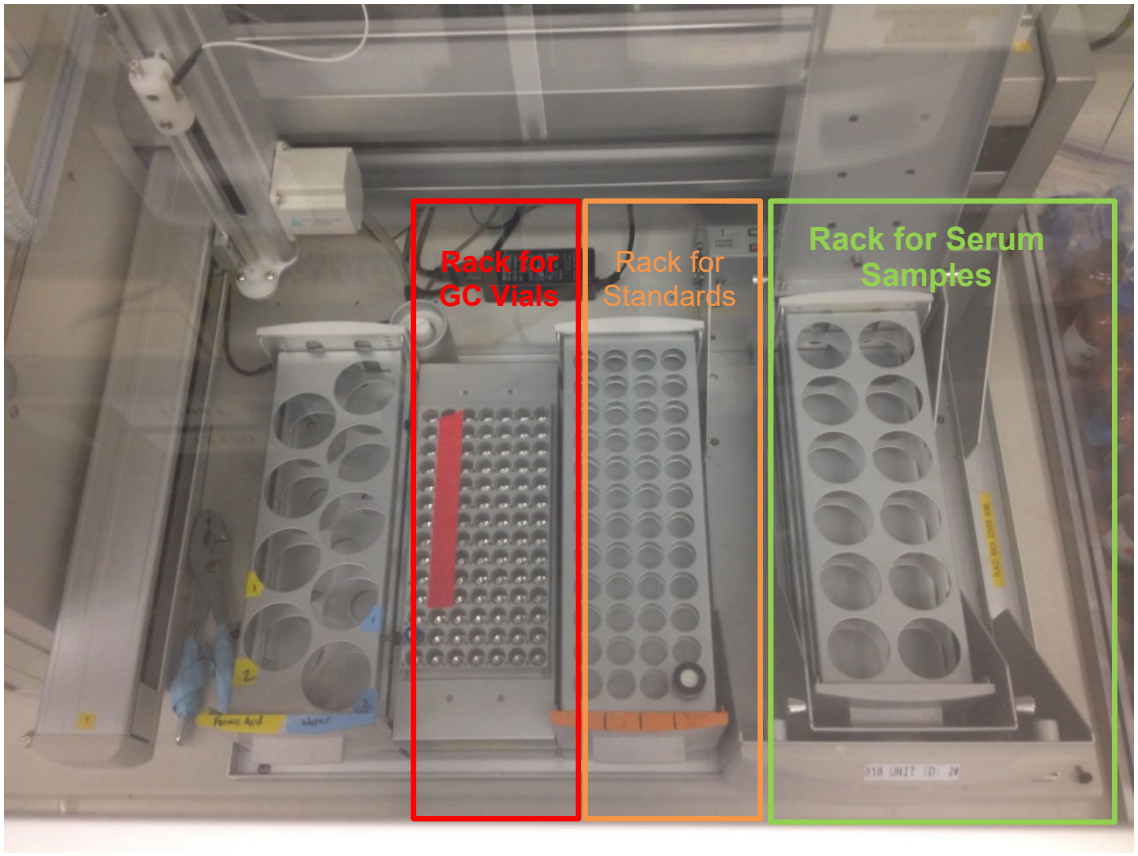

Figure 6.4.2.1. Gilson 215 Liquid Handler used for automated additions of internal surrogate standards to the serum samples. This equipment also adds recovery standard to GC vials.

A Gilson GX281 Liquid Handler is used for automated liquid-liquid extraction. Place the samples in the rack to the far right in Figure 6.4.2.2. Empty 20x150mm test tubes are placed in the rack on the left.

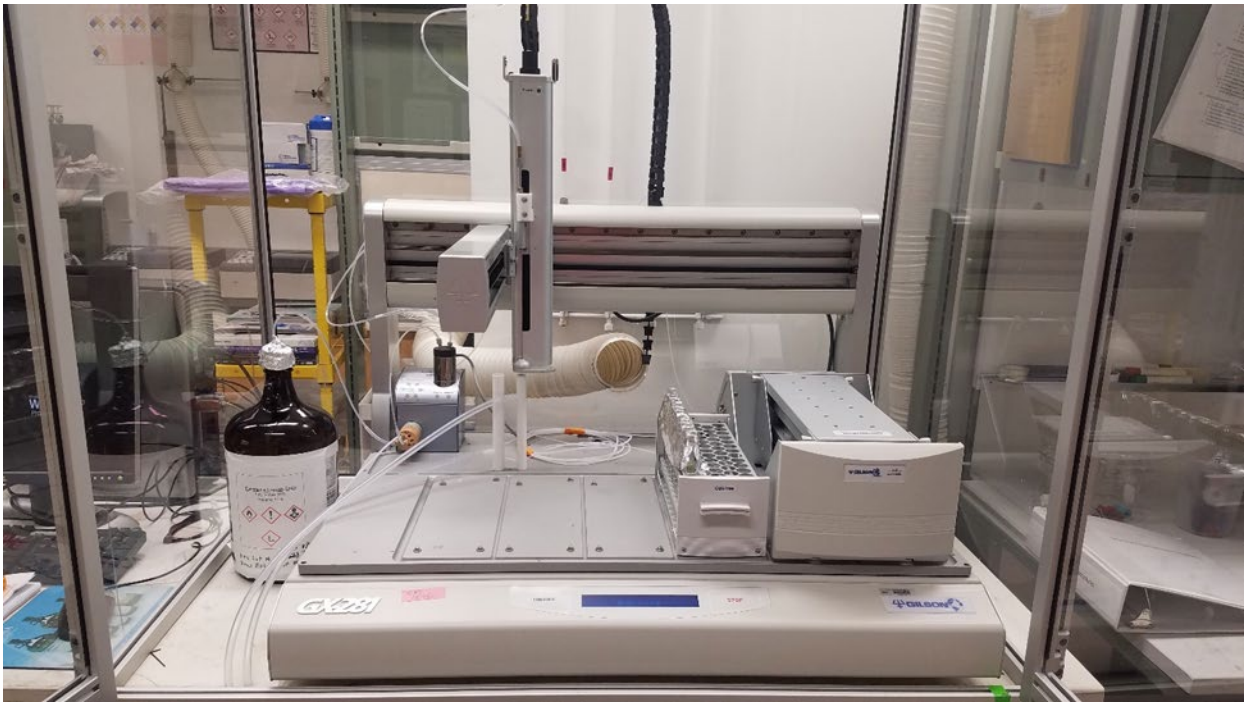

Figure 6.4.2.2. Gilson GX281 Liquid Handler used for automated liquid-liquid extraction.

6.4.3 Biotage TuboVap Evaporator

A Biotage Turbovap LV and Turbovap II, shown in Figure 6.4.3, are used for solvent evaporization. The TurboVap uses a combination of a heated water bath and a gentle flow of nitrogen to increase the speed of solvent evaporization steps in this procedure.

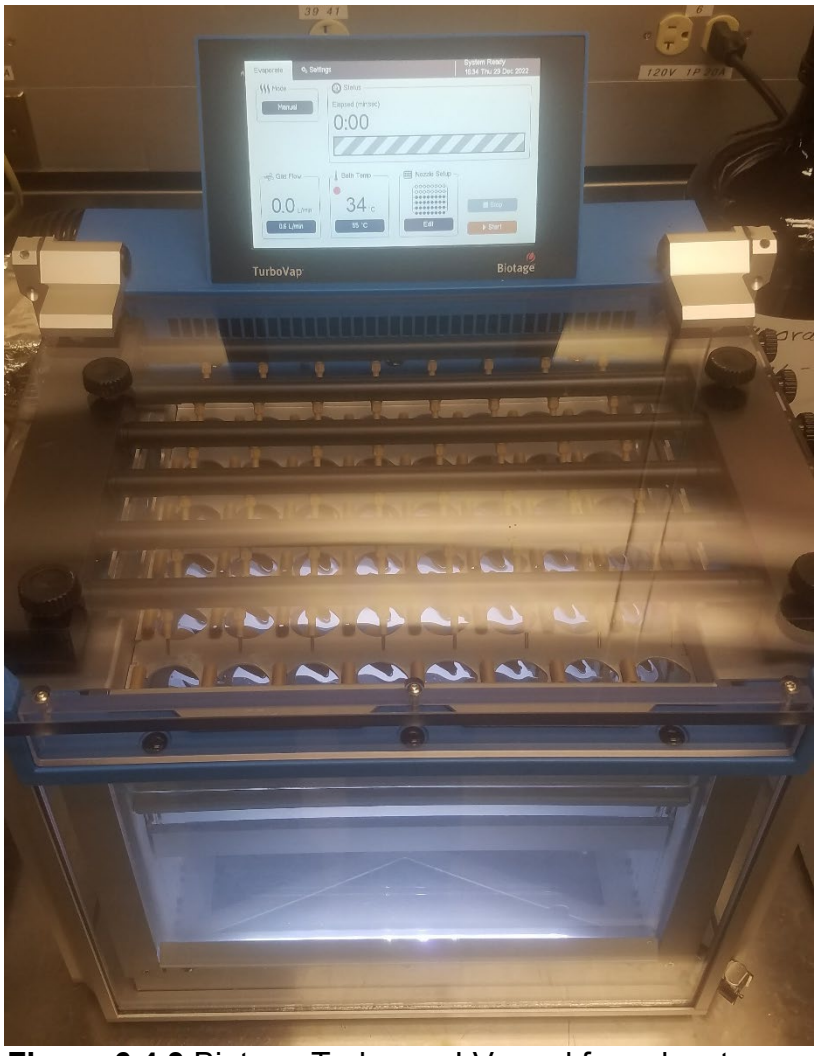

Figure 6.4.3 Biotage Turbovap LV used for solvent evaporization.

6.4.4 High resolution gas chromatograph- high resolution mass spectrometry (HRGC/HRMS)

Analysis of extracts is performed using:

1. Double Focusing Sector Mass Spectrometer (DFS) (ThermoFisher, Bremen, Germany).
    a. Operating Manual, Revision B, February 2011.
    b. Hardware Manual, Revision E, 2010.
2. TRACE 1310 Gas Chromatograph (GC) (ThermoFisher, Bremen, Germany) .
    a. User Guide, Revision B, September 2012.
3. TriPlus RSH Robotic Sample Handling (Thermo Scientific, Bremen, Germany).

   a. User Guide, Fifth Edition, March 2013.
4. Software:
   a. Individual components are programed and controlled using Thermo Xcalibur Software (Thermo Scientific, Bremen, Germany).
   b. Integration is performed using DFS TargetQuan Software (Thermo Scientific, Bremen, Germany)
      i. Operating Manual, Revision C, August 2014

Figure 6.4.4.1. Thermo Electron Double Focusing Sector (DFS) instrument.

6.5 Procedures for preparing quality control materials

The QC material for this assay is bovine serum or a comparable serum matrix in which the concentrations of the target analytes have been certified. One QC sample is analyzed in every set of 6 samples to ensure comparability and reliability between different sets of samples over time.

In addition to the QC sample, a bovine blank or a comparable serum matrix is analyzed in every set of 6 samples. This matrix should be diluted with water such that any measured analyte is representative of chemicals, materials, and/or the laboratory environment rather than a component of the “blank” serum itself.

Specific, predefined rules 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, then that set must be reanalyzed or the data set to Non-Reportable. For further details, see data handling section below and refer to the DLS Policies and Procedures Manual.

6.5.1 General Procedure for Preparing QC Materials

Day 1 Label the vials with computer-generated labels. This label should contain a unique name, constructed from the page number in the QC Pool notebook. 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 raw serum by submerging the container in water (37 ^{o}C) until the serum is completely thawed. Pour the serum into a large, clean beaker (4 L) containing a stir bar. Spike with native, ^{12}C, analytes to the appropriate concentration level (e.g., 500 pg/mL), cover, and stir the solution overnight using a magnetic stirrer.

Day 2: While still stirring the solution, transfer serum in 6.1 mL aliquots to each of the vials. Cap the vials and then place them in an appropriate freezer-safe container/ organizer. Place an identifying label on the edge of the container and store in a -70 ^{o}C freezer until needed for analysis.

Refer to DLS Policies and Procedures Manual for requirements to characterize concentrations in QC materials. Specific predefined rules 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. Some example QC rules are listed below:

- The QC determination must not deviate more than 3 times the standard deviation from the mean value of previous determinations of the same QC pool, and
- No more than ten consecutive QC samples may fall either above or below the mean value of previous determinations of the same pool after one data point has fallen outside of +/- 2SD.

All QC rules are checked by the DLS QC program available in StarLIMs. If the QC sample fails any of these tests the set of unknown study samples must be reanalyzed.

7. Calibration and Calibration Verification

7.1 Calibration of Mass Spectrometer

- Magnetic calibration (MCAL) of the DFS mass spectrometer is achieved by comparing the fragmentation of the reference compound (high boiling perfluorokerosene PFK) to the known fragmentation. Procedures to perform the MCAL are documented in the DFS Operating Manual Revision B (Feb 2011). The calibration should be performed monthly, or after a significant maintenance event on the instrument. The analyst will use their best judgement for performing this procedure and may delay the procedure in consultation with laboratory management.

- Electric Calibration (ECAL) of the mass spectrometer calibrates the acceleration voltage changes required to scan all the ions within an MID window. Procedures to perform the ECAL are documented in the DFS Operation Manual Revision B (Feb 2011). The calibration should be performed monthly, or after a significant maintenance event on the instrument. The analyst will use their best judgement for performing this procedure and may delay the procedure in consultation with laboratory management.

7.2 Creation of Calibration Curve

- At least five calibration standards are analyzed with each analytical run. A calibration curve is generated using the ratio of the peak area of the analyte to the labeled internal standard.

- The r^2 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 1st and 2nd quarter of 2017. Analytes with a determined 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).

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

Training in the use of a high resolution mass spectrometer is necessary for all GC/HRMS operators. Operators are required to read the operation manuals and must demonstrate safe techniques in performing the method. New operators must be evaluated by the supervisor to certify that they are appropriately qualified to perform the assay. Operators are, then, re-evaluated 6 months after their initial training. Thereafter, 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. Laboratory technicians are required to read the operation manuals for laboratory instrumentation and must demonstrate safe techniques in performing the method. New technicians must be evaluated by the supervisor to certify that they are appropriately qualified to perform the assay. Technicians are, then, re-evaluated 6 months after their initial training. Thereafter, the re-certification is performed annually.

8.1 Sending aliquot of serum for lipid determination

Serum lipid concentration in serum is determined by the Clinical Chemistry Branch (CCB). Total cholesterol (TCHOL) and triglycerides (TRIG) are measured for each serum sample. These measurements are used to calculate the total lipid concentration for each sample.

Generally, either the CDC Sample Logistics Laboratory or the Principle Investigator will prepare a separate aliquot of each serum sample for lipid measurements prior to samples being sent to the POPs laboratory. In this case, no additional lipid aliquot needs to be made prior to POPs analysis and the Sample Logistics will transfer this separate aliquot to CCB.

However, occasionally, there may not be a separate aliquot already available for lipid analysis. In this case, the POPs Laboratory will have to provide serum to CCB for lipid analysis, prior to any analysis for POPs.

There are three procedures allowed for the POPs Laboratory to provide serum to CCB for lipid analysis. The POPs Laboratory management staff will decide which of the three procedures to use for any set of samples.

In all cases, samples are transferred between the POPs laboratory and the CCB while frozen and in accordance with safety protocols outlined in the CDC Chemical Hygiene Plan and the CDC Intrafacility Specimen and Sample Transfer Policy.

The procedures allowed for providing serum samples to CCB for lipid analysis are as follows:

1. The entire POPs serum sample, in its original sample vial, is transferred to CCB for lipid analysis. After lipid measurements are completed and the results are reported by CCB, the serum samples will be transferred back to the POPs Laboratory for POPs analysis.

2. **The POPs laboratory will prepare a separate aliquot for CCB**.
    a. Aliquot 150µL of each sample into polypropylene vials (ie. Cryovials) after mixing the thawed serum samples. Make sure to use a new pipette tip for every sample to eliminate cross contamination.
    b. Samples are labeled with the correct DLS Specimen ID, as listed in the DLS StarLIMS system.
    c. Samples are frozen and then transferred to CCB.

3. **The POPs laboratory will prepare a separate, diluted aliquot for CCB**
    a. Aliquot 50µL of each sample into polypropylene vials (ie. Cryovials) after mixing the thawed serum samples. Make sure to use a new pipette tip for every sample to eliminate cross contamination.
    b. To each sample is added 100µL of 0.9% saline solution.
    c. Samples are labeled with the correct DLS Specimen ID, as listed in the DLS StarLIMS system.
    d. Samples are mixed well, frozen, and then transferred to CCB.

8.2 Thawing and weighing samples

Store samples in a -70^{o} 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 before proceeding.

For each batch of samples, complete the run sheet. On the run sheet, enter ALL requested information including, the analyst's name or initials, the date and run number.

Balance Function Check

To ensure optimum performance of the balance used for weighing serum samples:

1. First verify that the balance is level by checking the "bubble level" indicator on the balance and, if necessary, making any adjustments according to the manufacturer's instructions.
    a. If, for any reason, the balance cannot be made level, stop the procedure and resolve any problems before proceeding.
2. Next, verify the balance calibration using certified calibration weights spanning the range 10.000 g and 20.000 g before weighing each batch of samples. Calibration weights are placed on the balance after taring, and the reading is recorded on the run sheet.

3. Document the recorded weights on the run sheet in the “Balance Calibration” section.
4. The difference from the certified value may not exceed +/- 0.01 g.
    a. If this limit is exceeded, any problems must be resolved before proceeding. This may include 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 2oz bottles. Record all sample weights on the run sheet in the “Sample Weight” column.

The target sample weight of spiked Quality Control (QC) samples (ie. SSP:01:14 etc.) is approximately 3 grams, unless otherwise noted. Record the mass of the QC samples appropriately on the run sheet. The QC samples are then diluted up to 20mL with water.

Method blanks are made using 0.5 mL bovine “blank” serum unless otherwise noted. It is not necessary to record the mass of the bovine serum used. The blanks are then diluted up to 20mL with water.

The target sample weight of study samples (Unknowns) is to be specified on a per-study or per-run basis. Record the mass of the study samples appropriately on the run sheet. The maximum sample weight allowed in this method is 20g (~20mL). If the sample weight is less than 20g, then the sample must be diluted up to 20mL with water after recording the sample weight.

8.3 Sample pretreatment using Gilson 215 Liquid Handler

The Internal Standard fortification procedure is automated using the Gilson 215® Liquid Handler

The software controlling the Gilson Liquid Handler is called Trilution LH and a shortcut/icon is located on the desktop. After launching the software, the main menu is displayed (Figure 8.3.1). To set up the software for internal standard fortification, first click on “Applications” button in the menu. In the Application Menu (Figure 8.3.2) select the application named “**New Dioxin Internal Standard Spiking App**”. Make sure that number of samples to be fortified is correct for each method in the application.

8.3.1 Procedure

1. Adjust the volume of the sample to 20mL with water, if less serum was available for the measurement.
2. Record the weights of any specified internal standard vials before use and record their weight as the “Initial Weight” on the runsheet.
3. Place the internal standard vials on the Gilson 215 in the appropriate rack.
4. Place serum samples in the appropriate rack on the Gilson 215. Make sure that each sample is in the correct position in the rack. Leave the sample bottles un-capped during the spiking procedure.

5. After launching the software, the main menu is displayed (Figure 8.3.1). For setting up the software for extraction, first click on “Applications” button in the menu. In the Application Menu (Figure 8.3.2) select the application named “**New Dioxin Internal Standard Spiking App**”. Make sure that number of samples to be spiked is correct for each method in the Application window.
6. Briefly re-inspect the Gilson bed and the entries in the Trilution application to ensure that all bottles, tubes and vials are in the correct positions and that the correct number of samples are listed in the software.

7. **Spiking Gilson 215 Function Checks**:
    a. Ensure that sufficient quantities of all solvents and reagents are present in containers attached to the Gilson 215 instrument and that all solvent lines are kept at the bottom of each container by an attached weight at the end of the solvent line.
    b. If necessary, empty waste containers by replacing the container with an empty one.
    c. Begin the Gilson Spiking Application in Trilution LH. After the system initializes, inspect the syringes during the first “Rinse Probes” steps. Check to ensure that the syringes are pumping solvent correctly. If, for any reason, there is a problem with the syringes or solvent flow, stop the procedure and resolve any problems before proceeding. Record any Corrective Actions taken in the **POPs Spiking Gilson LH Instrument Logbook.**

8. During the procedure all samples are fortified with the internal surrogate standards (Approximately 20 minutes).
9. After the Gilson spiking application is complete, weigh the Internal Standard vials and record their weight as the “Final Weight” on the runsheet.
a. Check the accuracy of the internal standard spike as calculated on the run sheet, and if outside the acceptable range (95-105%, applicable for automated spiking on the Gilson 215 only) consult laboratory supervisor for how to proceed.
10. The samples are removed from the Gilson and mixed/vortexed manually for at least 10 seconds, each.

11. Next, add 5mL of 6M Hydrochloric Acid to the serum and cap the sample bottle. Mix the serum/acid mixture using a rocker or vortexer at a slow speed that does not result in a vortex greater than 1½ inch. Mix each sample for at least 10 seconds.

12. Then, add 20mL of Ethanol to the serum/acid mixture. Mix the samples by vortex or shaker for at least 10 seconds, each.

Figure 8.3.1. Detail of the Trilution Main Menu. A: The Application Menu button.

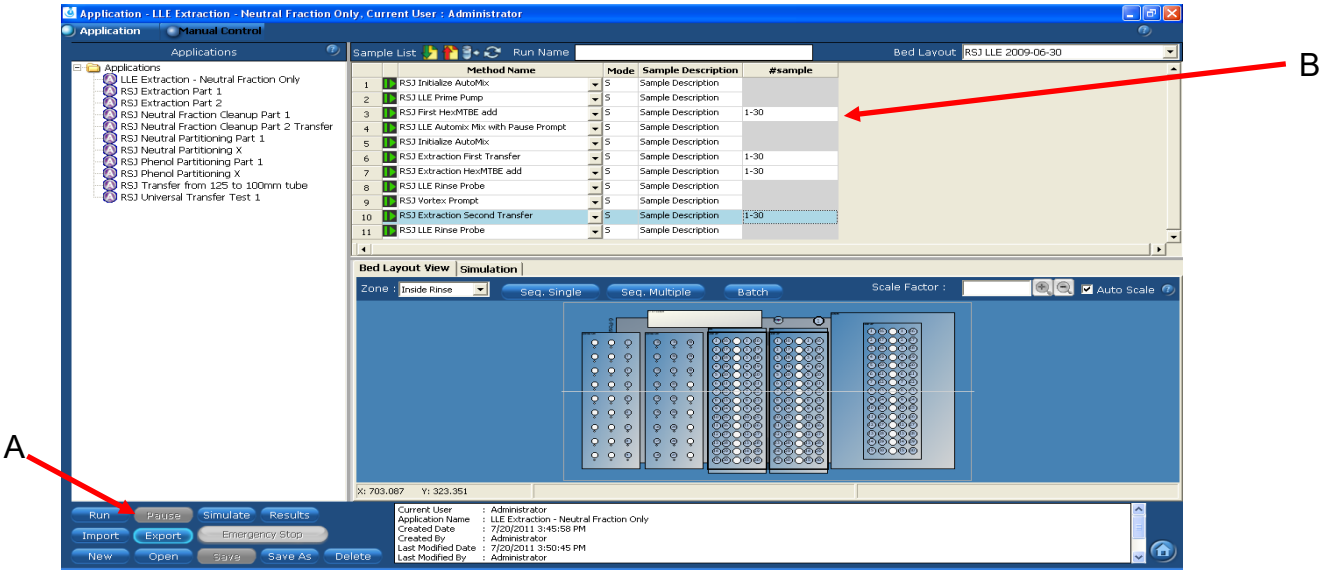

Figure 8.3.2. Detail of the Application Menu in Trilution LH. A: The Application Run Button. B: The column where the number of samples to be spiked is entered

8.3.2 Spike Recovery Standard to GC Vials using Gilson 215 - Liquid handler

The Recovery Standard fortification procedure is automated using the Gilson 215® Liquid Handler

The software controlling the Gilson Liquid Handler is called Trilution LH and a shortcut/icon is located on the desktop. After launching the software, the main menu is displayed (Figure 8.3.1). To set up the software for Recovery standard fortification, first click on “Applications” button in the menu. In the Application Menu (Figure 8.3.2) select the application named “**New Dioxin Recovery Spiking App**”. Make sure that number of samples to be fortified is correct for each method in the application.

Procedure

1. Weigh a new Recovery Standard vial and record its weight as the “Initial Weight” on the runsheet.
2. Place the new Recovery Standards vial Place in the rack on the Gilson 215.

3. Place clean, empty GC vials in the rack on the Gilson 215. Be sure to inspect the vials for cracks and debris before placing them into the rack.
4. **Spiking Gilson 215 Function Checks**:
    a. Ensure that sufficient quantities of all solvents and reagents are present in containers attached to the Gilson 215 instrument and that all solvent lines are kept at the bottom of each container by an attached weight at the end of the solvent line.
    b. If necessary, empty waste containers by replacing the container with an empty one.
    c. Begin the Gilson Recovery Spiking Application in Trilution LH. After the system initializes, inspect the syringes during the first “Rinse Probes” steps. Check to ensure that the syringes are pumping solvent correctly. If, for any reason, there is a problem with the syringes or solvent flow, stop the procedure and resolve any problems before proceeding. Record any Corrective Actions taken in the **Dioxin Spiking Gilson LH Instrument Logbook.**
5. During the procedure all GC vials are fortified with the Recovery standards (takes approximately 20 minutes).
6. After the Gilson Recovery spiking application is complete, the GC vials are removed from the Gilson, capped, and labeled.
7. Record the weight of the Recovery Standard vial as the “Final Weight” in the runsheet

8.4 Liquid-Liquid Extraction

8.4.1 LLE on the Gilson GX281 Liquid Handler.

The extraction procedure is automated using the Gilson GX281 Liquid Handler®

The software controlling the Gilson Liquid Handler is called Trilution LH and a shortcut/icon is located on the desktop. After launching the software, the main menu is displayed (Figure 8.3.1). For setting up the software for extraction, first click on “Applications” button in the menu. In the Application Menu (Figure 8.3.2) select the application named “**GX281 Dox Extraction App**”. Make sure that number of samples to be extracted is correct for each method in the application.

Then click the “Run” button to begin the extraction procedure outlined below.

After the first mixing step, the samples will be centrifuged (>5min, @2000rpm) to separate the organic/aqueous phases. Then, the samples are placed in the sample rack and the Application proceeds with the transfers of the organic phase.

Alternatively, if the Gilson is unavailable, the sample mixing and the transfer of the hexane/diethyl ether layer may be done manually. Mixing may be done using a shaking style of mixer. Transfer of the hexane/diethyl ether layer may be done using a borosilicate Pasteur pipette. However, care should be taken to avoid transferring water/ethanol to the collection tube.

Procedure - Extraction

1. Add **18-20mL Hexane/Diethyl Ether (1:1)** in each sample and then re-cap samples. This can be done using a repeating pipettor. Leave ~1cm headspace at the top of the bottle.
2. Place samples on a large orbital shaker to efficiently extract the aqueous phase. Samples should be mixed for >5 minutes.
3. Place samples into the centrifuge to separate the phases.
4. After centrifugation, carefully place the sample tubes in positions 1-12 in the rack in the 818 AutoMix.
5. Place empty 20x150mm tubes in positions 1-12 in the "Sample Extract" rack on the tray.
6. **Extraction Gilson GX281 Function Checks**:
    a. Ensure that sufficient quantities of all solvents and reagents are present in containers under the Gilson GX281 instrument and that all solvent lines are kept at the bottom of each container by an attached weight at the end of the solvent line.
    b. If necessary, empty waste containers by replacing the container with an empty one.
    c. Begin the Gilson Extraction Application named "**GX281 DOX Extraction App**" in Trilution LH.
    d. After the system initializes, inspect the syringes during the first "Rinse Probes" steps. Check to ensure that the syringes are pumping solvent correctly. If, for any reason, there is a problem with the syringes or solvent flow, stop the procedure and resolve any problems before proceeding. Record any Corrective Actions taken in the **Dioxin Extraction Gilson LH Instrument Logbook.**
7. The Gilson will then transfer the organic phase from the original sample tube to the corresponding 20x150mm tube.
8. After transferring all samples, the Gilson will add more hexane/diethyl ether solution to each original sample tube.
9. The Gilson will then transfer the organic phase from the original sample tube to the corresponding 20x150mm tube. Then the Application will end.

8.5 Cleanup and Isolation of PCDD/Fs and cPCBs

8.5.1 Acid Silica Column for Removing Polar Fats

An acid silica column (1.0g) is used to remove polar fats and acid-labile contaminants from the sample. This includes (but is not limited to) steroids, such as cholesterol, and free fatty acids, such as stearic and oleic acid. This also serves to filter the samples and to remove any small amounts of water or alcohol carried over from the extraction step.

The composition of the acid silica is 33% (w/w) sulfuric acid mixed with neutral silica. These components should be mixed together in a glass tube with a PTFE-lined cap, and it should be mixed by rotation for at least 24 hours prior to use. The thoroughly mixed acid silica should appear free-flowing without any visible clumping in the silica.

Procedure for preparation of Silica Gel:Sulfuric Acid Mixture

1. See section 6 for Manufacturer, grade and brand for all chemicals used.
2. Activate silica gel in oven at 250 ºC for at least 12 hours.
3. Using calibrated laboratory balance, add 6.6 g Silica gel and add 3.3 g of concentrated sulfuric acid to a 50-mL glass tube fitted with Teflon lined cap.
4. After adding the acid, vigorously shake mixture to break up any large clumps.
    a. Standard laboratory Personal Protective Equipment must be used, such as lab coat, safety glasses and gloves. See Section 2.2 for additional safety precautions when handling concentrated acids.
5. Allow the mixture to rotate for at least 12 hours using a rotating mixer. After mixing, confirm that no lumps are present in mixture before using the acidified silica mixture.

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8.5.2 Acid Silica Column Procedure

1. Make an acid silica column for each sample by weighing out 1.0g of 33% (w/w) sulfuric acid mixed in silica mixture into an empty 3mL SPE cartridge with a polyethylene frit at the bottom.
    a. The SPE cartridges packed a with Silica and Silica:Sulfuric acid have a shelf life of 1 day when stored in plastic re-sealable bag and hence must be prepared directly prior to use.
2. Add one void volume of hexane (1-2mL) to the top of each column and let elute through the column. Thereafter, condition each column with 10mL of hexane and then discard the used hexane.
3. The serum samples are evaporated to dryness after the extraction procedure using a Biotage TurboVap LV.
    a. Set the TurboVap to 40℃ until at least half of the volume (the diethyl ether) has evaporated. Then, if desired, the temperature can be increased up to 55℃ for the remainder of the evaporization.
    b. Co-extracted lipids will serve as a “keeper” for Dioxins and POPs in the sample. However, take care to remove the samples from the evaporator as soon as the solvent has been removed.

4. Reconstitute the samples with 0.5-1.0mL of hexane and lightly vortex to thoroughly mix the sample.
5. Using a Pasteur pipette transfer the sample to the acid silica column (1.0g) and allow the sample to drain completely into the acid silica.
6. Rinse the original sample tube with 0.5-1.0mL of hexane and transfer it to the silica column. Allow the sample to drain completely into the silica.
7. Elute each column with an additional 4mL of hexane. The total collected volume should be 5-6mL.

8.5.3 Principle of Power-Prep Sample Cleanup Procedure:

The FMS Power-Prep NG system is used to remove lipids and other biogenic compounds as well as other interfering chemicals that are present in the serum extract. The extracts are pumped through an acid/neutral/base silica column, alumina column and an AX-21 carbon column. The silica column serves to remove most biogenic and polar chemical background by oxidative degradation. The alumina is used to separate the aromatic PCDD/Fs and cPCBs from non-aromatic chemical interferences. Lastly, the planar PCDD/Fs and cPCBs are separated from non-planar compounds via the AX-21 carbon column.

The program used on the FMS PowerPrep NG is given in Table 8.5.1 including valve positions, solvents used and volumes used.

22_DIOXIN FMS DOX ONLY Method:

Step	Description	Columns In-line	Solvent	Volume	Destination
1	Hexane Prime	Bypass All	Hexane	10	Waste
2	Condition Silica w/ Hexane	Silica Only	Hexane	70	Waste
3	Hexane Bypass to Waste	Bypass All	Hexane	15	Waste
4	Condition Alumina w/ Hexane	Alumina Only	Hexane	50	Waste
5	Swap to Toluene	Bypass All	Toluene	15	Waste
6	Condition Carb w/ Toluene	Carbon (F)	Toluene	50	Waste
7	Swap to 5050	Bypass All	50/50 DCM/Hex	15	Waste
8	Condition Carb w/ 5050	Carbon (F)	50/50 DCM/Hex	30	Waste
9	Swap to Hexane	Bypass All	Hexane	15	Waste
10	Condition Carb w/ Hexane	Carbon (F)	Hexane	30	Waste
11	Add Sample	Silica-Alumina	Hexane	10	Waste
12	Elute Sil-Alu	Silica-Alumina	Hexane	70	Waste
13	Elute Alumina Column	Alumina Only	Hexane	30	Waste
14	Swap to 50/50	Bypass ALL	50/50 DCM/Hex	15	Waste
15	Elute Alu-Carb (Load Carbon)	Alumina-Carbon (F)	50/50 DCM/Hex	80	Waste
16	Elute Carbon to Waste	Carbon (F)	50/50 DCM/Hex	50	Waste
17	Swap to Toluene	Bypass ALL	Toluene	15	Waste
18	Elute Dioxins from Carb (Rev)	Carbon (R)	Toluene	45	Dioxin Fraction (Coplanar Fraction)
19	Swap to Hexane	Bypass ALL	Hexane	15	Waste
20	Flush Carbon with Hex	Carbon (R)	Hexane	10	Dioxin Fraction (Coplanar Fraction)
21	ALL OFF	Bypass ALL	-	-	-

Table 8.5.1. Program for Clean-up of a Sample for PCDD/Fs and cPCBs through the FMS PowerPrep NG System (22DIOXIN.stp).

8.5.4 FMS PowerPrep 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.

1. Make certain that sufficient quantities of all solvents are present in the solvent bottles attached to the FMS instrument and that all solvent lines are kept at the bottom of the containers by an attached weight at the end of each solvent line.
2. If necessary, empty waste containers by replacing the container with an empty one.
3. Run the RINSE function check programs.
    a. RINSE_1 is run with 15mL of 50% DCM in Hexane in clean 20x150mm test tube.

b. RINSE_2 is run with 15mL of Hexane in a clean 20x150mm test tube.

4. Check valves and fittings for leaks.
5. Inspect the volume aspirated from the 20x150mm test tube. Make sure that the FMS aspirates the correct volume of solvent from the test tube.
    a. If the pump volume calibration appears to be incorrect, calibrate the pump according to the manufacturer's instructions.
6. If, for any reason, there is a problem with the pump or solvent flow, stop the procedure and resolve any problems before proceeding.
7. If applicable (e.g., change valve) maintenance is performed, record in the **FMS PowerPrep Maintenance Logbook**.

8.5.5 Sample Cleanup Procedure

1. If the sample extracts were not passed through the acid silica column described in Section 8.5.1 and 8.5.2, then the sample extracts are evaporated to dryness and then reconstituted with 15mL of Hexane.

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

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

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

5. For a "Dioxin-only" run, start file "**22DIOXIN.stp**".

    a. Check all fittings for possible leaks as the program conditions the columns. If necessary, pause the program by pressing the "Halt" button on the view screen before tightening leaky fittings.
    b. Watch for excessive column backpressure during the conditioning steps and make any corrections to fittings or columns as necessary.
    c. Watch the sample test tube during the "Add Sample" step and make sure that the entire sample is loaded onto the silica column.

6. Allow the FMS to complete the program.
    a. This will collect 45 mL of toluene in the 200 mL TurboVap tube. This contains PCDD/F and cPCBs.

8.5.6 Evaporation and transfer to final GC-vial

1. Samples from the FMS cleanup step are evaporated to approximately 0.5 mL using the Caliper TurboVap II evaporator.

    It is imperative that the samples are not evaporated to dryness at this step, since all volatile analytes would be lost.

a. For the Dioxin fraction in toluene, start the evaporation with the following settings as a general guide: 60 °C water bath temperature and ~5psi line pressure.

2. Transfer the Dioxin fraction sample to the GC vial that was spiked with 100uL of Dioxin Recovery Standard from Section 8.3. **MAKE CERTAIN THAT THE SAMPLES ARE TRANSFERRED TO THE CORRECT VIAL !!!**

3. Rinse the sample TurboVap tube with ~0.5mL of dichloromethane and transfer to the corresponding GC-vial.

4. Evaporate all samples until <1uL remains using the Caliper TurboVap LV evaporator.
   a. The TurboVap LV for GC vials should not contain water.
   b. Start nitrogen flow at zero and slowly increase until a ripple is seen in the GC vials. Care must be taken not to increase the nitrogen flow too much since this could blow the sample out of the GC vials.

5. After evaporization, adjust the final volume in each GC vial to 5-7µL with nonane using either a GC syringe or a pipettor.

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

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

8.6 HRGC/HRMS Analysis

Analysis of the extracts is performed on a Double Focusing Sector Mass Spectrometer (DFS) (ThermoFisher, Bremen, Germany) instrument coupled to a Trace 1310 gas chromatograph (GC) (ThermoFisher, Bremen, Germany) equipped with a Programable Temperature Vaporizing Injector (PTV) module, using a TriPlus RSH Autosampler (ThermoFisher, Bremen, Germany) for injection. All components are controlled using the Xcalibur software provided by Thermo. Extracts are analyzed for BFRs first and for PCB/PSTs second in a separate analytical run with a different instrument method. Settings for each instrument method are detailed below.

8.6.1 GC Preparation

If a new GC column is required for analysis, it should be conditioned first. The front end of the column is installed in the GC inlet following procedures in the Trace 1310 User Guide, Revision B (Sept 2012). To prevent excess silica fouling the ion source of the DFS, the back end of the column is left uninstalled until after conditioning. Conditioning is accomplished with successive loops of the GC ramp used for each method. The GC can be set to run automatically and allowed to loop overnight. A minimum of three loops through the GC ramp should be accomplished before the back of the column is installed in the DFS following procedures detailed in the DFS Hardware Manual, Revision E (Feb 2010).

Maintenance of a GC column will be required when there is excessive tailing of peaks in the chromatograms or if analytes can no longer be resolved from each other. The analyst will use their best judgement when the column needs to be cut, or if a new column needs to be installed. When a new column is installed, or if the current column required cutting, new analyte retention times should be established, and the MID windows of the MS method adjusted accordingly.

New inlet septa and liner will be installed following procedures detailed in the Trace 1310 User Guide, Revision B (Sept 2012). The analyst will use their best judgement if new septa and liner are needed.

8.6.2 Autosampler Preparation

The injection syringe may need to be replaced after excessive use, if it is found to not be delivering the programmed volume, or if the needle has been damage. The analyst will use their best judgement to determine if the syringe should be replaced. The syringe is replaced following procedures in the User Guide, Fifth Edition (Mar 2013).

The analyst will ensure there is sufficient toluene (position A) and nonane (position B) in the solvent reservoirs used for rinsing the syringe between injections. Distilled nonane is used for the reservoir. Solvent in the reservoirs should be completely replaced weekly to prevent buildup of potential interfering compounds. Used solvent in the waste reservoir should be disposed of as hazardous waste.

8.6.3 DFS Preparation

The DFS is operated in Electron Ionization Mode. Before beginning analysis, ensure the DFS system is up to date with calibration procedures including MCAL, ECAL, MDAC, EDAC, and multiplier gain determination. Each of these procedures should be performed monthly, or after a significant maintenance event on the instrument. The analyst will use their best judgement for performing these procedures and may delay the procedure in consultation with laboratory management. Details on performing these procedures can be found in the DFS Operating Manual, Revision B (Feb 2011).

Before beginning an analytical run, the DFS is tuned to 10,000 mass resolution (5% peak height definition). A small amount (~1µL) of reference compound is introduced into the DFS using the reference inlet. For PCDD/F analysis, perflourokerosene (PFK) is used. The instrument is tuned on an appropriate mass of the reference compound using the Tune application in the Xcalibur software. Details on tuning the instrument may be found in the DFS Operating Manual, Revision B (Feb 2011).

If the instrument cannot achieve the required sensitivity or maintain the mass resolution of 10,000, the analyst will need to troubleshoot and fix the system before beginning an analytical run. The analyst, in consultation with laboratory management and vendor service engineers, will use their best judgment to determine the troubleshooting process necessary.

8.6.4 Analytical Runs

After the instrument has been tuned, an analytical run may proceed. Using the Xcalibur software, the analyst creates a sequence file for each analytical run which details the specific sample information and instrument method for each extract. A typical analytical run will begin with a sensitivity check, followed by a calibration curve, followed by sample extracts. Runs of nonane blanks using distilled nonane with no analyte or matrix are distributed within the analytical run to monitor for instrument carryover, and/or to clear the GC column of potential high-boiling compounds. The analyst will use their best judgement on how the nonane runs will be distributed. At the completion of the analytical run, the raw data is integrated using the TargetQuan software following guidelines from the DLS Policies and Procedures Manual, Version 7.3.1 (Feb 2022). Details for the operation of the TargetQuan software may be found in the Operating Manual, Revision C, (Aug 2014).

8.6.5 Documentation and Data Files

All maintenance completed on an instrument is documented in either a hardcopy Instrument Logbook, or electronically in the Instrument Maintenance Logbook Excel file. All runs performed on each instrument should be documented in the Instrument Logbook. The electronic logbook is archived in the data management system.

Each analytical run produces a log file and a raw data file. The log file details the specific settings used for analysis. Both the log and raw data file are archived in the data management system. Upon integration, each analytical run has an associated QuanAscii file which is transferred to the SAS system for quantitation. Each integrated file also produces a report file in pdf format which displays the integrated peaks. This file is archived in the data management system.

8.6.6 PCDD/F and cPCB Analysis

A. Autosampler Settings

- 5 nonane rinses before injection
- 3 sample pumps
- Injection volume: 4.0 µL with 1.0 µL air gap.
- Pre-injection delay for 1 seconds
- Post-injection delay for 1 seconds
- Injection depth: 45mm
- Penetration speed: 100mm/sec

- Injection Speed 50µL/sec
- 10 toluene rinses followed by 10 nonane rinses after injection
- Cool Tray temperature: 10°C

B. PTV Inlet Settings

- Splitless Mode
    1. Splitless for 3 minutes
    2. Split flow at 70 mL/min
    3. Septum purge flow at 5.0 mL/min
- Constant Flow mode at 1.0 mL/min
- Siltek deactivated metal liner for PTV (2.0mm x 2.75 mm x 120mm) with deactivated glass wool from Restek (Bellefonte, PA)
- Temperature Program

#	Rate (°C/min)	Temperature (°C)	Time (min)
Injection		140	0.5
Transfer	10	290	3.00
Cleaning	14.5	350	20.0
Post Cycle		140	

C. Oven Settings

- Column Phase: DB5-MS UI (Agilent, Hanover, PA) or equivalent 5% phenyl phase
- Column Dimensions: 60m, 0.25 mm I.D x 0.25 µm film
- Temperature program

#	Rate (°C/min)	Temperature (°C)	Hold Time (min)
Initial		150	4.0
1	35	230	15
2	3.4	330	9.0

- Transfer line temperature: 275°C

D. Mass Spectrometer Settings

- Source Settings:
    1. Source Temperature: 275°C ± 5°C
    2. Emission Current: 1.0 mA
    3. Electron Energy: 45eV
    4. Reference Inlet Temperature: 90°C
- MID Settings for all MID window:
    1. MID mode: Lock
    2. Data Type: Centroid
    3. Width 1st lock: 0.25amu
    4. Sweep Peak Width: 3.0
    5. Offset: 20µV
    6. Measure/Lock Ratio: 1
    7. Magnetic Delay: 60ms

8. Electric Delay: 10ms

- Cycle Time is adjusted for each window to ensure enough scans over the peak. In Table 8.6.6 the cycle time is given in the header of each window.
- The Intensity (Int) setting establishes the dwell time for each ion in the MID window based on the total cycle time. Table 8.6.6 below details the ions in each MID window, the specific lock and calibration masses used, and the intensity assigned to each ion.
- MID windows 1 and 8 have only lock and calibration masses acquired and are not used for quantitation. These windows help reset the instrument after a run allowing it to easily achieve lock on the next run.
- Detailed information on accurate masses for each analyte are given in Appendix B.

E. PCDD/F and cPCB Peak Identification

Proper peak identification is required to set up MID windows and integration. Figure 8.6.6 below shows the elution order of all peaks in the current analytical standards. The MID windows of the MS method are shown in boxes at the top. Not all identified peaks are quantitated.

F. TCDD Sensitivity Check

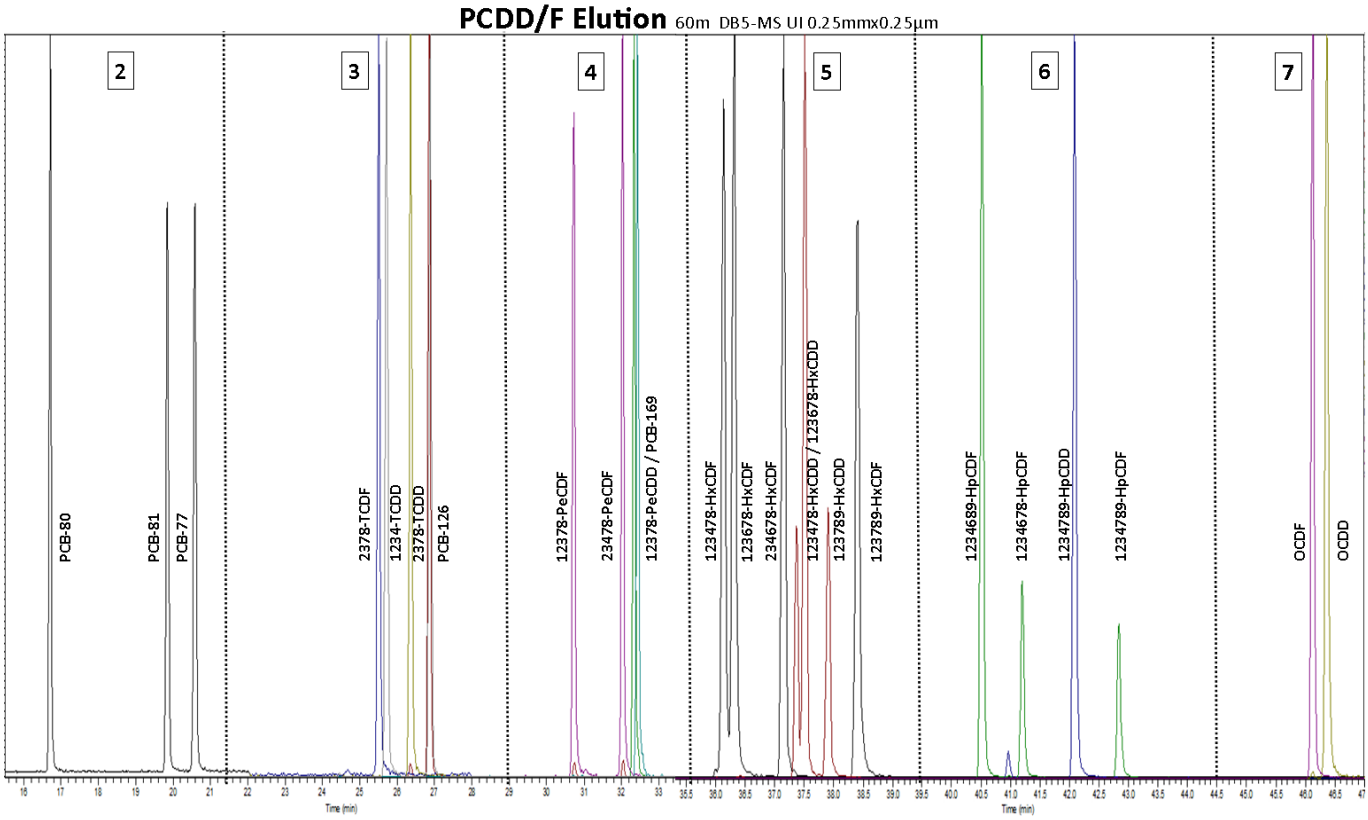
The daily function check will be evaluated using the first calibration standard injected, which will be the CDF4 standard. In this standard, the signal-to-noise (S/N) ratio of the 2378-TCDD peak should be at least 30:1. This is evaluated using the Signal-to-Noise application in the Xcalibur software using the m/z 321.8936 and a 20 second noise window.

Table 8.6.6 MID Window Setup of PCDD/F and cPCB

Window 1 10.s		Window 2 0.6s		Window 3 1.0s		Window 4 1.0s	
mass	Int	mass	Int	mass	Int	mass	Int
292.9819 l	25	289.9224	1	303.9016	1	330.9787 l	20
304.9819 c	25	291.9194	1	304.9819 l	25	337.8627	1
		292.9819 l	40	305.8987	1	339.8597	1
		301.9626	7	315.9419	4	349.9029	4
		301.9928	1	317.9389	4	351.9	4
		303.9597	7	319.8965	1	353.8576	1
		304.9819 c	40	321.8936	1	355.8546	1
				325.8804	1	359.8415	1
				327.8775	1	361.8385	1
				327.9137	4	365.8978	4
				331.9368	4	367.8949	4
				333.9338	4	371.8817	1
				337.9207	4	373.8788	1
				339.9177	4	380.9755 c	20
				342.9787 c	25	409.7974	4
				375.8364	4		

Window 5 1.25s		Window 6 0.65s		Window 7 0.65s		Window 8 1.0s	
mass	Int	mass	Int	mass	Int	mass	Int
371.8237	1	404.9755 l	25	441.7428	1	280.9819 l	25
373.8207	1	407.7818	1	442.9723 l	25	292.9819 c	25
380.9755 l	45	409.7788	1	443.7398	1		
385.861	11	419.822	5	453.783	5		
387.858	11	421.8191	5	455.7801	5		
389.8156	1	423.7767	1	457.7377	1		
391.8127	1	425.7737	1	459.7348	1		
401.8559	11	435.8169	5	469.778	5		
403.853	11	437.814	5	471.775	5		
442.9723 c	45	442.9723 c	25	480.9691 c	25		
445.7555	4	479.7165	4	513.6775	4		

Figure 8.6.6 PCDD/F and C PCB Elution Order



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 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 made between February, 2021 and August, 2022. 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 typically less than a coefficients of variance (CV) less than 10%. Precision data is given in Appendix A.

9.4 Analytical specificity

1. High Mass Resolution: This analysis is performed at 10,000 mass resolution (5% peak height definition) which provides excellent specificity.
2. Ion Ratios: Two ions are monitored for each native analyte and 13C-labeled internal standard. For each measurement, the ratio between these two ions is verified to be with +/- 26% from the theoretical isotope ratio.
3. Relative Retention Time: The relative retention time of native compound divided with its ^{13}C-internal standard is verified for each measurement.

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.:

The following QA/QC criteria is confirmed to be met for all analytical runs by DLS QC program:

- 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.
    - **3σ**, Average of three QCs is outside of the 3σ limit.
    - **2σ**, 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.

Further, every measurement of a set of samples must fulfill the following criteria given in appendix B with respect to recovery, relative retention time and isotopic ratio to be considered a valid measurement.

10.2 Proficiency testing (PT)

The only established external PT program for this assay was the Arctic Monitoring and Assessment program (AMAP) that was discontinued in the year 2020. Our laboratory participated in this program up to the discontinuation of this program.

Due to this the PT program used for this method is the Division of Laboratory Sciences (DLS) developed internal PT program in this program two challenges per annual year is conducted. In each challenge 5 PT samples from 3 pools are selected by the DLS statistician. The laboratory is blinded to the identity of the PT samples until the

measurement data has been evaluated by the DLS statistician. A report is provided after each challenge by the DLS statistician and 80% passing frequency by analyte is considered acceptable.

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 $\pm$ 20 ^{o}C.

The method is designed to run on a HRGC/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 electronic file format. 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 $\pm$ 20 °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.

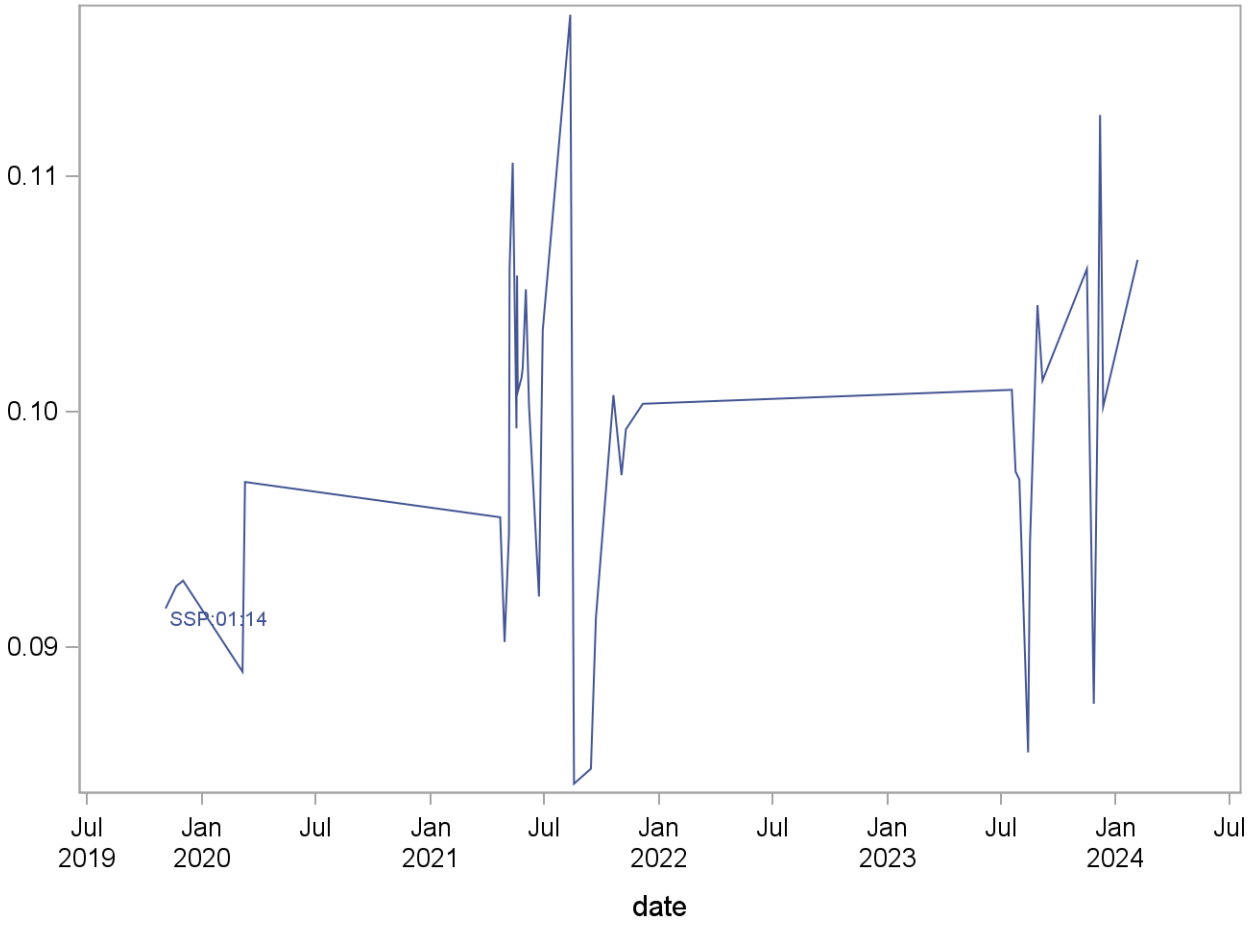
Standard record keeping formats (e.g., database, notebooks, and data files) are used to track specimens. Specimens will only be transferred or referred to other DLS Branch laboratories or, if required, to CLIA certified laboratories. Specimens may be stored at the CDC specimen handling and storage facility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

19. Summary Statistics and QC Graphs

Please see following pages.

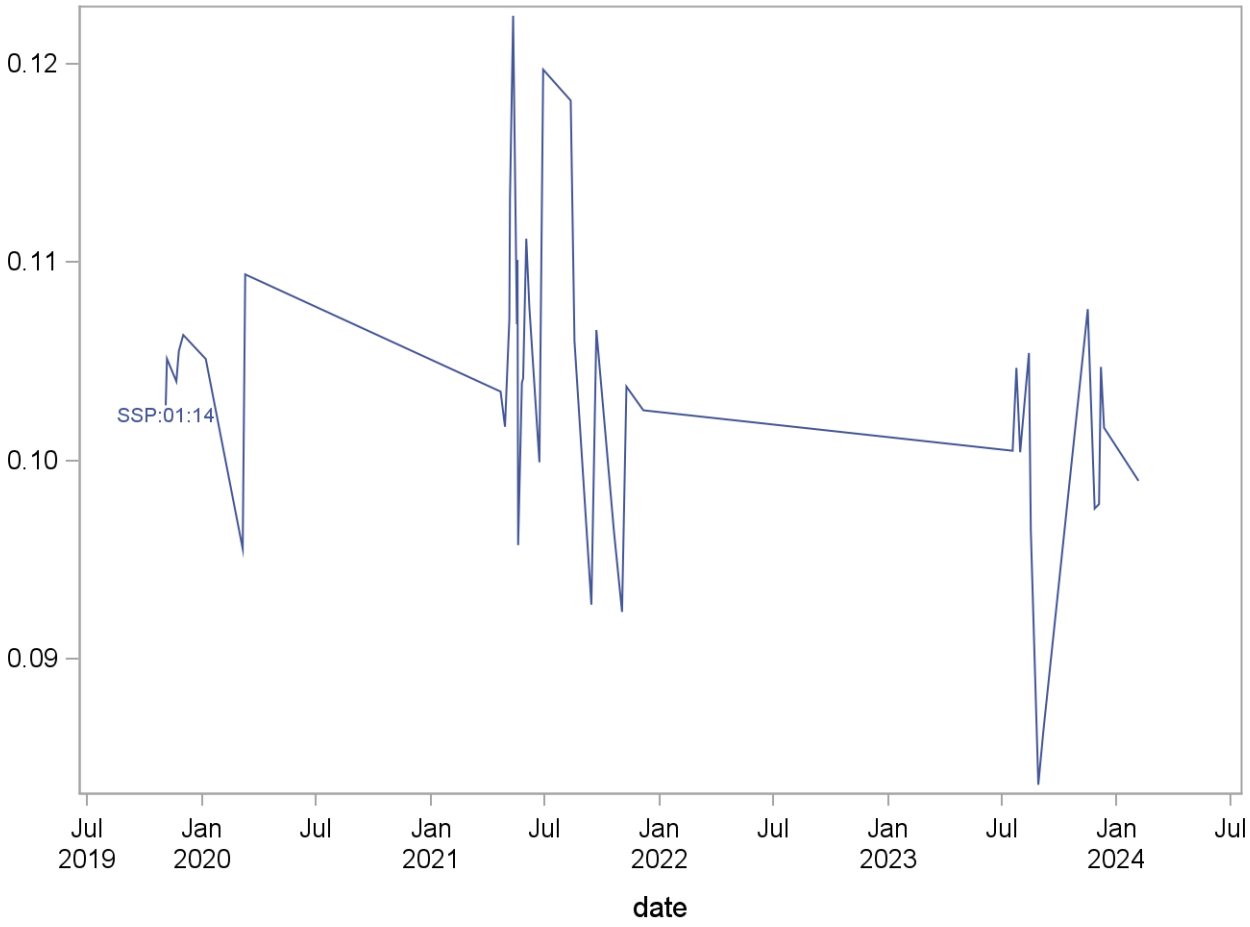
2015-2016 Summary Statistics and QC Chart
LBCD01 (1,2,3,7,8-pncdd (fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	40	04NOV19	05FEB24	0.098533	0.007474	7.6



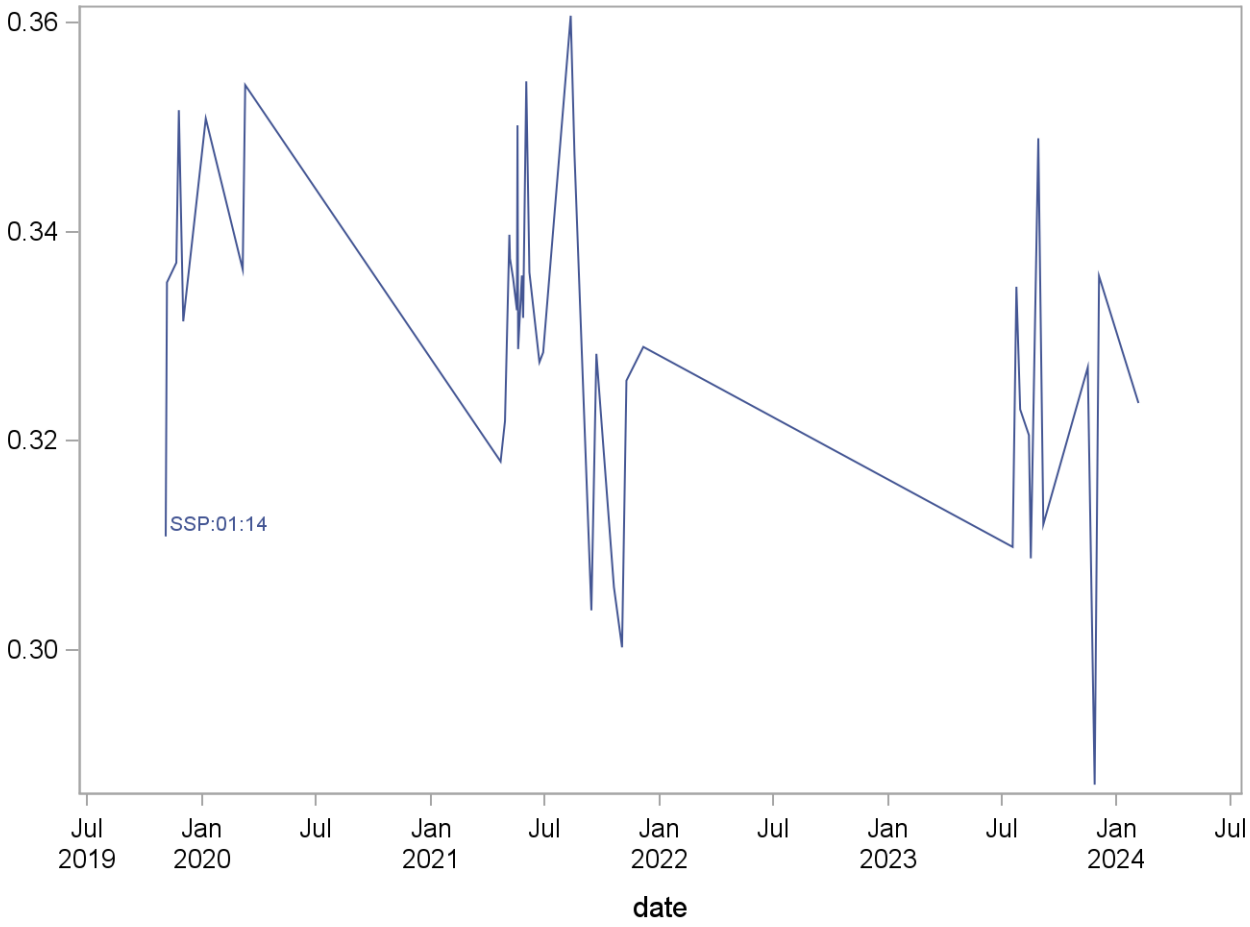
2015-2016 Summary Statistics and QC Chart
LBCD02 (1,2,3,4,7,8-hxcdd (fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	43	04NOV19	05FEB24	0.103369	0.007630	7.4



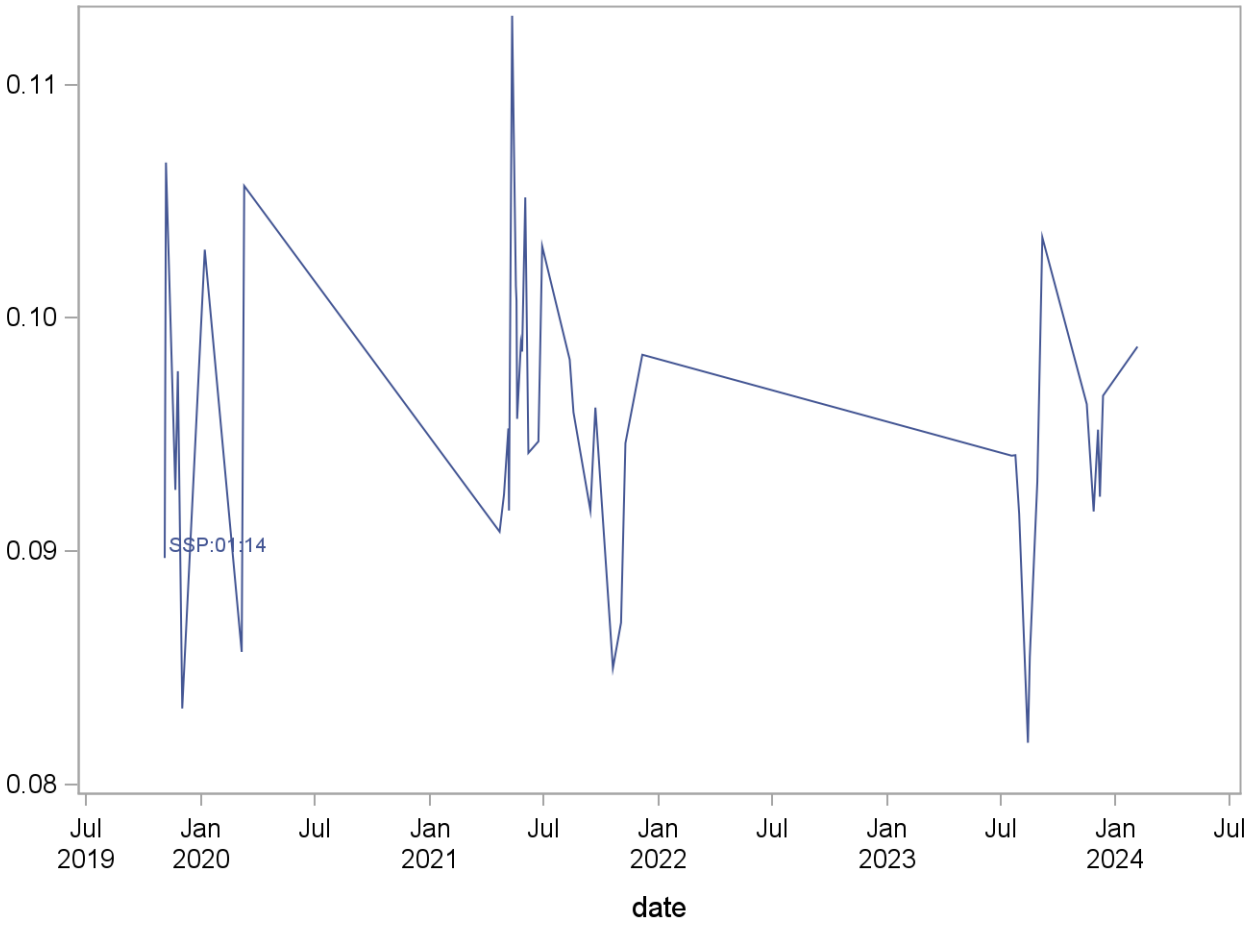
2015-2016 Summary Statistics and QC Chart
LBCD03 (1,2,3,6,7,8-hxcdd (fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	41	04NOV19	05FEB24	0.329701	0.016165	4.9



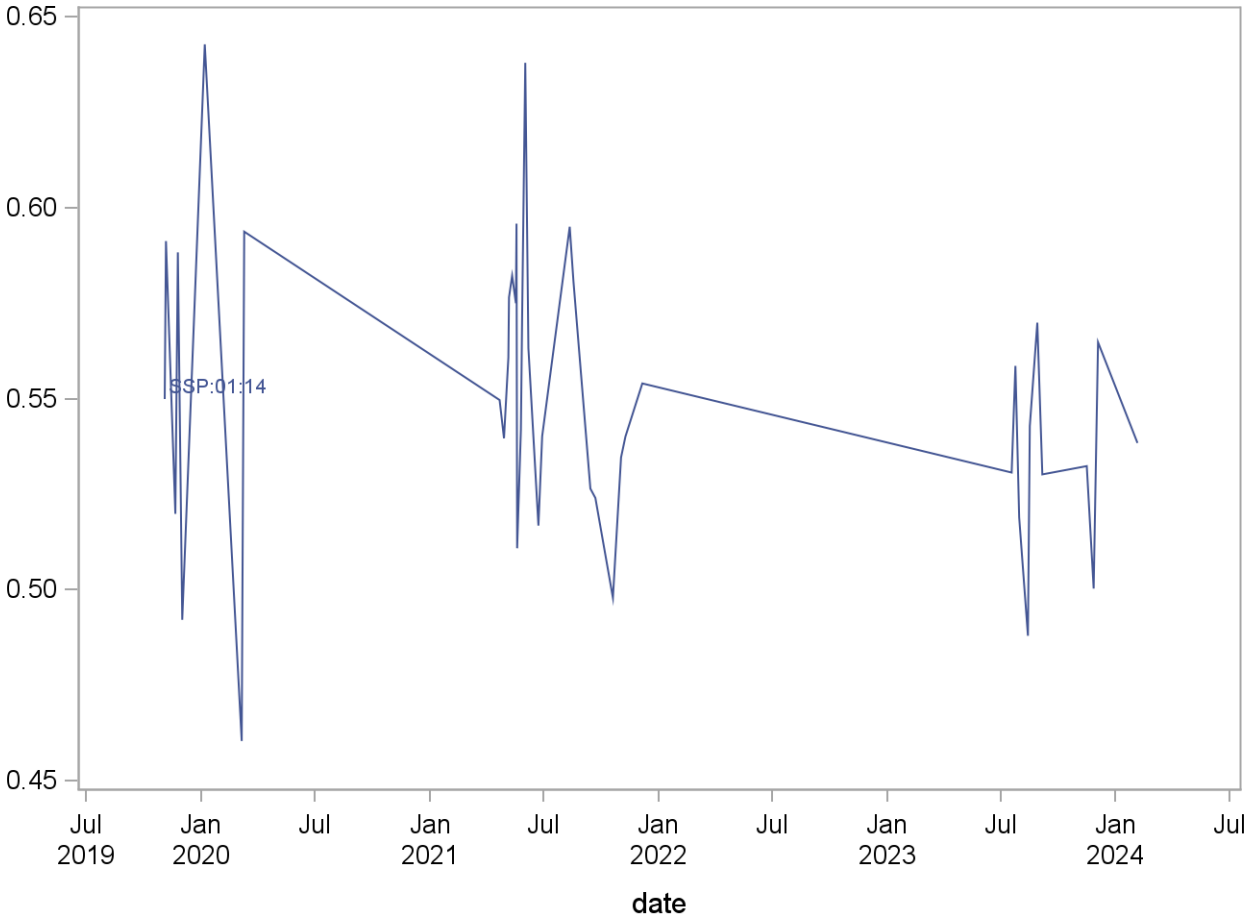
2015-2016 Summary Statistics and QC Chart
LBCD04 (1,2,3,7,8,9-hxcdd (fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	43	04NOV19	05FEB24	0.095383	0.006527	6.8



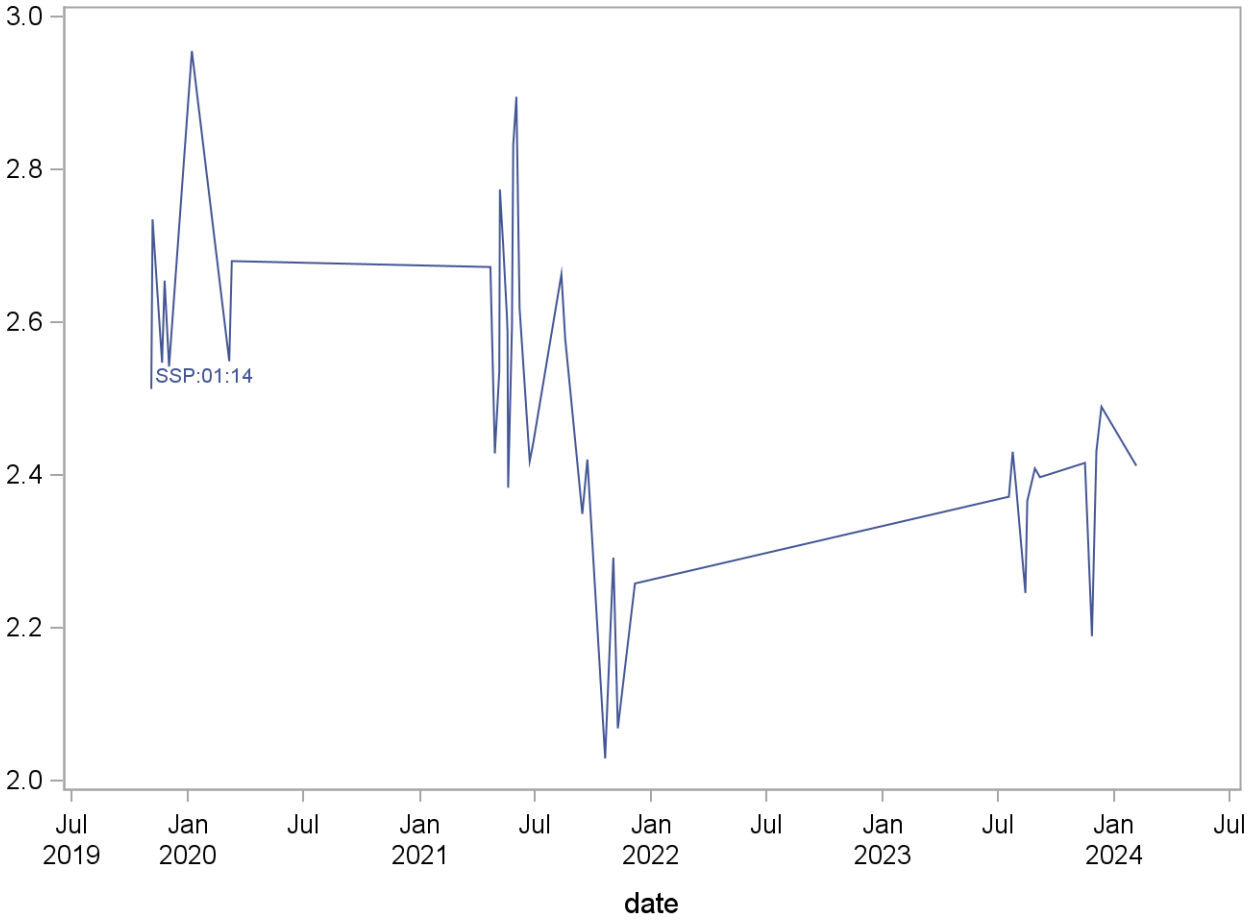
2015-2016 Summary Statistics and QC Chart
LBCD05 (1,2,3,4,6,7,8-hpcdd (fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	41	04NOV19	05FEB24	0.549446	0.038178	6.9



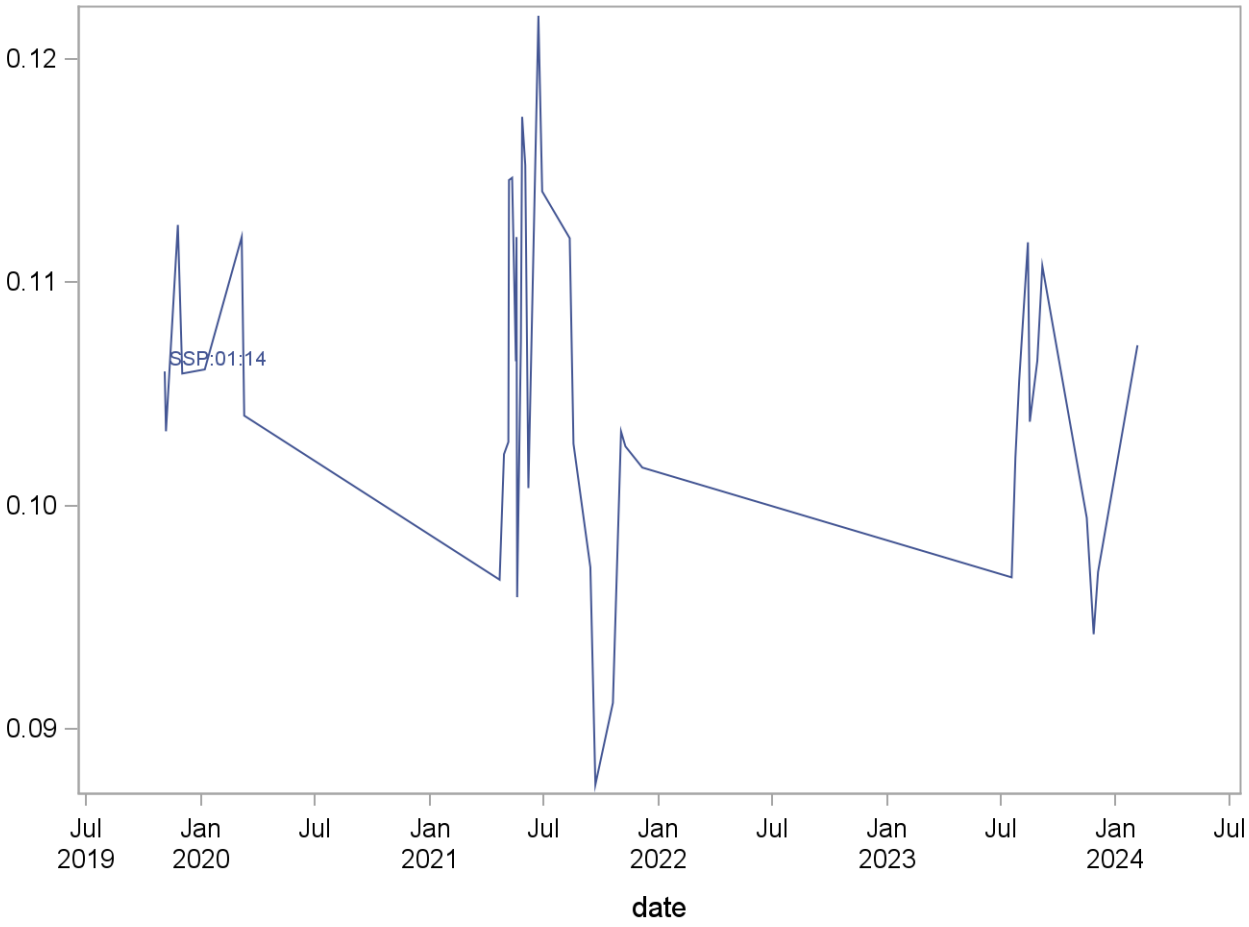
2015-2016 Summary Statistics and QC Chart
LBCD07 (1,2,3,4,6,7,8,9-ocdd (fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	42	04NOV19	05FEB24	2.496727	0.199594	8.0



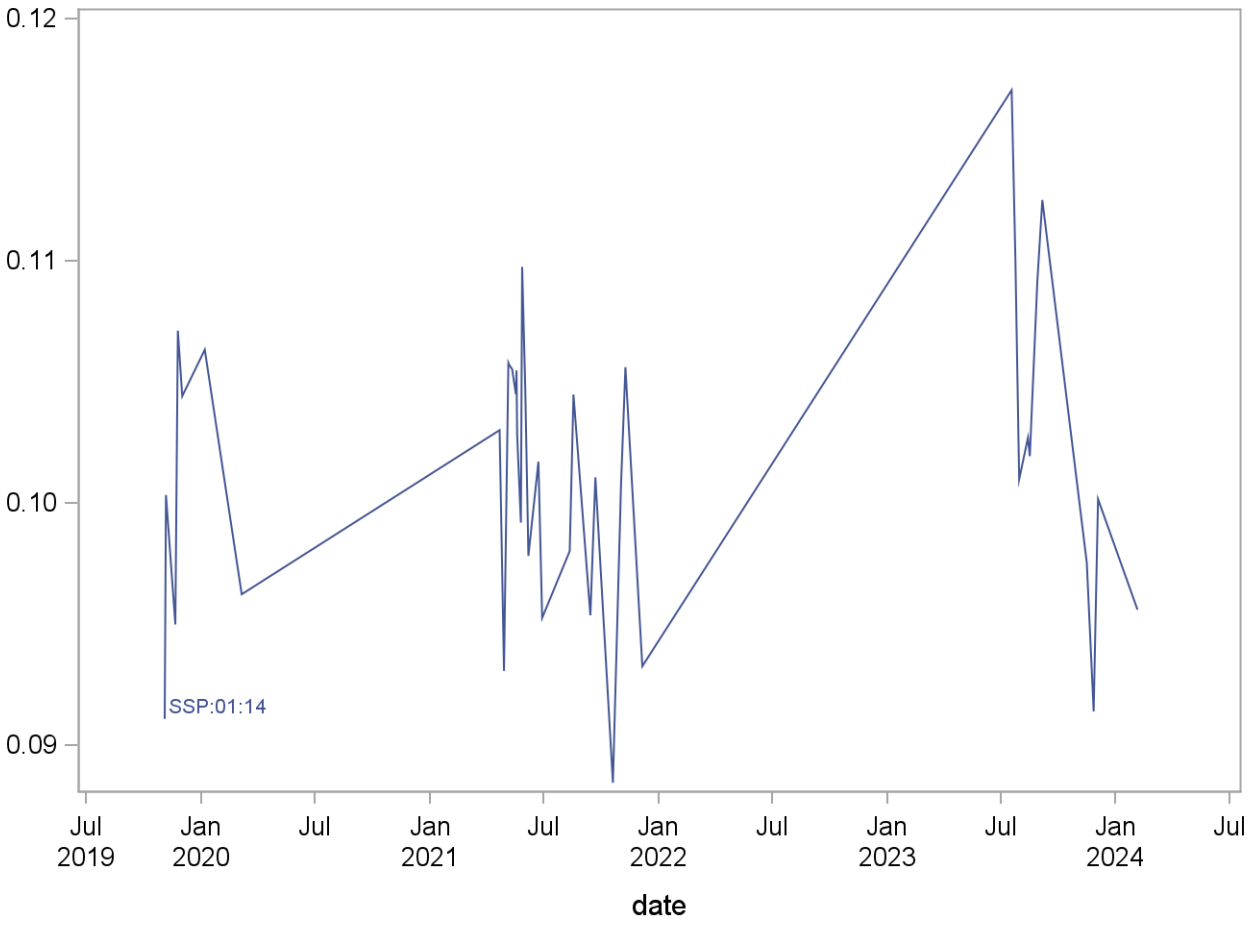
2015-2016 Summary Statistics and QC Chart
LBCF01 (2,3,7,8-Tetrachlorofuran (tcdf) (fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	40	04NOV19	05FEB24	0.105145	0.007459	7.1



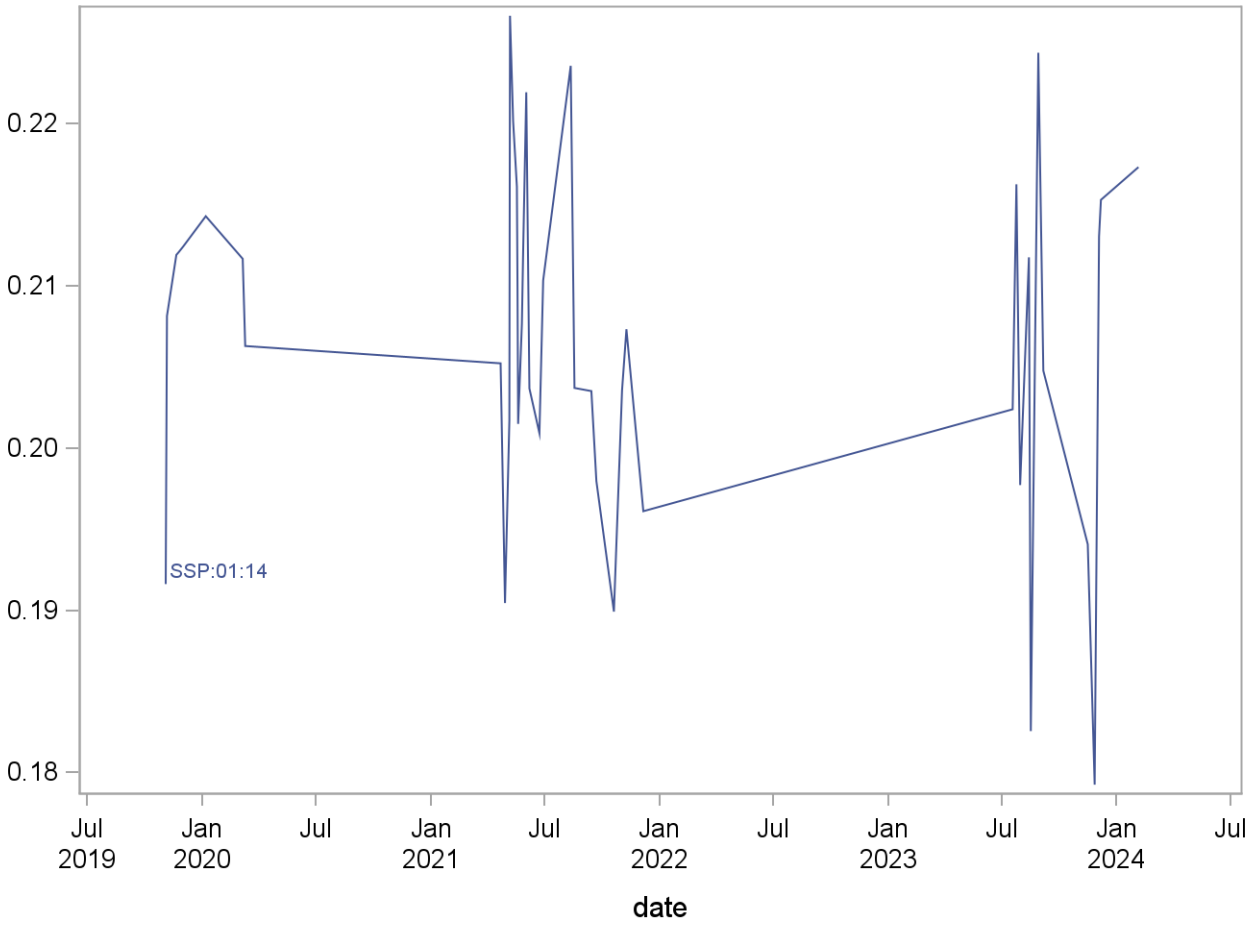
2015-2016 Summary Statistics and QC Chart
LBCF02 (1,2,3,7,8-Pentachlorofuran(pncdf)(fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	40	04NOV19	05FEB24	0.101506	0.006200	6.1



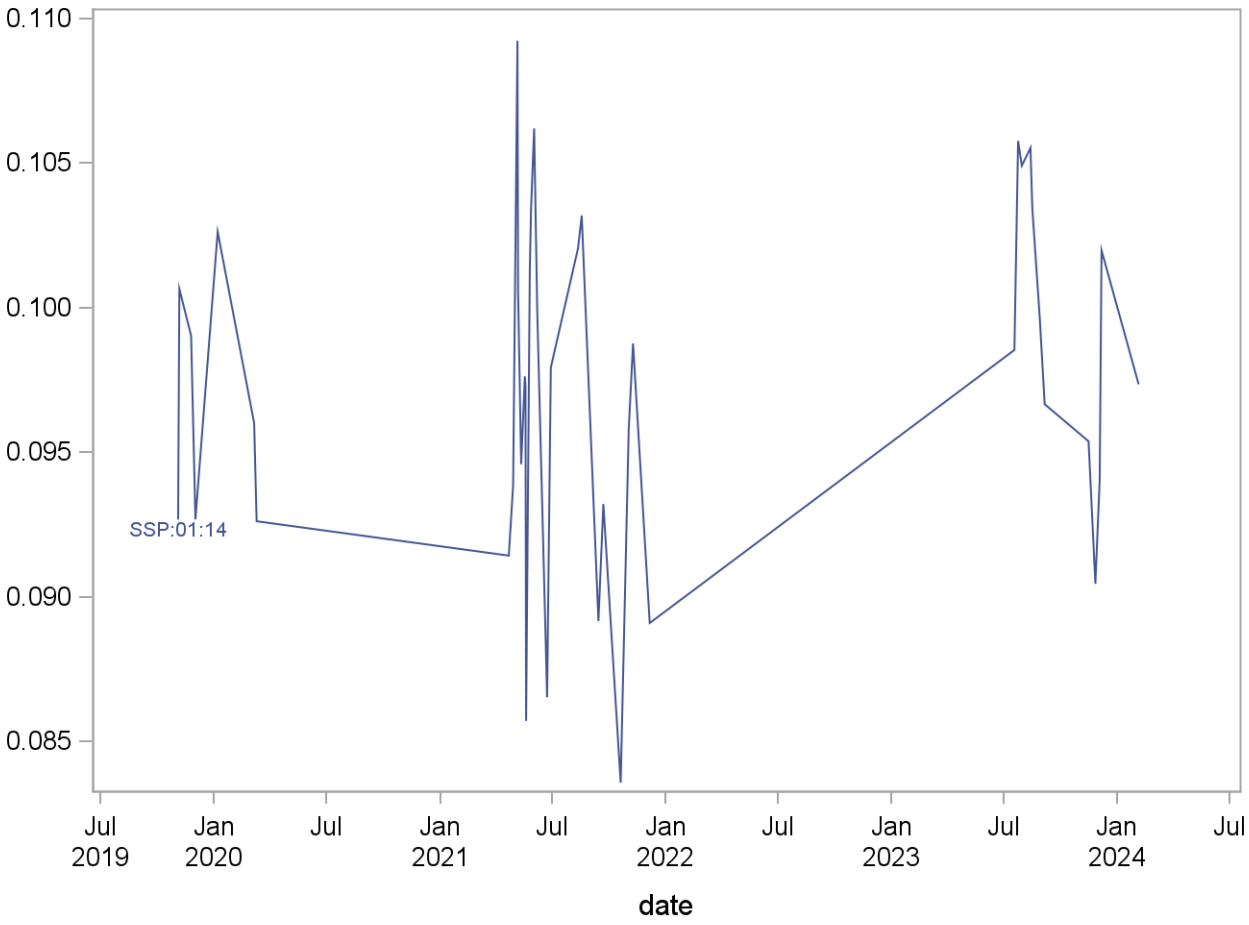
2015-2016 Summary Statistics and QC Chart
LBCF03 (2,3,4,7,8-Pentachlorofuran(pncdf)(fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	42	04NOV19	05FEB24	0.206690	0.010770	5.2



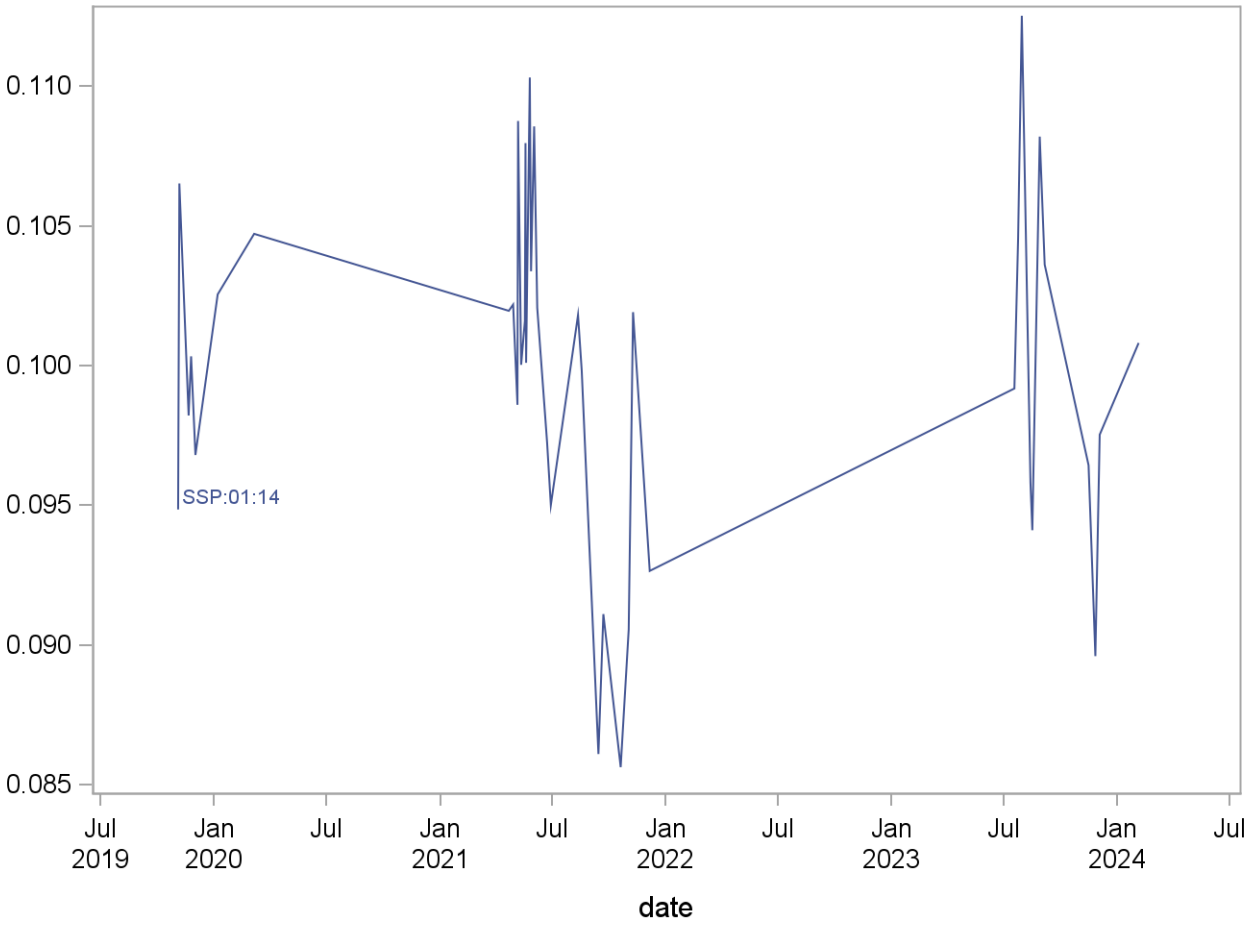
2015-2016 Summary Statistics and QC Chart
LBCF04 (1,2,3,4,7,8-hcxdf(fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	41	04NOV19	05FEB24	0.097333	0.005981	6.1



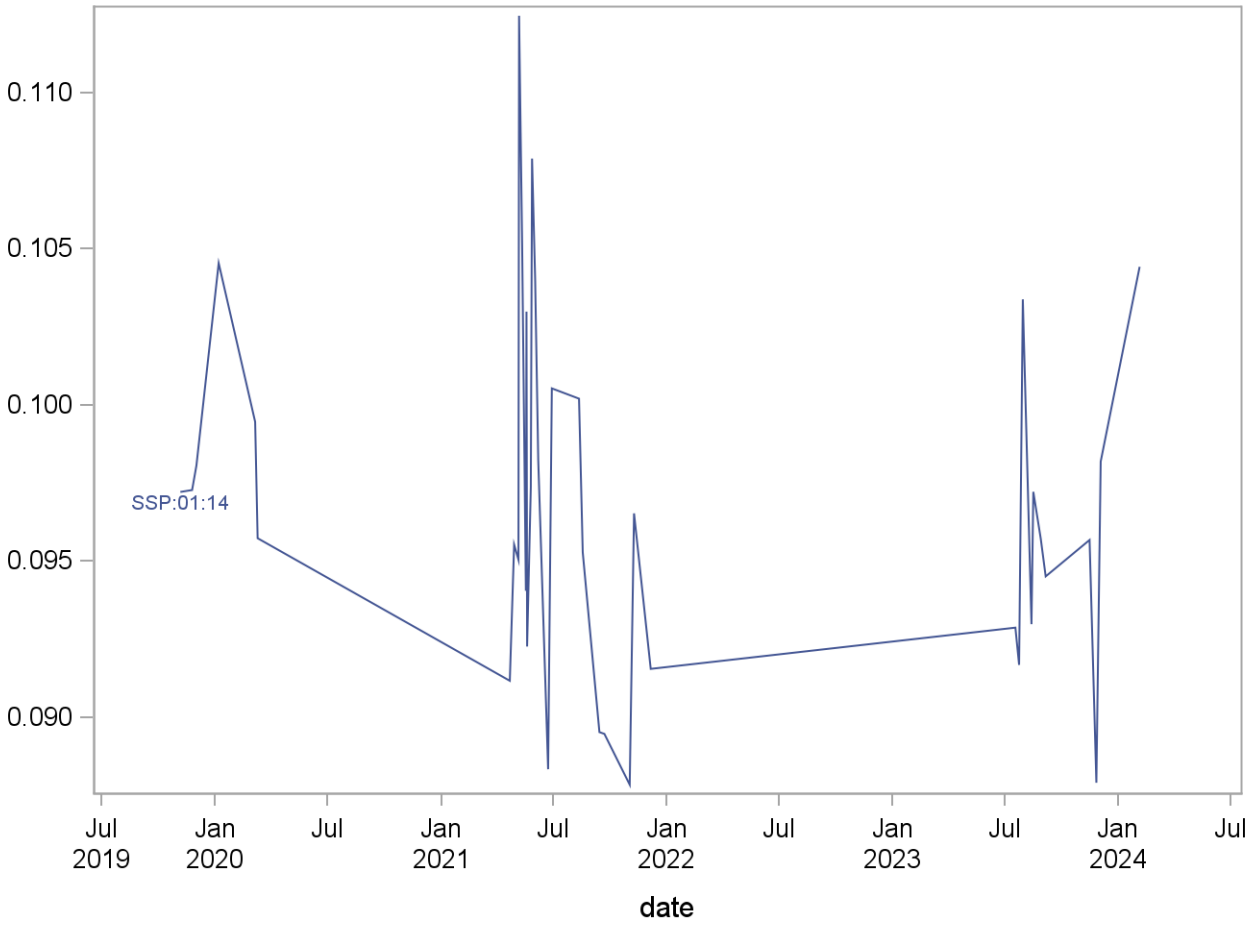
2015-2016 Summary Statistics and QC Chart
LBCF05 (1,2,3,6,7,8-hxcdf(fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	40	04NOV19	05FEB24	0.099837	0.006320	6.3



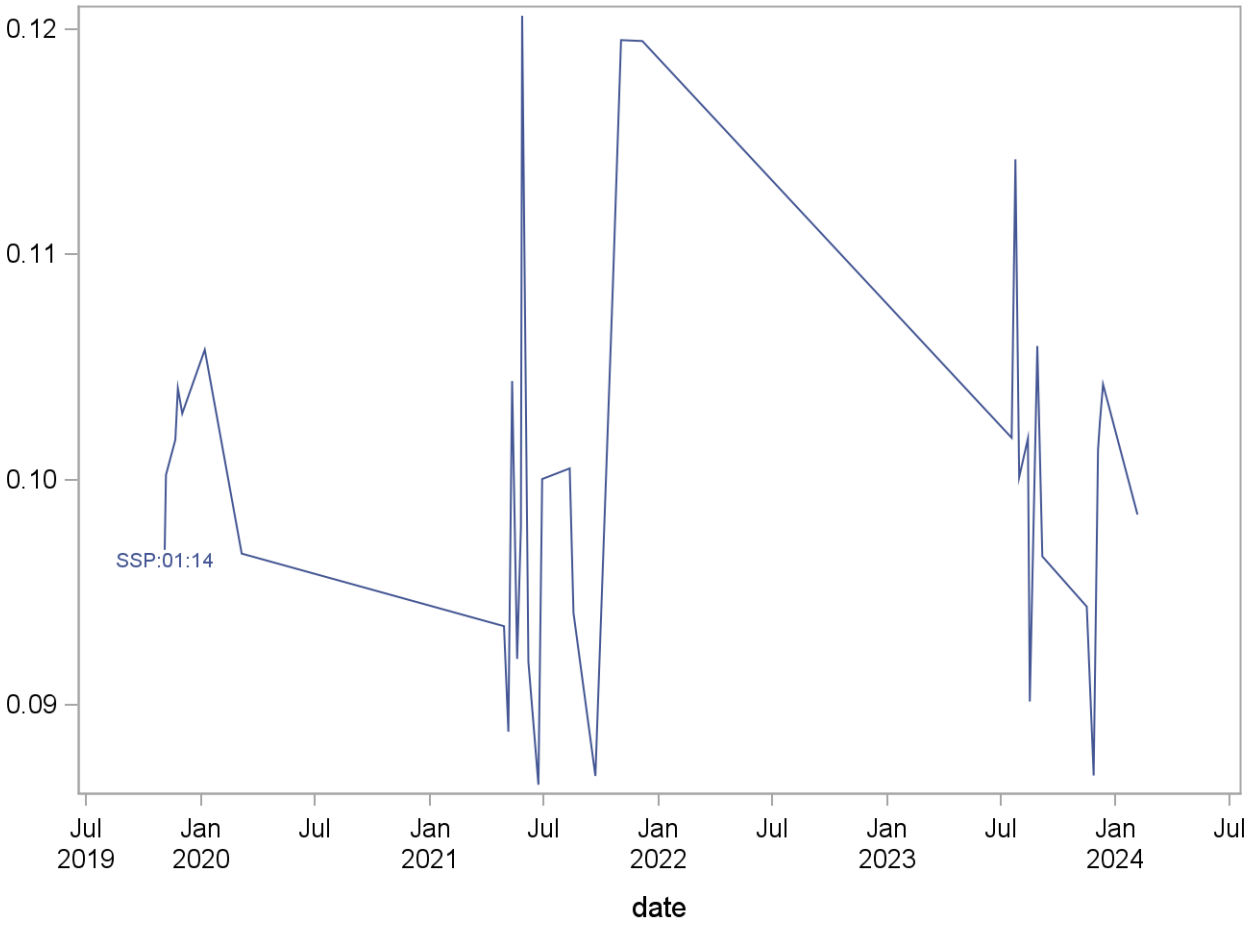
2015-2016 Summary Statistics and QC Chart
LBCF06 (1,2,3,7,8,9-Hexachlorodifuran(fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	38	06NOV19	05FEB24	0.096890	0.005753	5.9



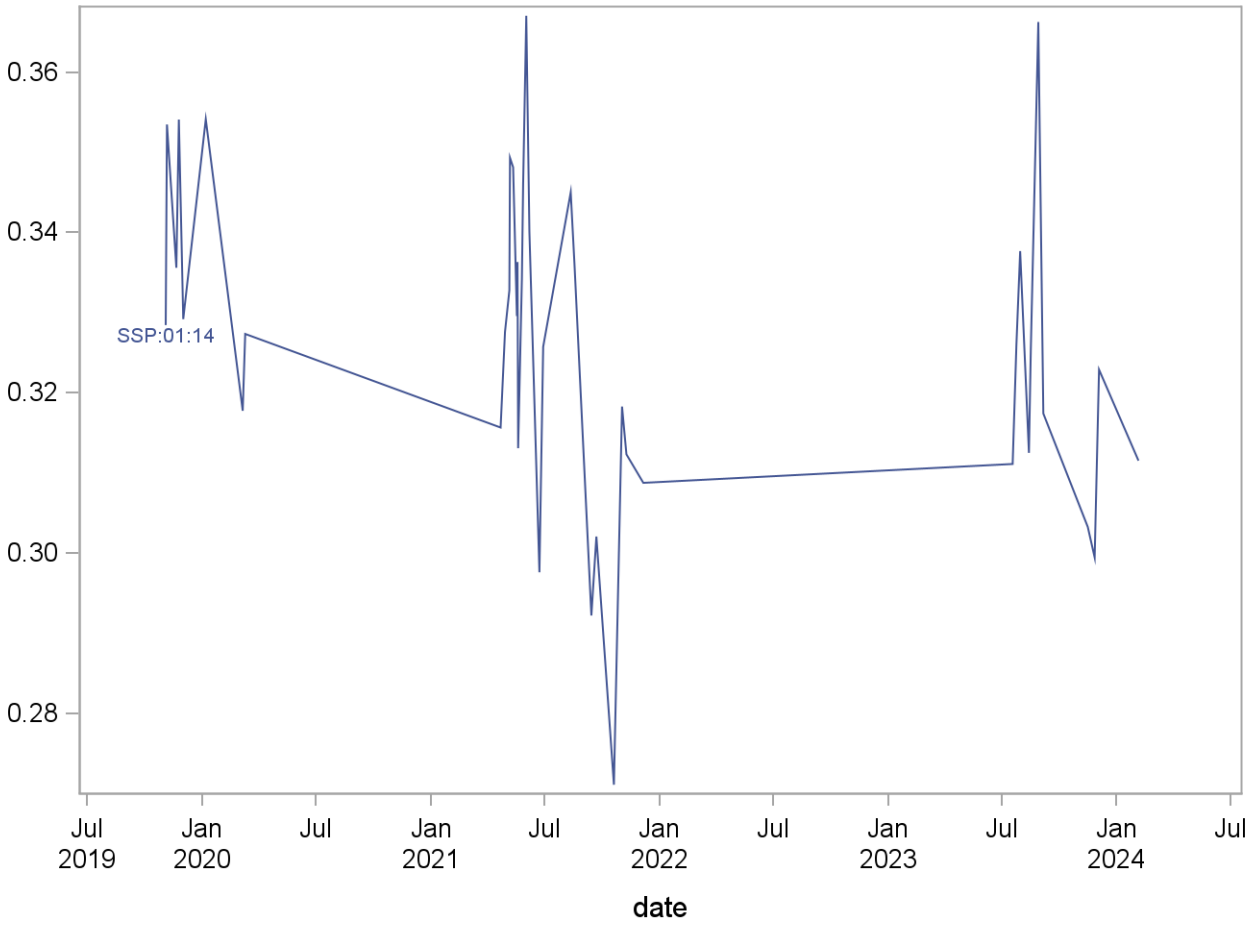
2015-2016 Summary Statistics and QC Chart
LBCF07 (2,3,4,6,7,8-Hexchlorofuran(hxcdf)(fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	35	04NOV19	05FEB24	0.100324	0.008804	8.8



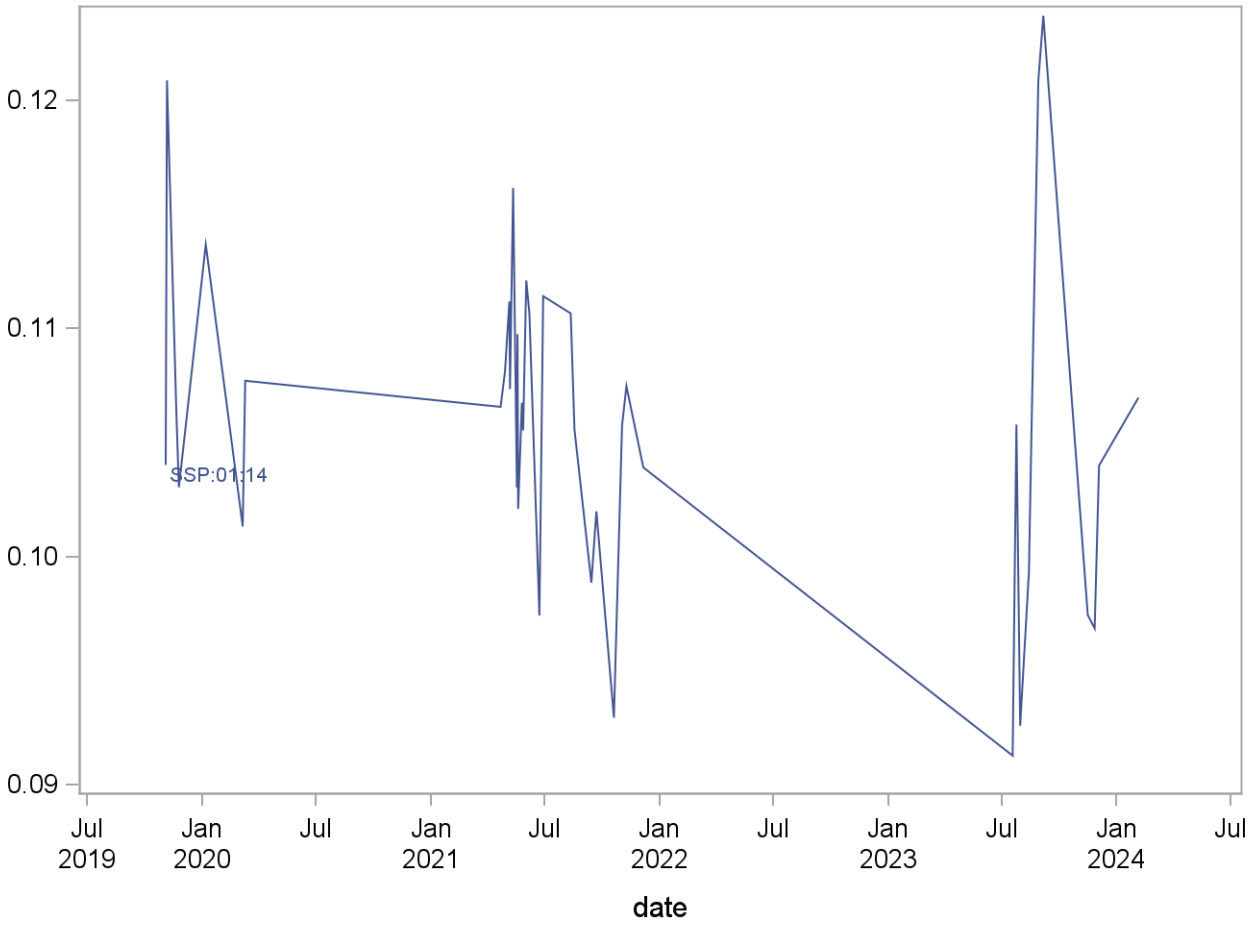
2015-2016 Summary Statistics and QC Chart
LBCF08 (1,2,3,4,6,7,8-Heptachlorodifuran (fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	41	04NOV19	05FEB24	0.326390	0.020349	6.2



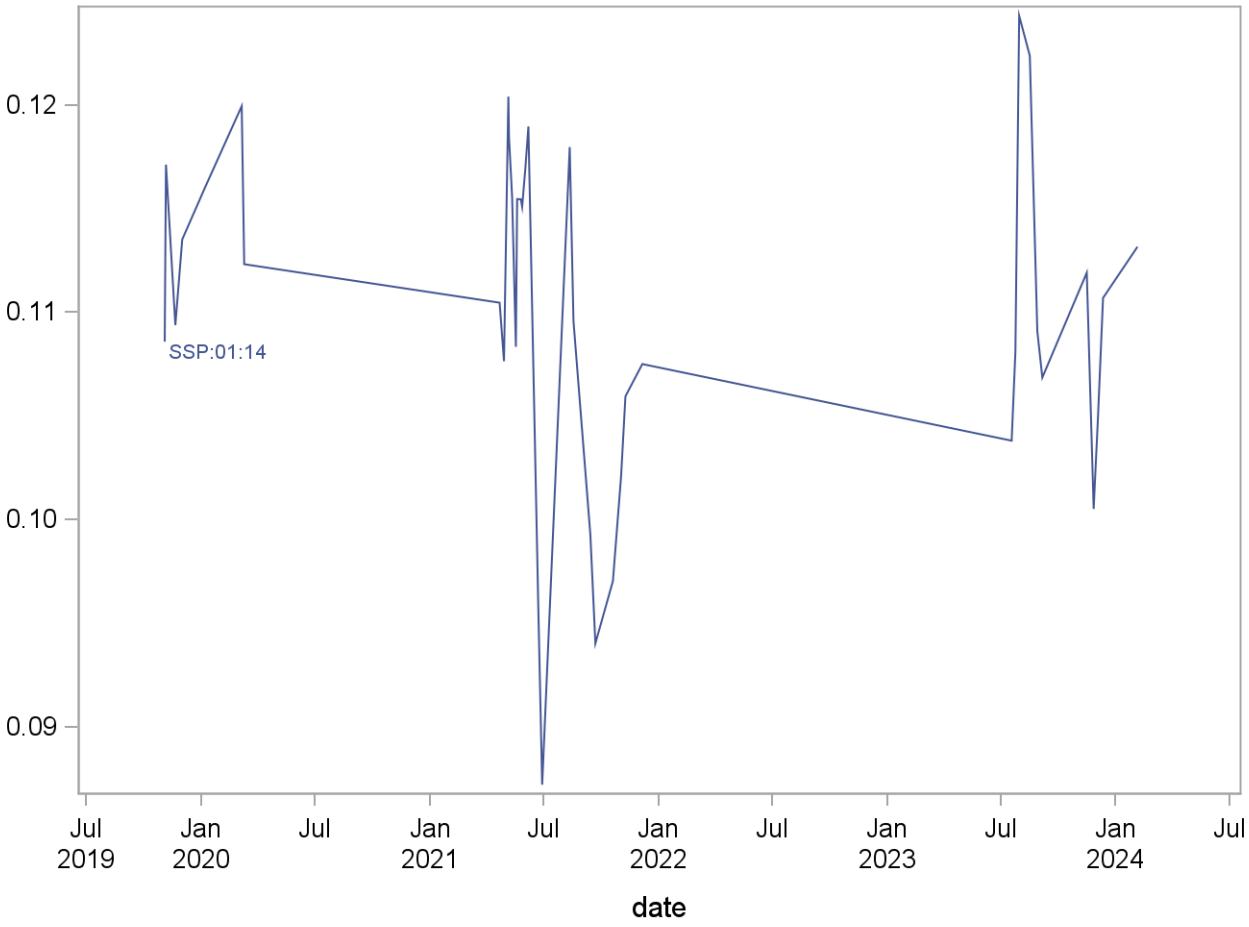
2015-2016 Summary Statistics and QC Chart
LBCF09 (1,2,3,4,7,8,9-Heptachlorodifuran (fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	41	04NOV19	05FEB24	0.105838	0.007181	6.8



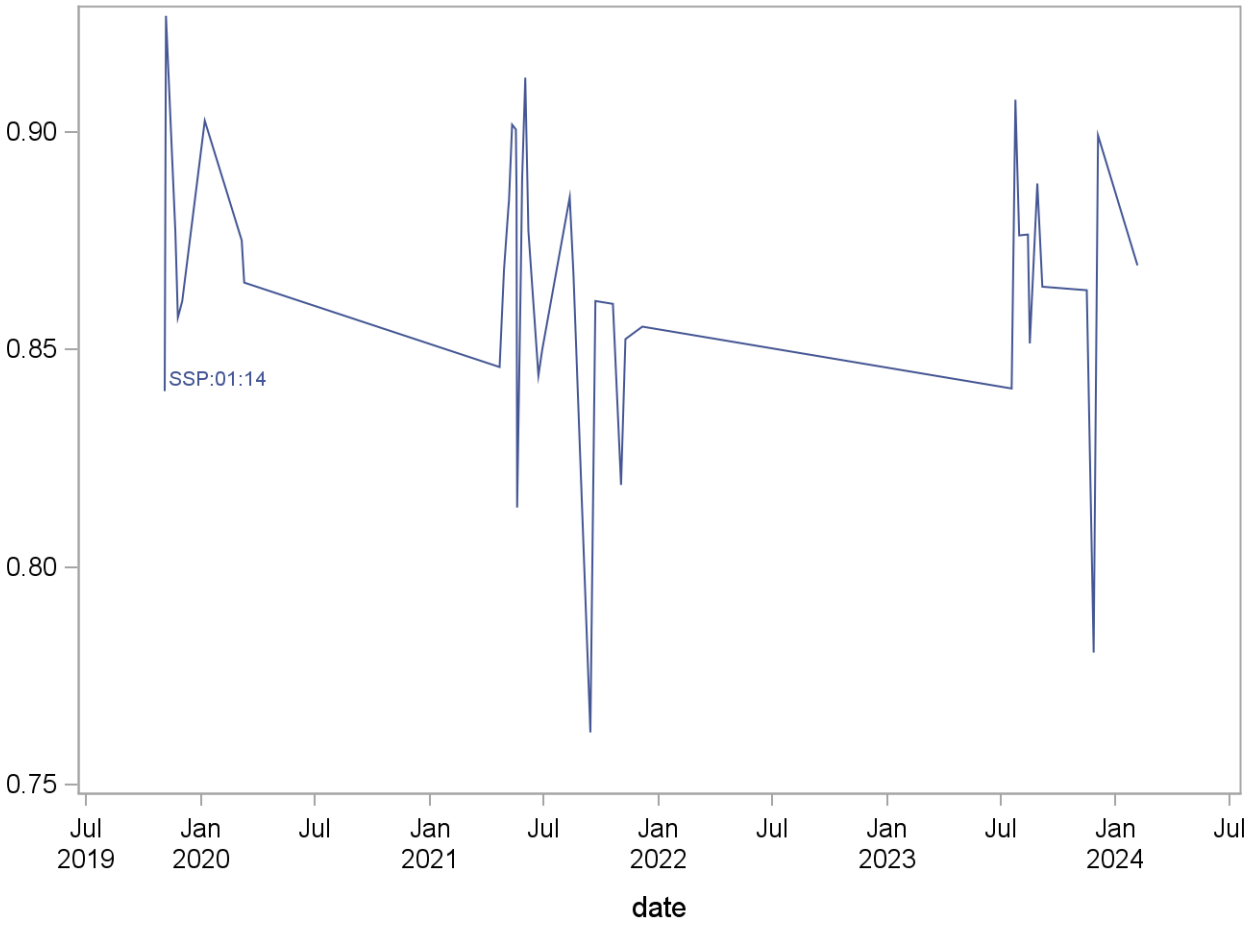
2015-2016 Summary Statistics and QC Chart
LBCF10 (1,2,3,4,6,7,8,9-Octachlorodifuran(fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	40	04NOV19	05FEB24	0.110074	0.008158	7.4



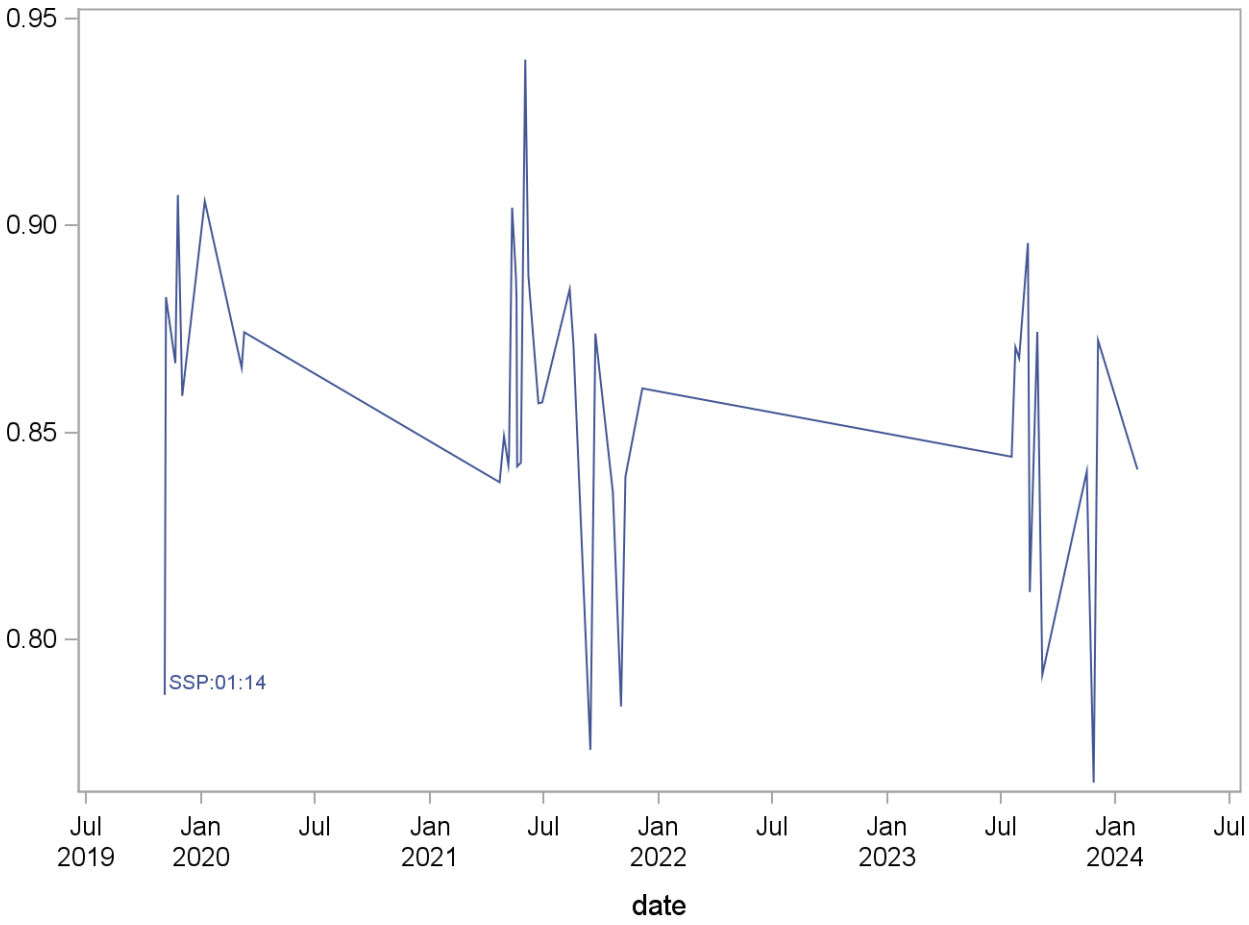
2015-2016 Summary Statistics and QC Chart
LBCHXC (3,3',4,4',5,5'-hexachlorobiphenyl(fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	41	04NOV19	05FEB24	0.866095	0.032265	3.7



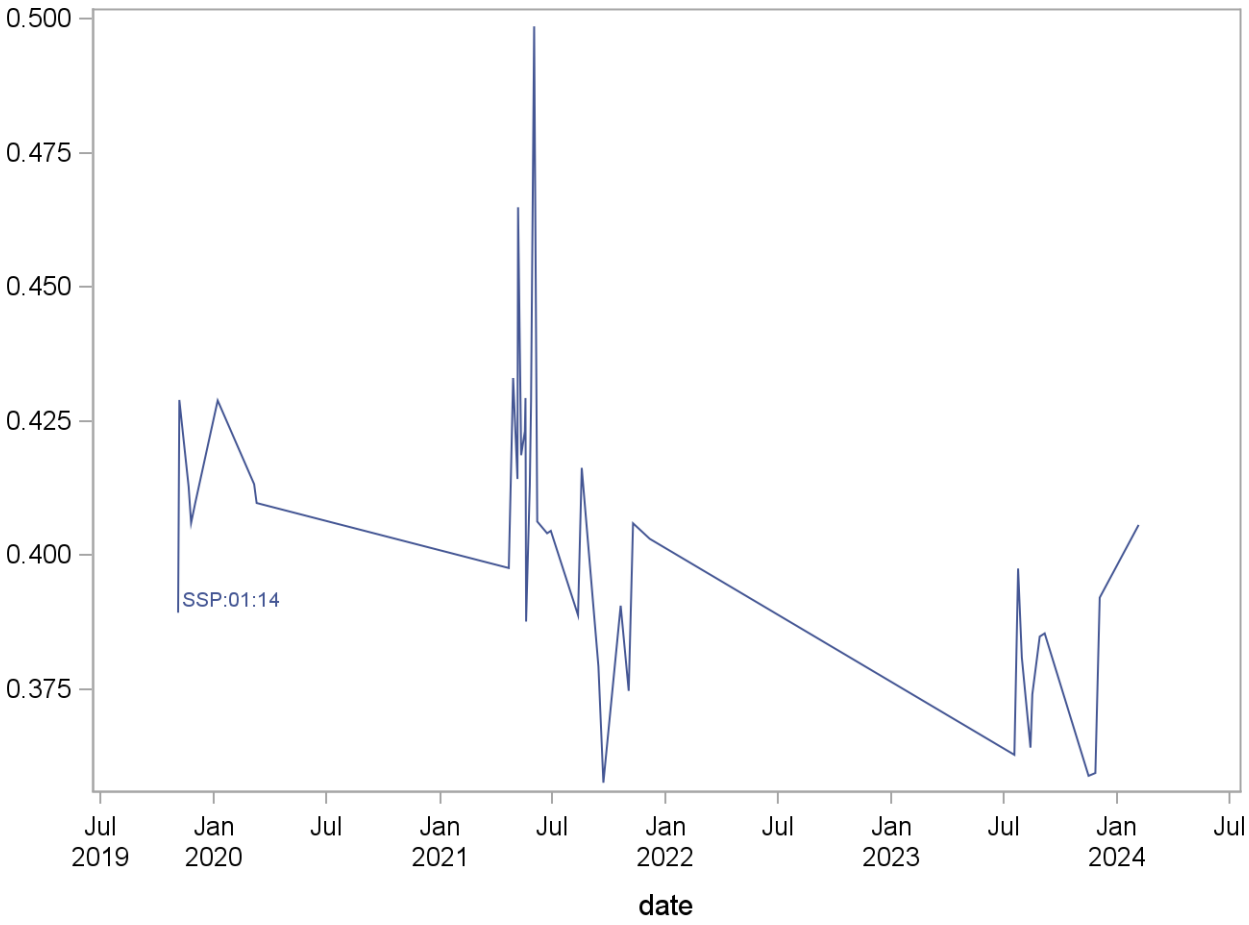
2015-2016 Summary Statistics and QC Chart
LBCPCB (3,3',4,4',5-Pentachlorobiphenyl (fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	41	04NOV19	05FEB24	0.855969	0.037524	4.4



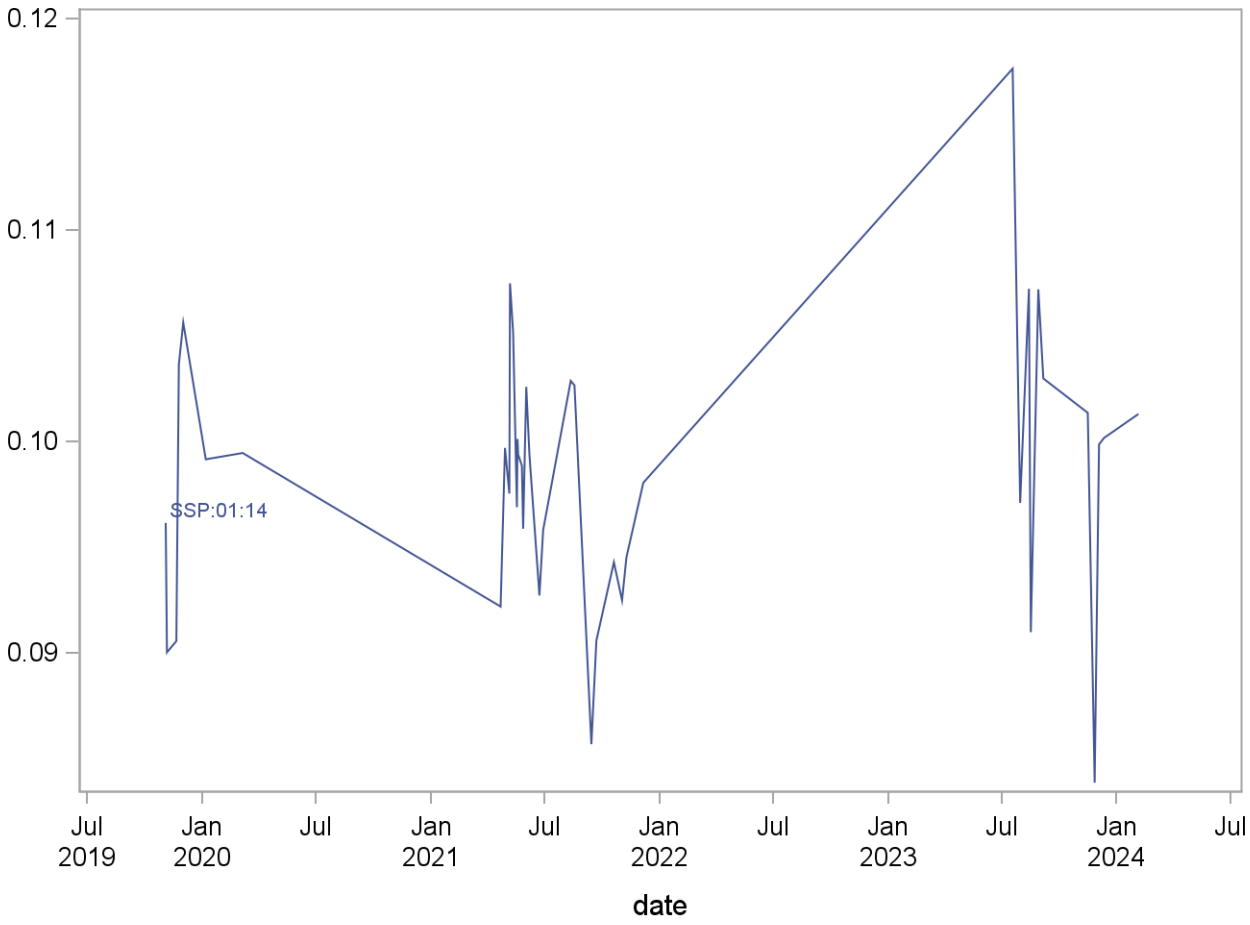
2015-2016 Summary Statistics and QC Chart
LBCTC2 (3,4,4',5-Tetrachlorobiphenyl (tcb)(fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	41	04NOV19	05FEB24	0.402537	0.027940	6.9



2015-2016 Summary Statistics and QC Chart
LBCTCD (2,3,7,8-Tetrachloro-p-dioxn(tcdd)(fg/g))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
SSP:01:14	41	04NOV19	05FEB24	0.098689	0.006511	6.6



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Appendix A: Limit of detection (LOD), specificity, fit for intended use, accuracy, precision and stability for each analyte included in the method

LOD, specificity and fit for intended use

Method name:	PCDD/F and cPCBs
Method #:	6501
Matrix:	Serum or plasma
Units:	fg/g

Analytes	Limit of Detection (LOD) [a,b]	Interferences successfully checked in at least 50 human samples	Accuracy, precision, LOD, specificity and stability meet performance specifications for intended use
2378-TeCDD	2.64	YES	YES
12378-PeCDD	3.86	YES	YES
123478-HxCDD	0.930	YES	YES
123678-HxCDD	0.733	YES	YES
123789-HxCDD	0.478	YES	YES
1234678-HpCDD	15.1	YES	YES
OCDD	81.1	YES	YES
2378-TeCDF	1.60	YES	YES
12378-PeCDF	2.90	YES	YES
23478-PeCDF	3.17	YES	YES
123478-HxCDF	1.85	YES	YES
123678-HxCDF	0.630	YES	YES
123789-HxCDF	1.26	YES	YES
234678-HxCDF	0.702	YES	YES
1234678-HpCDF	6.39	YES	YES
1234789-HpCDF	1.19	YES	YES
OCDF	8.51	YES	YES
PCB81	11.6	YES	YES
PCB126	8.20	YES	YES
PCB169	8.28	YES	YES

[a] LOD based on a serum or plasma amount of 20 gram
[b] LOD given as determined by Taylor. LOD defined as 3 standard deviations of method blanks may be higher for certain analytes.

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **2378-TeCDD**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		95.1	4.3
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	2.73	2.6	2.6	2.6	93.8	7.5	6.4	7.8	7.3	97.3		
	2		2.6	2.5				6.0	10.3				
	3	Pool: PTP1	2.6	2.5				7.2	6.1				
Sample + Spike 2	1	1.37	1.27	1.38	1.3	97.0	25	27	26	24.8	99.1		
	2		1.36	1.35				21	26				
	3	Pool: PTP2	1.37	1.21				23	26				
Sample + Spike 3	1	1.09	0.97	0.92	1	86.9	75	68	76	72	96.3		
	2		0.94	0.91				72	76				
	3	Pool: PTP3	0.95	0.99				64	77				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **12378-PeCDD**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		97.0	6.5
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	2.73	2.5	2.5	2.5	90.1	7.5	10.7	3.9	7.5	100.4		
	2		2.5	2.5				10.8	8.1				
	3	Pool: PTP1	2.3	2.5				5.0	6.7				
Sample + Spike 2	1	1.37	1.38	1.43	1.4	99.9	25	23	25	25.8	103.0		
	2		1.35	1.40				25	27				
	3	Pool: PTP2	1.21	1.40				29	24				
Sample + Spike 3	1	1.09	0.94	0.96	1	87.4	75	71	73	76	101.3		
	2		0.93	1.00				87	75				
	3	Pool: PTP3	0.90	1.00				76	73				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **123478-HxCDD**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		100.7	4.8
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	2.73	2.6	3.0	2.7	99.1	7.5	9.8	4.0	7.5	99.9		
	2		2.6	2.7				9.7	7.1				
	3	Pool: PTP1	2.7	2.6				8.6	5.7				
Sample + Spike 2	1	1.37	1.48	1.42	1.4	105.6	25	27	20	24.7	98.8		
	2		1.43	1.48				26	23				
	3	Pool: PTP2	1.30	1.53				29	24				
Sample + Spike 3	1	1.09	1.04	1.04	1	93.9	75	95	72	80	106.9		
	2		0.99	1.10				78	76				
	3	Pool: PTP3	1.01	0.98				89	71				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **123678-HxCDD**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		101.9	2.9
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	8.19	8.5	8.8	8.5	103.9	75	73.8	73.0	75.0	99.9		
	2		8.2	8.7				75.1	78.8				
	3	Pool: PTP1	8.4	8.6				74.2	74.9				
Sample + Spike 2	1	4.10	4.37	4.62	4.4	106.5	250	260	254	254.8	101.9		
	2		4.37	4.36				267	244				
	3	Pool: PTP2	4.04	4.43				269	235				
Sample + Spike 3	1	3.28	3.37	3.18	3	98.6	750	761	716	756	100.7		
	2		3.25	3.30				776	748				
	3	Pool: PTP3	3.21	3.09				785	748				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **123789-HxCDD**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		90.9	13.3
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	2.73	2.7	2.7	2.6	95.9	7.5	7.1	3.5	4.9	64.8		
	2		2.6	2.5				5.4	3.0				
	3	Pool: PTP1	2.6	2.7				7.1	3.2				
Sample + Spike 2	1	1.37	1.37	1.41	1.3	98.7	25	26	18	24.1	96.6		
	2		1.34	1.34				30	19				
	3	Pool: PTP2	1.20	1.42				32	20				
Sample + Spike 3	1	1.09	0.99	0.97	1	89.3	75	87	69	75	100.2		
	2		0.96	0.97				80	68				
	3	Pool: PTP3	0.97	1.00				78	70				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **1234678-HpCDD**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		90.7	6.8
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	16.44	15.5	15.7	15.4	93.6	75	57.0	59.8	58.6	78.1		
	2		15.1	15.6				55.3	56.5				
	3	Pool: PTP1	14.8	15.6				63.1	59.9				
Sample + Spike 2	1	8.22	7.72	8.11	7.8	94.3	250	219	230	232.2	92.9		
	2		7.71	7.86				236	230				
	3	Pool: PTP2	6.86	8.24				246	232				
Sample + Spike 3	1	6.58	6.51	5.67	6	88.5	750	755	711	728	97.1		
	2		5.80	5.51				736	723				
	3	Pool: PTP3	5.66	5.75				716	729				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **OcCDD**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		98.1	25.6
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	65.96	72.3	84.7	75.5	114.4	750	399.4	340.1	401.5	53.5		
	2		68.3	81.2				381.2	355.8				
	3	Pool: PTP1	62.0	84.2				576.4	356.0				
Sample + Spike 2	1	32.98	41.06	40.77	40.6	123.1	2500	2162	2194	2213.0	88.5		
	2		43.94	39.32				2306	2089				
	3	Pool: PTP2	37.35	41.18				2519	2008				
Sample + Spike 3	1	26.38	32.04	30.71	30	115.4	7500	7330	6663	7018	93.6		
	2		30.95	32.16				7261	6796				
	3	Pool: PTP3	26.82	29.99				7174	6885				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name:	PCDD/F and cPCBs
Method #:	6501
Matrix:	Serum
Units:	fg/g
Analyte:	**2378-TeCDF**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		101.9	3.9
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	2.73	2.8	2.8	2.8	103.0	7.5	8.6	6.4	7.6	101.6		
	2		2.8	2.7				8.0	5.6				
	3	Pool: PTP1	2.9	2.9				9.2	8.0				
Sample + Spike 2	1	1.37	1.44	1.42	1.4	103.9	25	26	26	26.4	105.7		
	2		1.41	1.42				29	26				
	3	Pool: PTP2	1.36	1.47				26	26				
Sample + Spike 3	1	1.09	1.08	1.01	1	94.5	75	80	73	77	102.6		
	2		1.06	0.98				76	74				
	3	Pool: PTP3	1.04	1.03				79	80				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **12378-PeCDF**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		93.9	3.4
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	2.73	2.5	2.7	2.6	95.5	7.5	8.6	5.8	6.9	92.6		
	2		2.5	2.7				7.0	7.3				
	3	Pool: PTP1	2.6	2.6				6.2	6.8				
Sample + Spike 2	1	1.37	1.30	1.36	1.3	98.5	25	22	24	23.2	92.6		
	2		1.31	1.36				25	22				
	3	Pool: PTP2	1.24	1.50				23	22				
Sample + Spike 3	1	1.09	0.94	0.97	1	88.5	75	72	73	72	95.8		
	2		0.93	1.00				75	69				
	3	Pool: PTP3	0.98	1.00				69	73				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **23478-PeCDF**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		101.3	7.7
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	5.46	5.1	5.5	5.2	95.2	7.5	7.5	8.9	7.4	99.2		
	2		5.1	5.3				6.9	9.4				
	3	Pool: PTP1	5.0	5.3				3.2	8.8				
Sample + Spike 2	1	2.73	2.73	2.87	2.9	104.7	25	30	26	28.7	114.8		
	2		2.79	2.86				34	26				
	3	Pool: PTP2	2.75	3.16				32	24				
Sample + Spike 3	1	2.19	2.02	2.09	2	93.7	75	85	75	75	100.4		
	2		2.01	2.09				73	70				
	3	Pool: PTP3	1.94	2.15				77	72				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **123478-HxCDF**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		99.5	6.2
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	2.73	2.7	2.7	2.7	97.1	7.5	8.0	7.2	7.9	105.2		
	2		2.6	2.7				8.0	8.7				
	3	Pool: PTP1	2.6	2.7				7.9	7.6				
Sample + Spike 2	1	1.37	1.38	1.30	1.3	98.2	25	24	26	25.8	103.2		
	2		1.36	1.35				28	25				
	3	Pool: PTP2	1.19	1.47				27	25				
Sample + Spike 3	1	1.09	0.99	0.97	1	88.8	75	80	74	78	104.5		
	2		0.97	0.95				82	78				
	3	Pool: PTP3	0.97	0.98				79	77				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **123678-HxCDF**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		97.2	2.9
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	2.73	2.7	2.7	2.6	96.7	7.5	8.1	5.6	7.1	94.7		
	2		2.7	2.6				6.8	7.2				
	3	Pool: PTP1	2.5	2.7				8.3	6.6				
Sample + Spike 2	1	1.37	1.35	1.39	1.4	101.1	25	24	23	24.5	98.1		
	2		1.37	1.44				26	24				
	3	Pool: PTP2	1.29	1.44				27	23				
Sample + Spike 3	1	1.09	1.03	1.03	1	93.1	75	80	71	74	99.2		
	2		1.05	0.99				74	74				
	3	Pool: PTP3	1.02	0.98				73	73				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **123789-HxCDF**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		99.6	7.3
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	2.73	2.4	2.6	2.5	91.2	7.5	6.9	7.5	8.0	107.1		
	2		2.3	2.5				9.2	8.8				
	3	Pool: PTP1	2.4	2.6				8.4	7.3				
Sample + Spike 2	1	1.37	1.35	1.36	1.3	98.8	25	27	24	26.5	106.1		
	2		1.37	1.39				27	26				
	3	Pool: PTP2	1.21	1.42				29	25				
Sample + Spike 3	1	1.09	1.08	0.96	1	90.6	75	80	76	78	103.8		
	2		1.01	0.91				80	76				
	3	Pool: PTP3	0.97	1.00				79	77				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **234678-HxCDF**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		98.1	4.6
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	3.63	3.5	3.6	3.5	96.0	7.5	7.1	7.7	7.6	101.1		
	2		3.4	3.5				7.8	8.0				
	3	Pool: PTP1	3.4	3.5				7.0	7.9				
Sample + Spike 2	1	1.82	1.78	1.76	1.8	97.7	25	24	26	25.0	99.9		
	2		1.78	1.79				25	27				
	3	Pool: PTP2	1.62	1.91				24	24				
Sample + Spike 3	1	1.45	1.39	1.29	1	90.4	75	79	74	78	103.6		
	2		1.34	1.26				78	75				
	3	Pool: PTP3	1.33	1.27				79	81				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **1234678-HpCDF**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		104.8	3.5
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	8.19	8.7	9.3	8.8	107.7	75	76.2	77.6	76.8	102.4		
	2		8.6	8.9				75.8	77.2				
	3	Pool: PTP1	8.5	8.8				75.0	79.1				
Sample + Spike 2	1	4.10	4.47	4.70	4.5	110.5	250	256	261	258.7	103.5		
	2		4.51	4.59				252	251				
	3	Pool: PTP2	4.08	4.81				267	266				
Sample + Spike 3	1	3.28	3.42	3.31	3	101.3	750	783	769	774	103.2		
	2		3.37	3.25				762	784				
	3	Pool: PTP3	3.37	3.20				775	771				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **1234789-HpCDF**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		105.6	5.1
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	2.73	3.0	3.0	2.9	105.6	7.5	7.8	6.5	7.8	103.4		
	2		2.9	2.8				8.6	8.5				
	3	Pool: PTP1	2.8	2.9				7.5	7.6				
Sample + Spike 2	1	1.37	1.49	1.65	1.6	115.4	25	25	26	25.3	101.2		
	2		1.55	1.67				25	25				
	3	Pool: PTP2	1.35	1.75				26	25				
Sample + Spike 3	1	1.09	1.15	1.03	1	102.6	75	87	77	79	105.4		
	2		1.14	1.17				77	76				
	3	Pool: PTP3	1.10	1.14				80	77				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **OcCDF**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		104.5	6.9
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	2.73	3.2	3.1	3.0	110.8	7.5	7.5	6.5	7.2	95.7		
	2		2.8	3.0				9.9	5.9				
	3	Pool: PTP1	3.0	3.0				6.8	6.6				
Sample + Spike 2	1	1.37	1.50	1.62	1.5	109.7	25	27	24	23.9	95.8		
	2		1.50	1.50				27	24				
	3	Pool: PTP2	1.36	1.51				18	23				
Sample + Spike 3	1	1.09	1.35	1.05	1	108.6	75	80	73	80	106.6		
	2		1.24	1.10				102	71				
	3	Pool: PTP3	1.19	1.19				79	74				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **PCB-81**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		96.8	4.7
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	10.93	10.2	10.3	10.2	93.7	75	73.3	67.7	78.0	104.0		
	2		10.0	10.2				81.8	92.7				
	3	Pool: PTP1	10.2	10.6				77.8	74.9				
Sample + Spike 2	1	5.46	5.49	5.31	5.3	96.4	250	251	244	248.3	99.3		
	2		5.24	5.35				249	248				
	3	Pool: PTP2	4.79	5.42				254	244				
Sample + Spike 3	1	4.37	3.42	4.25	4	90.2	750	715	741	728	97.1		
	2		3.86	4.18				710	747				
	3	Pool: PTP3	3.76	4.20				737	720				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **PCB-126**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		101.0	2.5
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	29.05	28.7	28.8	28.4	97.9	75	80.7	74.6	76.5	102.0		
	2		27.9	28.2				77.3	75.8				
	3	Pool: PTP1	29.1	28.0				80.6	69.9				
Sample + Spike 2	1	14.53	14.96	14.77	14.8	101.6	250	257	253	260.6	104.3		
	2		15.74	14.69				477	241				
	3	Pool: PTP2	13.09	15.33				93	242				
Sample + Spike 3	1	11.62	14.40	10.92	11	98.1	750	814	730	766	102.2		
	2		10.70	10.64				794	708				
	3	Pool: PTP3	10.90	10.86				825	726				

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: PCDD/F and cPCBs
Method #: 6501
Matrix: Serum
Units: fg/g
Analyte: **PCB-169**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0	0	0.0		0	0	0	0.0		97.9	3.1
	2		0	0				0	0				
	3		0	0				0	0				
Sample + Spike 1	1	29.05	27.5	28.6	27.9	95.9	75	76.0	76.4	75.1	100.1		
	2		27.1	28.0				76.2	78.0				
	3	Pool: PTP1	27.1	28.9				71.0	73.0				
Sample + Spike 2	1	14.53	14.74	14.48	14.5	99.8	250	254	235	246.7	98.7		
	2		14.58	14.56				254	239				
	3	Pool: PTP2	13.53	15.10				252	246				
Sample + Spike 3	1	11.62	10.97	10.71	11	92.6	750	770	735	752	100.3		
	2		10.62	10.76				772	732				
	3	Pool: PTP3	10.54	10.98				770	734				

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**2378-TeCDD**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	2.21	2.26	2.23	0.000538219	0.000538219	9.985249103
2	2.15	2.26	2.20	0.002896988	0.002896988	9.719744185
3	2.31	2.22	2.27	0.001994148	0.001994148	10.29265194
4	2.45	2.48	2.46	0.000221258	0.000221258	12.14232562
5	2.30	2.27	2.28	0.000153657	0.000153657	10.44167854
6	2.22	2.87	2.54	0.106338773	0.106338773	12.9322836
7	3.15	2.50	2.83	0.106362189	0.106362189	15.9644999
8	3.28	2.82	3.05	0.053901877	0.053901877	18.6311938
9	2.40	2.53	2.46	0.003926257	0.003926257	12.14019285
10	2.07	2.27	2.17	0.00992344	0.00992344	9.38229636

Grand sum	49.01265449	**Grand mean**	2.450632724

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.572513611	0.057251361	0.239272566	9.76
Between Run	1.520100903	0.1689001	0.236271813	9.64
Total	2.092614514		0.33626735	**13.72**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	1.19	1.14	1.16	0.000562003	0.000562003	2.697349433
2	0.88	0.82	0.85	0.000735198	0.000735198	1.439994295
3	1.18	1.24	1.21	0.001191739	0.001191739	2.930264321
4	1.19	0.96	1.07	0.012323005	0.012323005	2.306934189
5	1.03	0.99	1.01	0.00026263	0.00026263	2.040062624
6	0.89	0.89	0.89	8.65247E-07	8.65247E-07	1.586718261
7	1.02	1.01	1.02	3.86247E-06	3.86247E-06	2.067168031
8	0.97	0.97	0.97	7.0525E-06	7.0525E-06	1.876589129
9	1.07	1.08	1.08	4.93586E-05	4.93586E-05	2.318204639
10	0.93	0.95	0.94	0.000200601	0.000200601	1.768957567

Grand sum	20.39467852	**Grand mean**	1.019733926

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.030672629	0.003067263	0.055382875	5.43
Between Run	0.235096898	0.026121878	0.107365298	10.53
Total	0.265769526		0.120807989	**11.85**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**12378-PeCDD**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	2.22	2.48	2.35	0.016910476	0.016910476	11.06472154
2	2.40	2.39	2.40	2.46562E-05	2.46562E-05	11.51808837
3	2.52	2.39	2.45	0.00426389	0.00426389	12.02800467
4	2.33	2.41	2.37	0.001394114	0.001394114	11.22801409
5	2.46	2.43	2.44	0.000342533	0.000342533	11.95140492
6	2.31	3.35	2.83	0.268164761	0.268164761	15.99957054
7	3.17	2.69	2.93	0.057614501	0.057614501	17.17613122
8	2.96	2.56	2.76	0.039330897	0.039330897	15.20635015
9	2.74	2.49	2.61	0.016175791	0.016175791	13.6680846
10	2.60	2.30	2.45	0.022000696	0.022000696	12.00748988

Grand sum	51.1978558	**Grand mean**	2.55989279

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.85244463	0.085244463	0.291966544	11.41
Between Run	0.786838044	0.087426449	0.033030186	1.29
Total	1.639282673		0.293828957	**11.48**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	1.25	1.10	1.17	0.005300186	0.005300186	2.751027917
2	0.92	0.98	0.95	0.00079695	0.00079695	1.807995938
3	1.34	1.32	1.33	0.000111842	0.000111842	3.530537327
4	1.17	1.05	1.11	0.003702929	0.003702929	2.481809303
5	1.00	0.90	0.95	0.002272017	0.002272017	1.804382494
6	0.98	1.18	1.08	0.010061081	0.010061081	2.323867315
7	1.00	1.08	1.04	0.001751927	0.001751927	2.158567534
8	1.03	1.00	1.02	0.000188614	0.000188614	2.06681998
9	1.02	1.06	1.04	0.000313244	0.000313244	2.172133717
10	0.96	0.95	0.95	5.39926E-05	5.39926E-05	1.815531523

Grand sum	21.2886767	**Grand mean**	1.064433835

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.049105565	0.004910556	0.070075363	6.58
Between Run	0.252285261	0.028031696	0.107520089	10.10
Total	0.301390826		0.128339885	**12.06**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**123478-HxCDD**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	2.40	2.43	2.41	0.000163042	0.000163042	11.6385771
2	2.11	1.95	2.03	0.006549949	0.006549949	8.238401215
3	2.16	2.08	2.12	0.001803959	0.001803959	8.987166787
4	2.30	2.36	2.33	0.000879876	0.000879876	10.85654976
5	2.46	2.32	2.39	0.004965477	0.004965477	11.41527496
6	2.33	3.02	2.67	0.118164654	0.118164654	14.29354279
7	2.84	2.19	2.51	0.105467345	0.105467345	12.61005878
8	3.05	2.60	2.83	0.050074205	0.050074205	16.00378589
9	2.20	2.26	2.23	0.00085737	0.00085737	9.914132803
10	2.33	2.17	2.25	0.006406919	0.006406919	10.14740453

Grand sum	47.54532836	**Grand mean**	2.377266418

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.590665592	0.059066559	0.243036127	10.22
Between Run	1.07698217	0.119664686	0.174066261	7.32
Total	1.667647763		0.298940834	**12.57**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	1.07	1.06	1.06	5.73008E-06	5.73008E-06	2.260038652
2	0.91	0.85	0.88	0.00113405	0.00113405	1.547834724
3	1.14	1.16	1.15	9.04421E-05	9.04421E-05	2.625365675
4	1.03	1.07	1.05	0.000516739	0.000516739	2.209522942
5	0.90	0.94	0.92	0.000487238	0.000487238	1.688282642
6	0.91	0.94	0.93	0.00021413	0.00021413	1.718746841
7	1.15	1.17	1.16	9.03715E-05	9.03715E-05	2.687988014
8	1.16	1.16	1.16	1.03171E-06	1.03171E-06	2.687750772
9	1.34	1.33	1.33	3.15426E-05	3.15426E-05	3.55939224
10	1.12	1.06	1.09	0.000962576	0.000962576	2.372499287

Grand sum	21.45422481	**Grand mean**	1.07271124

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.007067702	0.00070677	0.02658515	2.48
Between Run	0.343233684	0.038137076	0.136803337	12.75
Total	0.350301387		0.13936256	**12.99**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**123678-HxCDD**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	7.18	6.91	7.04	0.017419473	0.017419473	99.22867675
2	7.00	6.81	6.90	0.008689177	0.008689177	95.31155392
3	7.15	7.51	7.33	0.032281389	0.032281389	107.4223575
4	7.54	7.08	7.31	0.053167168	0.053167168	106.9326662
5	7.00	7.69	7.35	0.119243059	0.119243059	107.9688735
6	6.68	9.02	7.85	1.369376884	1.369376884	123.3485507
7	9.82	7.40	8.61	1.467547508	1.467547508	148.3471366
8	10.24	8.12	9.18	1.131081745	1.131081745	168.5731529
9	7.48	7.76	7.62	0.020038217	0.020038217	116.217373
10	6.93	7.19	7.06	0.017047723	0.017047723	99.65296282

Grand sum 152.5269997 **Grand mean** 7.626349986

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	8.471784685	0.847178469	0.920422983	12.07
Between Run	9.779021717	1.086557969	0.345962064	4.54
Total	18.2508064		0.983294574	**12.89**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	3.48	3.29	3.39	0.008361078	0.008361078	22.92770268
2	2.77	2.90	2.83	0.004117399	0.004117399	16.04185889
3	3.81	3.84	3.82	0.000194569	0.000194569	29.22319229
4	3.41	3.19	3.30	0.012788379	0.012788379	21.77309422
5	3.00	2.97	2.99	0.000170075	0.000170075	17.84815309
6	2.81	2.94	2.88	0.004545209	0.004545209	16.55162228
7	3.65	3.73	3.69	0.001820589	0.001820589	27.24509029
8	3.38	3.49	3.43	0.003064561	0.003064561	23.54536433
9	3.55	3.56	3.56	3.03809E-05	3.03809E-05	25.29194274
10	3.25	3.32	3.28	0.001397445	0.001397445	21.57261279

Grand sum 66.332821 **Grand mean** 3.31664105

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.072979374	0.007297937	0.085427966	2.58
Between Run	2.018476498	0.224275166	0.329376099	9.93
Total	2.091455872		0.340274231	**10.26**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**123789-HxCDD**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	2.10	2.16	2.13	0.000834841	0.000834841	9.083837443
2	2.15	2.13	2.14	0.000119239	0.000119239	9.151579936
3	2.08	2.13	2.10	0.000448989	0.000448989	8.860615026
4	2.24	2.16	2.20	0.00147913	0.00147913	9.703657693
5	2.28	2.32	2.30	0.00048436	0.00048436	10.5985198
6	2.20	2.85	2.53	0.105120493	0.105120493	12.78492357
7	2.78	2.26	2.52	0.069648283	0.069648283	12.70193157
8	2.82	2.42	2.62	0.039734361	0.039734361	13.73325866
9	2.23	2.27	2.25	0.000325422	0.000325422	10.11273423
10	2.29	2.18	2.23	0.003314788	0.003314788	9.973620188

Grand sum	46.06087746	**Grand mean**	2.303043873

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.443019811	0.044301981	0.210480358	9.14
Between Run	0.624456513	0.069384057	0.111986776	4.86
Total	1.067476324		0.238417741	**10.35**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	1.12	1.04	1.08	0.001341838	0.001341838	2.328171569
2	0.79	0.76	0.78	0.00019106	0.00019106	1.207019699
3	1.20	1.12	1.16	0.001895671	0.001895671	2.687876174
4	1.02	0.97	1.00	0.000772086	0.000772086	1.988268281
5	0.88	0.91	0.89	0.000158291	0.000158291	1.600159916
6	0.87	0.92	0.90	0.000621775	0.000621775	1.604221125
7	0.99	0.99	0.99	1.10853E-06	1.10853E-06	1.962281081
8	0.96	1.00	0.98	0.000428138	0.000428138	1.923125674
9	1.03	1.03	1.03	1.95426E-07	1.95426E-07	2.134109783
10	0.99	0.96	0.97	0.000142975	0.000142975	1.898128048

Grand sum	19.56102505	**Grand mean**	0.978051252

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.011106275	0.001110627	0.033326078	3.41
Between Run	0.201676308	0.022408479	0.103193632	10.55
Total	0.212782582		0.108441473	**11.09**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**1234678-HpCDD**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	13.86	13.75	13.81	0.003106677	0.003106677	381.3070826
2	13.16	11.69	12.42	0.537434755	0.537434755	308.7520027
3	12.36	12.46	12.41	0.002362239	0.002362239	308.0931375
4	12.60	13.59	13.09	0.246784886	0.246784886	342.9068919
5	14.35	12.59	13.47	0.781059409	0.781059409	362.8662468
6	12.69	16.77	14.73	4.163262944	4.163262944	434.1787714
7	17.66	13.14	15.40	5.09903638	5.09903638	474.4172401
8	18.54	15.48	17.01	2.354228169	2.354228169	578.7011806
9	13.99	13.97	13.98	0.000187927	0.000187927	390.9143443
10	12.68	12.93	12.80	0.015699275	0.015699275	327.8018024

Grand sum	278.2733043	**Grand mean**	13.91366521

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	26.40632532	2.640632532	1.625002318	11.68
Between Run	38.13710681	4.237456312	0.893538969	6.42
Total	64.54343213		1.854466075	**13.33**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	6.16	6.21	6.18	0.000617547	0.000617547	76.50777699
2	5.05	5.11	5.08	0.00094587	0.00094587	51.56981154
3	6.39	6.66	6.53	0.018087891	0.018087891	85.19637401
4	5.93	6.09	6.01	0.006717726	0.006717726	72.20859478
5	5.63	5.43	5.53	0.010327577	0.010327577	61.21856416
6	4.77	4.53	4.65	0.01408113	0.01408113	43.18880822
7	5.80	6.00	5.90	0.009431545	0.009431545	69.65644582
8	5.53	5.56	5.54	0.000293971	0.000293971	61.46890303
9	6.92	6.70	6.81	0.011463144	0.011463144	92.72715145
10	5.60	5.55	5.57	0.000565907	0.000565907	62.1465496

Grand sum	115.613291	**Grand mean**	5.780664552

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.145064619	0.014506462	0.120442774	2.08
Between Run	7.567326274	0.84081403	0.642770398	11.12
Total	7.712390892		0.653957373	**11.31**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**OcCDD**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	60.11	58.90	59.51	0.368142616	0.368142616	7082.032188
2	57.96	57.79	57.88	0.007732586	0.007732586	6699.063393
3	58.82	58.57	58.69	0.016031323	0.016031323	6890.100509
4	66.29	58.81	62.55	14.00207179	14.00207179	7825.15754
5	60.22	61.50	60.86	0.407828195	0.407828195	7408.34074
6	54.01	71.42	62.71	75.77570659	75.77570659	7865.707431
7	81.24	61.15	71.19	100.8971776	100.8971776	10137.03519
8	77.96	64.93	71.45	42.40460058	42.40460058	10209.2773
9	58.60	60.38	59.49	0.793236274	0.793236274	7077.281001
10	60.76	61.25	61.01	0.059929518	0.059929518	7443.994984

Grand sum	1250.672052	**Grand mean**	62.53360258

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	469.4649141	46.94649141	6.851750974	10.96
Between Run	428.9612376	47.66235973	0.598275991	0.96
Total	898.4261516		6.877821281	**11.00**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	28.11	28.21	28.16	0.00242832	0.00242832	1585.811444
2	22.19	22.43	22.31	0.014603019	0.014603019	995.4985983
3	31.35	31.74	31.54	0.039422343	0.039422343	1990.083115
4	27.97	27.25	27.61	0.132535721	0.132535721	1524.699063
5	23.75	23.97	23.86	0.0127546	0.0127546	1138.424097
6	25.42	23.92	24.67	0.560595221	0.560595221	1217.342999
7	27.16	27.85	27.50	0.118579575	0.118579575	1512.635752
8	24.87	24.14	24.50	0.133390648	0.133390648	1200.775175
9	28.95	29.82	29.39	0.191486428	0.191486428	1727.024734
10	25.89	25.50	25.70	0.037727559	0.037727559	1320.733435

Grand sum	530.4809855	**Grand mean**	26.52404928

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	2.487046866	0.248704687	0.498703004	1.88
Between Run	142.5246126	15.83606806	2.79171662	10.53
Total	145.0116594		2.835910149	**10.69**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**2378-TeCDF**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	2.67	2.63	2.65	0.000440788	0.000440788	14.051616
2	2.43	2.32	2.38	0.00268108	0.00268108	11.28428444
3	2.81	2.62	2.72	0.009397574	0.009397574	14.76207149
4	2.55	2.69	2.62	0.005338766	0.005338766	13.74085447
5	2.78	2.87	2.83	0.002378215	0.002378215	15.96620804
6	2.53	3.11	2.82	0.083511941	0.083511941	15.86437
7	3.52	2.90	3.21	0.094750827	0.094750827	20.58991451
8	3.58	3.30	3.44	0.020474154	0.020474154	23.68756558
9	2.47	2.56	2.51	0.001827528	0.001827528	12.62354392
10	2.53	2.32	2.43	0.011020761	0.011020761	11.78202748

Grand sum 55.1905445 **Grand mean** 2.759527225

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.463643266	0.046364327	0.215323772	7.80
Between Run	2.052645843	0.22807176	0.301419503	10.92
Total	2.516289109		0.370429539	**13.42**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	1.27	1.22	1.24	0.000624607	0.000624607	3.089026544
2	0.85	0.99	0.92	0.004877288	0.004877288	1.701697338
3	1.38	1.30	1.34	0.001785578	0.001785578	3.600922251
4	1.19	1.20	1.19	3.97327E-05	3.97327E-05	2.840326287
5	1.01	1.07	1.04	0.000856488	0.000856488	2.14549893
6	1.04	1.03	1.03	6.8692E-05	6.8692E-05	2.140061228
7	1.09	1.22	1.16	0.0036607	0.0036607	2.669439782
8	1.08	1.08	1.08	1.00736E-05	1.00736E-05	2.334338383
9	1.15	1.10	1.12	0.000524077	0.000524077	2.524274289
10	1.12	1.13	1.13	2.70315E-06	2.70315E-06	2.533836847

Grand sum 22.50711404 **Grand mean** 1.125355702

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.024899878	0.002489988	0.049899777	4.43
Between Run	0.250912754	0.027879195	0.112670331	10.01
Total	0.275812632		0.123225774	**10.95**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**12378-PeCDF**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	2.49	2.54	2.51	0.00067062	0.00067062	12.63765428
2	2.35	2.49	2.42	0.004841583	0.004841583	11.7182849
3	2.52	2.68	2.60	0.006416694	0.006416694	13.47039801
4	2.65	2.52	2.59	0.004624628	0.004624628	13.36953213
5	2.64	2.71	2.67	0.001180287	0.001180287	14.26825156
6	2.40	3.23	2.81	0.17140745	0.17140745	15.8120771
7	3.22	2.61	2.91	0.090976861	0.090976861	16.98813036
8	2.70	3.03	2.86	0.027355193	0.027355193	16.41258334
9	2.52	2.57	2.55	0.000676063	0.000676063	12.9588959
10	2.42	2.55	2.49	0.004265445	0.004265445	12.35987557

Grand sum	52.81660564	**Grand mean**	2.640830282

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.624829647	0.062482965	0.249965927	9.47
Between Run	0.515991596	0.0573324	0	0.00
Total	1.140821243		0.249965927	**9.47**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	1.20	1.21	1.21	1.79334E-05	1.79334E-05	2.911675202
2	0.92	0.97	0.94	0.000519926	0.000519926	1.785198909
3	1.37	1.31	1.34	0.000705691	0.000705691	3.598470219
4	1.25	1.20	1.22	0.000509365	0.000509365	2.998575917
5	1.03	0.97	1.00	0.000649453	0.000649453	1.998267709
6	1.09	1.06	1.08	0.00015739	0.00015739	2.311373144
7	1.07	1.20	1.14	0.004255608	0.004255608	2.576672831
8	1.09	1.07	1.08	8.76955E-05	8.76955E-05	2.339455541
9	1.10	1.09	1.09	4.03777E-05	4.03777E-05	2.392948323
10	1.10	0.99	1.05	0.002928976	0.002928976	2.189292055

Grand sum	22.2968753	**Grand mean**	1.114843765

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.019744832	0.001974483	0.044435157	3.99
Between Run	0.24439745	0.027155272	0.112206927	10.06
Total	0.264142282		0.120685035	**10.83**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**23478-PeCDF**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	5.20	5.37	5.29	0.006997011	0.006997011	55.89522533
2	4.70	5.75	5.22	0.278801387	0.278801387	54.58260464
3	6.73	5.56	6.14	0.343024511	0.343024511	75.44052402
4	7.81	6.33	7.07	0.548884669	0.548884669	100.0260227
5	4.79	4.61	4.70	0.008296753	0.008296753	44.12171401
6	4.87	4.93	4.90	0.001020646	0.001020646	48.01537923
7	5.47	5.63	5.55	0.006935094	0.006935094	61.62630995
8	5.94	5.71	5.82	0.013407451	0.013407451	67.80108789
9	5.45	5.07	5.26	0.037354917	0.037354917	55.36134931
10	5.43	5.76	5.59	0.028027019	0.028027019	62.5583933

Grand sum	111.0967833	**Grand mean**	5.554839164

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	2.545498915	0.254549891	0.504529376	9.08
Between Run	8.303847519	0.922649724	0.577970515	10.40
Total	10.84934643		0.767202586	**13.81**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	2.35	2.46	2.40	0.002993716	0.002993716	11.56728798
2	1.91	1.90	1.91	4.65836E-05	4.65836E-05	7.278848605
3	2.59	2.72	2.66	0.00449204	0.00449204	14.11869651
4	2.57	2.37	2.47	0.010226376	0.010226376	12.21346513
5	1.90	2.10	2.00	0.009652109	0.009652109	7.981124235
6	2.04	1.85	1.95	0.009600737	0.009600737	7.569484083
7	2.25	2.32	2.28	0.001210629	0.001210629	10.40487231
8	2.31	2.34	2.33	0.000252785	0.000252785	10.81585456
9	2.20	2.14	2.17	0.00101086	0.00101086	9.406647954
10	2.15	2.08	2.12	0.00113671	0.00113671	8.950552662

Grand sum	44.54886465	**Grand mean**	2.227443232

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.081245091	0.008124509	0.090136059	4.05
Between Run	1.076766961	0.119640773	0.236131599	10.60
Total	1.158012052		0.252750156	**11.35**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**123478-HxCDF**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	2.21	2.10	2.16	0.003431655	0.003431655	9.293675158
2	2.10	2.08	2.09	4.49515E-05	4.49515E-05	8.73867293
3	2.12	2.13	2.13	2.77788E-05	2.77788E-05	9.052381871
4	2.15	2.09	2.12	0.000932597	0.000932597	8.966282757
5	2.20	2.15	2.17	0.000673244	0.000673244	9.42892911
6	2.12	2.63	2.38	0.06437847	0.06437847	11.30455827
7	2.86	2.36	2.61	0.064713952	0.064713952	13.62091395
8	3.25	2.66	2.95	0.08737715	0.08737715	17.45918088
9	2.22	2.11	2.16	0.003056747	0.003056747	9.373249897
10	2.20	2.40	2.30	0.009815365	0.009815365	10.6014439

Grand sum	46.14194854	**Grand mean**	2.307097427

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.46890382	0.046890382	0.216541871	9.39
Between Run	1.385317966	0.153924218	0.231337239	10.03
Total	1.854221785		0.31687111	**13.73**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	1.01	1.00	1.01	1.29285E-05	1.29285E-05	2.028245449
2	0.82	0.83	0.83	1.64903E-05	1.64903E-05	1.364427885
3	1.16	1.19	1.18	0.000247906	0.000247906	2.76380606
4	1.05	1.05	1.05	4.11614E-06	4.11614E-06	2.197443707
5	0.90	0.91	0.91	1.04795E-05	1.04795E-05	1.645044594
6	0.81	0.84	0.83	0.000268555	0.000268555	1.363766996
7	1.09	1.07	1.08	8.22569E-05	8.22569E-05	2.322735649
8	0.93	0.94	0.93	2.70003E-05	2.70003E-05	1.737235283
9	1.04	1.01	1.02	0.000280642	0.000280642	2.099793563
10	1.07	1.04	1.05	0.000177614	0.000177614	2.224472438

Grand sum	19.75673959	**Grand mean**	0.987836979

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.002255977	0.000225598	0.015019912	1.52
Between Run	0.230533674	0.025614853	0.112670437	11.41
Total	0.232789652		0.113667169	**11.51**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**123678-HxCDF**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	2.38	2.32	2.35	0.000730163	0.000730163	11.06161003
2	2.27	2.19	2.23	0.001554648	0.001554648	9.960601021
3	2.43	2.24	2.33	0.009467189	0.009467189	10.88722837
4	2.38	2.40	2.39	7.30041E-05	7.30041E-05	11.40227823
5	2.49	2.33	2.41	0.006907943	0.006907943	11.63435193
6	2.21	2.70	2.45	0.062103581	0.062103581	12.04712418
7	2.76	2.33	2.55	0.046729136	0.046729136	12.96474755
8	3.52	2.85	3.18	0.110224457	0.110224457	20.27542319
9	2.27	2.29	2.28	6.4763E-05	6.4763E-05	10.40667918
10	2.41	2.62	2.51	0.010612989	0.010612989	12.64103217

Grand sum	49.3912755	**Grand mean**	2.469563775

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.496935747	0.049693575	0.222920557	9.03
Between Run	1.306171068	0.145130119	0.218445123	8.85
Total	1.803106815		0.31210871	**12.64**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	1.15	1.16	1.15	4.08445E-05	4.08445E-05	2.651432104
2	0.92	0.94	0.93	8.36257E-05	8.36257E-05	1.719630867
3	1.19	1.21	1.20	9.67024E-05	9.67024E-05	2.899528122
4	1.11	1.12	1.11	4.06875E-05	4.06875E-05	2.478409737
5	0.88	0.94	0.91	0.000754116	0.000754116	1.656508115
6	0.94	0.97	0.96	0.000223612	0.000223612	1.836147372
7	1.05	1.05	1.05	1.2311E-05	1.2311E-05	2.201642488
8	0.99	1.05	1.02	0.000648002	0.000648002	2.079431068
9	1.09	1.03	1.06	0.000837525	0.000837525	2.233318126
10	1.04	1.03	1.04	1.57897E-05	1.57897E-05	2.148389907

Grand sum	20.85236537	**Grand mean**	1.042618268

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.00550643	0.000550643	0.023465784	2.25
Between Run	0.16338084	0.018153427	0.093815733	9.00
Total	0.16888727		0.096705919	**9.28**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**123789-HxCDF**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	2.42	2.34	2.38	0.001623507	0.001623507	11.29000145
2	2.26	2.14	2.20	0.003119155	0.003119155	9.673624573
3	2.33	2.37	2.35	0.00036213	0.00036213	11.04325004
4	2.29	2.41	2.35	0.003884845	0.003884845	11.02738443
5	2.44	2.35	2.40	0.00203208	0.00203208	11.47638474
6	2.31	2.92	2.62	0.093677188	0.093677188	13.68372333
7	3.03	2.31	2.67	0.130938289	0.130938289	14.26002075
8	3.31	2.77	3.04	0.074486992	0.074486992	18.49810779
9	2.34	2.48	2.41	0.004898138	0.004898138	11.59065886
10	2.49	2.61	2.55	0.003267243	0.003267243	12.99302719

Grand sum	49.90378364	**Grand mean**	2.495189182

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.636579139	0.063657914	0.2523052	10.11
Between Run	1.016802075	0.112978008	0.157035178	6.29
Total	1.653381214		0.29718338	**11.91**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	1.20	1.12	1.16	0.001310349	0.001310349	2.691927994
2	0.90	0.96	0.93	0.00090604	0.00090604	1.719185358
3	1.24	1.19	1.21	0.000642167	0.000642167	2.950997946
4	1.10	1.04	1.07	0.000880069	0.000880069	2.279285228
5	1.01	0.96	0.99	0.000619781	0.000619781	1.955286906
6	0.97	0.95	0.96	8.92325E-05	8.92325E-05	1.841470431
7	1.02	1.10	1.06	0.001398423	0.001398423	2.241532958
8	1.10	1.06	1.08	0.000350284	0.000350284	2.324583011
9	1.05	0.97	1.01	0.001325658	0.001325658	2.04548004
10	0.99	0.99	0.99	4.78519E-08	4.78519E-08	1.953249896

Grand sum	20.9083141	**Grand mean**	1.045415705

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.015044101	0.00150441	0.038786726	3.71
Between Run	0.145119835	0.016124426	0.085498585	8.18
Total	0.160163936		0.093885132	**8.98**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**234678-HxCDF**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	3.11	3.05	3.08	0.000868916	0.000868916	18.98011282
2	3.02	2.77	2.90	0.015188695	0.015188695	16.78721062
3	3.13	2.94	3.03	0.009081117	0.009081117	18.4205818
4	3.05	3.03	3.04	0.000105589	0.000105589	18.47300929
5	3.18	3.21	3.19	0.000224945	0.000224945	20.36501035
6	3.12	3.96	3.54	0.173529029	0.173529029	25.04943308
7	3.91	3.16	3.54	0.140025994	0.140025994	25.04500089
8	4.32	3.49	3.91	0.168759115	0.168759115	30.50055823
9	3.19	3.17	3.18	0.000106587	0.000106587	20.24207879
10	3.17	3.68	3.42	0.064954106	0.064954106	23.40045377

Grand sum	65.65519492	**Grand mean**	3.282759746

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	1.145688182	0.114568818	0.338480159	10.31
Between Run	1.733218632	0.192579848	0.197498139	6.02
Total	2.878906814		0.391885612	**11.94**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	1.46	1.46	1.46	5.42233E-09	5.42233E-09	4.240430344
2	1.21	1.29	1.25	0.001327373	0.001327373	3.129304396
3	1.65	1.69	1.67	0.000290232	0.000290232	5.590188765
4	1.46	1.51	1.48	0.000656341	0.000656341	4.402633428
5	1.20	1.20	1.20	2.37564E-07	2.37564E-07	2.891283247
6	1.24	1.21	1.23	0.000278241	0.000278241	3.008179893
7	1.50	1.46	1.48	0.000524346	0.000524346	4.392853168
8	1.41	1.37	1.39	0.000354285	0.000354285	3.85164606
9	1.39	1.38	1.39	3.43675E-05	3.43675E-05	3.850692491
10	1.37	1.37	1.37	8.46591E-07	8.46591E-07	3.750962503

Grand sum	27.83616159	**Grand mean**	1.391808079

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.00693255	0.000693255	0.026329737	1.89
Between Run	0.365579697	0.040619966	0.141291739	10.15
Total	0.372512247		0.143724078	**10.33**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**234678-HxCDF**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	3.11	3.05	3.08	0.000868916	0.000868916	18.98011282
2	3.02	2.77	2.90	0.015188695	0.015188695	16.78721062
3	3.13	2.94	3.03	0.009081117	0.009081117	18.4205818
4	3.05	3.03	3.04	0.000105589	0.000105589	18.47300929
5	3.18	3.21	3.19	0.000224945	0.000224945	20.36501035
6	3.12	3.96	3.54	0.173529029	0.173529029	25.04943308
7	3.91	3.16	3.54	0.140025994	0.140025994	25.04500089
8	4.32	3.49	3.91	0.168759115	0.168759115	30.50055823
9	3.19	3.17	3.18	0.000106587	0.000106587	20.24207879
10	3.17	3.68	3.42	0.064954106	0.064954106	23.40045377

Grand sum	65.65519492	**Grand mean**	3.282759746

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	1.145688182	0.114568818	0.338480159	10.31
Between Run	1.733218632	0.192579848	0.197498139	6.02
Total	2.878906814		0.391885612	**11.94**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	1.46	1.46	1.46	5.42233E-09	5.42233E-09	4.240430344
2	1.21	1.29	1.25	0.001327373	0.001327373	3.129304396
3	1.65	1.69	1.67	0.000290232	0.000290232	5.590188765
4	1.46	1.51	1.48	0.000656341	0.000656341	4.402633428
5	1.20	1.20	1.20	2.37564E-07	2.37564E-07	2.891283247
6	1.24	1.21	1.23	0.000278241	0.000278241	3.008179893
7	1.50	1.46	1.48	0.000524346	0.000524346	4.392853168
8	1.41	1.37	1.39	0.000354285	0.000354285	3.85164606
9	1.39	1.38	1.39	3.43675E-05	3.43675E-05	3.850692491
10	1.37	1.37	1.37	8.46591E-07	8.46591E-07	3.750962503

Grand sum	27.83616159	**Grand mean**	1.391808079

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.00693255	0.000693255	0.026329737	1.89
Between Run	0.365579697	0.040619966	0.141291739	10.15
Total	0.372512247		0.143724078	**10.33**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**1234678-HpCDF**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	8.29	7.98	8.14	0.023548274	0.023548274	132.4145093
2	7.91	7.70	7.81	0.010886978	0.010886978	121.9622029
3	8.12	8.49	8.31	0.034488053	0.034488053	138.0158328
4	8.30	7.82	8.06	0.056891829	0.056891829	129.9916485
5	7.94	8.34	8.14	0.038305513	0.038305513	132.5070811
6	7.66	9.45	8.56	0.792382263	0.792382263	146.3790222
7	10.88	8.18	9.53	1.81627554	1.81627554	181.548908
8	10.95	8.87	9.91	1.08347139	1.08347139	196.2898822
9	8.06	7.95	8.01	0.003047276	0.003047276	128.2917836
10	8.60	8.91	8.76	0.024698726	0.024698726	153.3586883

Grand sum	170.4196012	**Grand mean**	8.520980058

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	7.767991686	0.776799169	0.88136211	10.34
Between Run	8.61753573	0.95750397	0.300586761	3.53
Total	16.38552742		0.931209734	**10.93**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	3.90	3.71	3.81	0.009400958	0.009400958	28.96308445
2	2.94	3.05	2.99	0.003454718	0.003454718	17.93033062
3	4.11	4.32	4.21	0.01178298	0.01178298	35.51064905
4	3.78	3.94	3.86	0.00637985	0.00637985	29.8615446
5	3.19	3.27	3.23	0.001373612	0.001373612	20.86264732
6	3.11	3.14	3.13	0.000163442	0.000163442	19.54693778
7	3.33	3.53	3.43	0.010372687	0.010372687	23.52603067
8	3.28	3.20	3.24	0.00161609	0.00161609	21.01083614
9	3.41	3.40	3.40	1.37339E-05	1.37339E-05	23.15826702
10	3.53	3.51	3.52	0.000113396	0.000113396	24.73421093

Grand sum	69.64767532	**Grand mean**	3.482383766

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.089342934	0.008934293	0.094521391	2.71
Between Run	2.564604681	0.284956076	0.371498171	10.67
Total	2.653947615		0.383334299	**11.01**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**1234789-HpCDF**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	2.17	2.20	2.19	0.000206985	0.000206985	9.566707445
2	2.22	2.11	2.16	0.003533345	0.003533345	9.372126276
3	2.37	2.51	2.44	0.00499355	0.00499355	11.89561569
4	2.32	2.00	2.16	0.025863662	0.025863662	9.324794874
5	2.28	2.50	2.39	0.011984491	0.011984491	11.44534014
6	2.31	2.72	2.51	0.041897487	0.041897487	12.62123253
7	2.92	2.27	2.59	0.106704228	0.106704228	13.46514936
8	2.98	2.48	2.73	0.060544442	0.060544442	14.90376235
9	2.17	2.26	2.22	0.001698987	0.001698987	9.819168376
10	2.29	2.48	2.39	0.009017572	0.009017572	11.37877885

Grand sum	47.55946284	**Grand mean**	2.377973142

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.532889498	0.05328895	0.230843995	9.71
Between Run	0.697550593	0.077505621	0.110037884	4.63
Total	1.230440091		0.25572893	**10.75**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	1.00	1.02	1.01	6.83159E-05	6.83159E-05	2.04202089
2	0.81	0.76	0.79	0.000562029	0.000562029	1.238951314
3	1.26	1.26	1.26	1.94688E-07	1.94688E-07	3.180143483
4	1.08	1.13	1.10	0.000456484	0.000456484	2.440131308
5	0.87	0.87	0.87	1.2974E-05	1.2974E-05	1.510016213
6	0.83	0.82	0.82	6.54079E-05	6.54079E-05	1.359311209
7	1.11	1.14	1.13	0.000280404	0.000280404	2.531892962
8	1.08	1.11	1.10	0.000343238	0.000343238	2.404063739
9	1.14	1.12	1.13	6.07434E-05	6.07434E-05	2.552430906
10	1.02	1.04	1.03	0.000104546	0.000104546	2.119093165

Grand sum	20.47388766	**Grand mean**	1.023694383

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.003908675	0.000390868	0.01977037	1.93
Between Run	0.419051386	0.046561265	0.151938141	14.84
Total	0.422960061		0.153219014	**14.97**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**OcCDF**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	2.51	2.53	2.52	0.000123589	0.000123589	12.70378382
2	2.34	2.31	2.32	0.000210058	0.000210058	10.78437856
3	2.32	2.50	2.41	0.0079524	0.0079524	11.62272181
4	2.40	2.60	2.50	0.010224888	0.010224888	12.53761247
5	2.67	2.66	2.67	4.20073E-06	4.20073E-06	14.22414506
6	2.39	3.09	2.74	0.125608847	0.125608847	15.01711355
7	3.51	2.58	3.05	0.215059522	0.215059522	18.54672035
8	3.72	2.99	3.36	0.134363315	0.134363315	22.5569026
9	2.78	2.56	2.67	0.011915868	0.011915868	14.29163631
10	2.67	2.86	2.77	0.009592482	0.009592482	15.30604921

Grand sum	54.01399468	**Grand mean**	2.700699734

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	1.03011034	0.103011034	0.320953321	11.88
Between Run	1.715482689	0.190609188	0.20928229	7.75
Total	2.745593029		0.383158076	**14.19**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	1.22	1.11	1.17	0.002856757	0.002856757	2.72925023
2	1.02	0.95	0.99	0.001283344	0.001283344	1.944916187
3	1.36	1.43	1.39	0.001211226	0.001211226	3.873731862
4	1.23	1.26	1.24	0.000240518	0.000240518	3.094289749
5	0.97	1.12	1.05	0.005171942	0.005171942	2.191455122
6	1.03	1.05	1.04	0.000100823	0.000100823	2.15674974
7	1.21	1.27	1.24	0.000900685	0.000900685	3.051582803
8	1.11	1.13	1.12	0.000181393	0.000181393	2.51387729
9	1.23	1.23	1.23	1.66371E-07	1.66371E-07	3.013225845
10	1.26	1.29	1.28	0.000206241	0.000206241	3.254705223

Grand sum	23.46912005	**Grand mean**	1.173456002

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.02430619	0.002430619	0.049301309	4.20
Between Run	0.283804265	0.031533807	0.120629988	10.28
Total	0.308110455		0.130315821	**11.11**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**PCB81**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	10.09	9.87	9.98	0.011873261	0.011873261	199.094245
2	9.56	9.69	9.62	0.003907535	0.003907535	185.2617331
3	10.26	9.76	10.01	0.062923358	0.062923358	200.4400687
4	10.03	10.33	10.18	0.02337516	0.02337516	207.1801505
5	10.02	10.29	10.16	0.017283089	0.017283089	206.2831072
6	9.63	12.49	11.06	2.041621823	2.041621823	244.5064148
7	13.15	10.33	11.74	1.992059613	1.992059613	275.6335792
8	13.71	10.90	12.31	1.976294206	1.976294206	302.9460544
9	9.71	9.98	9.84	0.018707926	0.018707926	193.8440893
10	9.42	9.61	9.52	0.008934527	0.008934527	181.1188676

Grand sum	208.8231386	**Grand mean**	10.44115693

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	12.313961	1.2313961	1.109682882	10.63
Between Run	15.95314833	1.772572037	0.520180708	4.98
Total	28.26710933		1.225554596	**11.74**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	4.54	4.53	4.54	2.73342E-05	2.73342E-05	41.20492888
2	3.49	3.74	3.61	0.014820946	0.014820946	26.11886181
3	5.18	4.97	5.07	0.011536467	0.011536467	51.46245071
4	4.79	4.49	4.64	0.02301987	0.02301987	43.04696513
5	3.96	4.02	3.99	0.001004095	0.001004095	31.87665533
6	3.70	3.68	3.69	4.02127E-05	4.02127E-05	27.22396384
7	4.39	4.59	4.49	0.009476515	0.009476515	40.36167617
8	3.94	3.91	3.93	0.000290297	0.000290297	30.85514364
9	3.93	4.07	4.00	0.005435923	0.005435923	31.98022035
10	3.99	3.95	3.97	0.000392566	0.000392566	31.48992988

Grand sum	83.86659731	**Grand mean**	4.193329866

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.132088454	0.013208845	0.114929741	2.74
Between Run	3.940488495	0.437832055	0.460772834	10.99
Total	4.072576949		0.474889935	**11.32**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**PCB126**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	25.36	25.79	25.58	0.046308323	0.046308323	1308.203531
2	24.77	25.66	25.22	0.197087736	0.197087736	1271.748653
3	25.87	24.06	24.97	0.824122588	0.824122588	1246.701062
4	25.61	25.84	25.73	0.01330762	0.01330762	1323.782771
5	25.91	26.32	26.12	0.041379958	0.041379958	1364.205566
6	24.90	33.11	29.00	16.8511173	16.8511173	1682.036343
7	34.22	27.10	30.66	12.68988378	12.68988378	1879.976227
8	35.77	29.91	32.84	8.593998888	8.593998888	2157.342591
9	25.15	26.38	25.77	0.382601287	0.382601287	1327.703051
10	23.69	24.83	24.26	0.328721889	0.328721889	1176.983528

Grand sum	540.2602684	**Grand mean**	27.01301342

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	79.93705873	7.993705873	2.827314251	10.47
Between Run	144.6254411	16.06949345	2.009451116	7.44
Total	224.5624998		3.468659635	**12.84**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	12.42	12.24	12.33	0.007386375	0.007386375	304.0285131
2	10.15	9.89	10.02	0.017024013	0.017024013	200.8379909
3	12.80	12.95	12.87	0.005329548	0.005329548	331.435874
4	13.12	10.77	11.95	1.382695912	1.382695912	285.4237733
5	9.89	9.84	9.87	0.000802608	0.000802608	194.6697053
6	9.76	9.78	9.77	8.83531E-05	8.83531E-05	191.0309902
7	12.10	12.56	12.33	0.052581815	0.052581815	304.0150325
8	11.82	11.71	11.76	0.002693345	0.002693345	276.8144566
9	11.96	11.94	11.95	8.90086E-05	8.90086E-05	285.6175325
10	11.62	11.40	11.51	0.01218247	0.01218247	265.0396615

Grand sum	228.7290331	**Grand mean**	11.43645166

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	2.961746895	0.29617469	0.54421934	4.76
Between Run	23.06499957	2.562777729	1.064566353	9.31
Total	26.02674646		1.195607046	**10.45**

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	PCDD/F and cPCBs
Method #:	6501.05
Matrix:	Serum/Plasma
Units:	pg/g
Analyte:	**PCB169**

Quality material 1 (Pool used: PTP1)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	26.17	26.37	26.27	0.00954503	0.00954503	1380.452749
2	25.73	26.00	25.86	0.018972113	0.018972113	1337.852933
3	27.25	25.66	26.46	0.636902426	0.636902426	1399.74932
4	26.61	26.37	26.49	0.013734152	0.013734152	1403.415481
5	26.94	26.84	26.89	0.002486353	0.002486353	1446.583114
6	26.21	33.89	30.05	14.73830551	14.73830551	1806.280685
7	34.31	27.14	30.73	12.84287528	12.84287528	1888.164564
8	34.56	28.58	31.57	8.953829424	8.953829424	1993.561125
9	25.71	26.38	26.05	0.109772858	0.109772858	1356.699521
10	27.22	26.42	26.82	0.160678928	0.160678928	1439.017458

Grand sum	554.3872389	**Grand mean**	27.71936194

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	74.97420414	7.497420414	2.738141781	9.88
Between Run	84.51641849	9.390713165	0.97295754	3.51
Total	159.4906226		2.905867648	**10.48**

Quality material 2 (Pool used PTP3)

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	12.48	12.41	12.45	0.001247396	0.001247396	309.9202782
2	10.24	10.32	10.28	0.001529382	0.001529382	211.2028305
3	13.51	13.61	13.56	0.002324945	0.002324945	367.5736807
4	12.55	11.37	11.96	0.351043415	0.351043415	286.1294309
5	10.22	10.25	10.23	0.000288058	0.000288058	209.424446
6	10.56	10.46	10.51	0.002590997	0.002590997	220.8988859
7	12.04	12.30	12.17	0.016749036	0.016749036	296.3994058
8	11.10	11.49	11.30	0.038767501	0.038767501	255.2171585
9	11.23	11.17	11.20	0.000944649	0.000944649	250.7602387
10	11.70	11.52	11.61	0.008088358	0.008088358	269.3828705

Grand sum	230.5156592	**Grand mean**	11.52578296

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.847147475	0.084714747	0.29105798	2.53
Between Run	20.0357692	2.226196578	1.034766116	8.98
Total	20.88291667		1.074921235	**9.33**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**2378-TeCDD**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	2.22	2.57	2.22	2.20	2.22	2.61	2.22	2.71
Replicate 2	2.87	2.57	2.87	2.17	2.87	2.48	2.87	2.68
Replicate 3	2.39	2.62	2.39	2.34	2.39	2.52	2.39	2.72
Mean	2.49	2.59	2.49	2.24	2.49	2.54	2.49	2.70
% difference from initial measurement	--	**3.8**	--	**-10.3**	--	**1.8**	--	**8.3**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	1.19	0.97	1.19	0.98	1.19	0.92	1.19	1.07
Replicate 2	1.14	0.94	1.14	1.01	1.14	0.91	1.14	1.08
Replicate 3	0.88	0.95	0.88	1.01	0.88	0.99	0.88	1.07
Mean	1.07	0.96	1.07	1.00	1.07	0.94	1.07	1.07
% difference from initial measurement	--	**-10.3**	--	**-6.4**	--	**-11.5**	--	**0.7**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**123478-HxCDD**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	2.84	2.64	2.84	2.51	2.84	2.98	2.84	3.21
Replicate 2	2.19	2.64	2.19	2.72	2.19	2.71	2.19	3.09
Replicate 3	3.05	2.68	3.05	2.92	3.05	2.60	3.05	2.92
Mean	2.69	2.65	2.69	2.72	2.69	2.76	2.69	3.07
% difference from initial measurement	--	**-1.4**	--	**1.0**	--	**2.6**	--	**14.2**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	1.14	1.04	1.14	1.22	1.14	1.04	1.14	1.34
Replicate 2	1.16	0.99	1.16	1.09	1.16	1.10	1.16	1.33
Replicate 3	1.03	1.01	1.03	1.14	1.03	0.98	1.03	1.12
Mean	1.11	1.01	1.11	1.15	1.11	1.04	1.11	1.26
% difference from initial measurement	--	**-8.4**	--	**3.8**	--	**-6.2**	--	**14.1**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
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Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**123478-HxCDD**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	9.82	8.50	9.82	7.63	9.82	8.80	9.82	9.66
Replicate 2	7.40	8.17	7.40	7.69	7.40	8.68	7.40	9.05
Replicate 3	10.24	8.38	10.24	9.04	10.24	8.56	10.24	9.58
Mean	9.16	8.35	9.16	8.12	9.16	8.68	9.16	9.43
% difference from initial measurement	--	**-8.8**	--	**-11.3**	--	**-5.2**	--	**3.0**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	3.48	3.37	3.48	3.94	3.48	3.18	3.48	3.55
Replicate 2	3.29	3.25	3.29	3.29	3.29	3.30	3.29	3.56
Replicate 3	2.77	3.21	2.77	3.39	2.77	3.09	2.77	3.85
Mean	3.18	3.28	3.18	3.54	3.18	3.19	3.18	3.65
% difference from initial measurement	--	**3.1**	--	**11.2**	--	**0.2**	--	**14.9**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**123789-HxCDD**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	2.20	2.69	2.20	2.23	2.20	2.68	2.20	2.69
Replicate 2	2.85	2.57	2.85	2.26	2.85	2.54	2.85	2.63
Replicate 3	2.18	2.55	2.18	2.64	2.18	2.69	2.18	2.59
Mean	2.41	2.60	2.41	2.38	2.41	2.64	2.41	2.64
% difference from initial measurement	--	**7.8**	--	**-1.4**	--	**9.4**	--	**9.3**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	1.12	0.99	1.12	1.13	1.12	0.97	1.12	1.03
Replicate 2	1.04	0.96	1.04	0.99	1.04	0.97	1.04	1.03
Replicate 3	0.79	0.97	0.79	1.01	0.79	1.00	0.79	1.03
Mean	0.98	0.97	0.98	1.04	0.98	0.98	0.98	1.03
% difference from initial measurement	--	**-1.0**	--	**6.0**	--	**-0.5**	--	**4.9**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**1234678-HpCDD**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	17.66	15.49	17.66	13.88	17.66	15.68	17.66	18.56
Replicate 2	13.14	15.11	13.14	14.58	13.14	15.62	13.14	17.21
Replicate 3	18.54	14.79	18.54	16.64	18.54	15.59	18.54	16.98
Mean	16.45	15.13	16.45	15.03	16.45	15.63	16.45	17.58
% difference from initial measurement	--	**-8.0**	--	**-8.6**	--	**-5.0**	--	**6.9**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	6.39	6.51	6.39	7.00	6.39	5.67	6.39	6.92
Replicate 2	6.66	5.80	6.66	5.65	6.66	5.51	6.66	6.70
Replicate 3	5.93	5.66	5.93	6.05	5.93	5.75	5.93	6.59
Mean	6.33	5.99	6.33	6.23	6.33	5.64	6.33	6.73
% difference from initial measurement	--	**-5.3**	--	**-1.5**	--	**-10.8**	--	**6.4**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**OcCDD**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	81.24	72.30	81.24	72.93	81.24	84.68	81.24	87.03
Replicate 2	61.15	68.34	61.15	75.06	61.15	81.22	61.15	84.01
Replicate 3	77.96	62.01	77.96	83.83	77.96	84.18	77.96	79.93
Mean	73.45	67.55	73.45	77.27	73.45	83.36	73.45	83.66
% difference from initial measurement	--	**-8.0**	--	**5.2**	--	**13.5**	--	**13.9**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	31.35	32.04	31.35	43.68	31.35	30.71	31.35	28.95
Replicate 2	31.74	30.95	31.74	28.30	31.74	32.16	31.74	29.82
Replicate 3	27.97	26.82	27.97	28.54	27.97	29.99	27.97	29.02
Mean	30.35	29.94	30.35	33.51	30.35	30.95	30.35	29.27
% difference from initial measurement	--	**-1.4**	--	**10.4**	--	**2.0**	--	**-3.6**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**2378-TeCDF**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	2.53	2.82	2.53	2.34	2.53	2.83	2.53	2.72
Replicate 2	3.11	2.78	3.11	2.47	3.11	2.72	3.11	2.54
Replicate 3	2.67	2.87	2.67	3.00	2.67	2.85	2.67	2.74
Mean	2.77	2.83	2.77	2.61	2.77	2.80	2.77	2.67
% difference from initial measurement	--	**2.1**	--	**-5.8**	--	**1.2**	--	**-3.6**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	1.27	1.08	1.27	1.26	1.27	1.01	1.27	1.15
Replicate 2	1.22	1.06	1.22	1.00	1.22	0.98	1.22	1.10
Replicate 3	0.85	1.04	0.85	1.08	0.85	1.03	0.85	1.07
Mean	1.11	1.06	1.11	1.11	1.11	1.01	1.11	1.11
% difference from initial measurement	--	**-5.0**	--	**-0.1**	--	**-9.5**	--	**-0.5**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**12378-PeCDF**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	2.40	2.52	2.40	2.23	2.40	2.69	2.40	2.65
Replicate 2	3.23	2.53	3.23	2.32	3.23	2.72	3.23	2.55
Replicate 3	2.52	2.56	2.52	2.77	2.52	2.63	2.52	2.68
Mean	2.72	2.54	2.72	2.44	2.72	2.68	2.72	2.63
% difference from initial measurement	--	**-6.6**	--	**-10.1**	--	**-1.3**	--	**-3.3**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	1.20	0.94	1.20	1.15	1.20	0.97	1.20	1.10
Replicate 2	1.21	0.93	1.21	1.04	1.21	1.00	1.21	1.09
Replicate 3	0.92	0.98	0.92	1.05	0.92	1.00	0.92	1.05
Mean	1.11	0.95	1.11	1.08	1.11	0.99	1.11	1.08
% difference from initial measurement	--	**-14.8**	--	**-2.5**	--	**-11.2**	--	**-2.9**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**23478-PeCDF**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	4.70	5.07	4.70	4.73	4.70	5.47	4.70	5.45
Replicate 2	5.75	5.05	5.75	4.73	5.75	5.28	5.75	5.07
Replicate 3	4.94	4.98	4.94	5.69	4.94	5.34	4.94	5.32
Mean	5.13	5.03	5.13	5.05	5.13	5.36	5.13	5.28
% difference from initial measurement	--	**-1.8**	--	**-1.5**	--	**4.6**	--	**3.0**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	2.35	2.02	2.35	2.35	2.35	2.09	2.35	2.20
Replicate 2	2.46	2.01	2.46	2.13	2.46	2.09	2.46	2.14
Replicate 3	1.91	1.94	1.91	2.12	1.91	2.15	1.91	2.18
Mean	2.24	1.99	2.24	2.20	2.24	2.11	2.24	2.17
% difference from initial measurement	--	**-11.2**	--	**-1.8**	--	**-6.0**	--	**-3.1**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**123478-HxCDF**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	2.12	2.65	2.12	2.31	2.12	2.69	2.12	2.59
Replicate 2	2.63	2.57	2.63	2.37	2.63	2.71	2.63	2.27
Replicate 3	2.44	2.56	2.44	2.71	2.44	2.73	2.44	2.49
Mean	2.40	2.59	2.40	2.46	2.40	2.71	2.40	2.45
% difference from initial measurement	--	**8.2**	--	**2.6**	--	**13.0**	--	**2.3**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	1.01	0.99	1.01	1.08	1.01	0.97	1.01	1.04
Replicate 2	1.00	0.97	1.00	1.00	1.00	0.95	1.00	1.01
Replicate 3	0.82	0.97	0.82	1.02	0.82	0.98	0.82	1.05
Mean	0.95	0.98	0.95	1.03	0.95	0.96	0.95	1.03
% difference from initial measurement	--	**3.3**	--	**9.0**	--	**2.0**	--	**9.1**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**123678-HxCDF**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	2.21	2.70	2.21	2.18	2.21	2.68	2.21	2.76
Replicate 2	2.70	2.65	2.70	2.33	2.70	2.60	2.70	2.40
Replicate 3	2.39	2.54	2.39	2.76	2.39	2.67	2.39	2.58
Mean	2.43	2.63	2.43	2.42	2.43	2.65	2.43	2.58
% difference from initial measurement	--	**8.2**	--	**-0.5**	--	**9.0**	--	**6.0**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	1.15	1.03	1.15	1.15	1.15	1.03	1.15	1.09
Replicate 2	1.16	1.05	1.16	0.95	1.16	0.99	1.16	1.03
Replicate 3	0.92	1.02	0.92	0.98	0.92	0.98	0.92	1.02
Mean	1.07	1.04	1.07	1.03	1.07	1.00	1.07	1.04
% difference from initial measurement	--	**-3.5**	--	**-4.3**	--	**-7.0**	--	**-2.8**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**123789-HxCDF**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	2.31	2.43	2.31	2.25	2.31	2.65	2.31	2.76
Replicate 2	2.92	2.33	2.92	2.42	2.92	2.52	2.92	2.55
Replicate 3	2.56	2.40	2.56	2.90	2.56	2.63	2.56	2.55
Mean	2.60	2.39	2.60	2.52	2.60	2.60	2.60	2.62
% difference from initial measurement	--	**-8.1**	--	**-2.9**	--	**0.1**	--	**1.0**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	1.20	1.08	1.20	1.18	1.20	0.96	1.20	1.05
Replicate 2	1.12	1.01	1.12	0.93	1.12	0.91	1.12	0.97
Replicate 3	0.90	0.97	0.90	0.96	0.90	1.00	0.90	0.96
Mean	1.07	1.02	1.07	1.02	1.07	0.96	1.07	0.99
% difference from initial measurement	--	**-4.8**	--	**-4.7**	--	**-10.6**	--	**-7.3**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**234678-HxCDF**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	3.12	3.55	3.12	3.03	3.12	3.59	3.12	3.54
Replicate 2	3.96	3.37	3.96	3.30	3.96	3.47	3.96	3.40
Replicate 3	3.22	3.42	3.22	3.75	3.22	3.53	3.22	3.41
Mean	3.43	3.45	3.43	3.36	3.43	3.53	3.43	3.45
% difference from initial measurement	--	**0.4**	--	**-2.1**	--	**2.9**	--	**0.6**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	1.46	1.39	1.46	1.54	1.46	1.29	1.46	1.39
Replicate 2	1.46	1.34	1.46	1.30	1.46	1.26	1.46	1.38
Replicate 3	1.21	1.33	1.21	1.35	1.21	1.27	1.21	1.37
Mean	1.38	1.36	1.38	1.39	1.38	1.27	1.38	1.38
% difference from initial measurement	--	**-1.4**	--	**1.4**	--	**-7.6**	--	**0.3**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**1234678-HpCDF**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	7.66	8.73	7.66	8.59	7.66	9.33	7.66	9.17
Replicate 2	9.45	8.63	9.45	8.08	9.45	8.86	9.45	8.72
Replicate 3	8.44	8.55	8.44	9.13	8.44	8.85	8.44	8.39
Mean	8.52	8.64	8.52	8.60	8.52	9.01	8.52	8.76
% difference from initial measurement	--	**1.4**	--	**1.0**	--	**5.8**	--	**2.9**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	3.90	3.42	3.90	3.83	3.90	3.31	3.90	3.41
Replicate 2	3.71	3.37	3.71	3.28	3.71	3.25	3.71	3.40
Replicate 3	2.94	3.37	2.94	3.39	2.94	3.20	2.94	3.25
Mean	3.52	3.39	3.52	3.50	3.52	3.25	3.52	3.35
% difference from initial measurement	--	**-3.7**	--	**-0.4**	--	**-7.5**	--	**-4.7**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**1234789-HpCDF**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	2.92	2.97	2.92	2.69	2.92	3.00	2.92	3.06
Replicate 2	2.27	2.90	2.27	2.78	2.27	2.82	2.27	2.83
Replicate 3	2.98	2.75	2.98	3.23	2.98	2.86	2.98	2.80
Mean	2.72	2.88	2.72	2.90	2.72	2.90	2.72	2.89
% difference from initial measurement	--	**5.6**	--	**6.6**	--	**6.4**	--	**6.4**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	1.26	1.15	1.26	1.32	1.26	1.03	1.26	1.14
Replicate 2	1.26	1.14	1.26	1.08	1.26	1.17	1.26	1.12
Replicate 3	1.08	1.10	1.08	1.17	1.08	1.14	1.08	1.10
Mean	1.20	1.13	1.20	1.19	1.20	1.11	1.20	1.12
% difference from initial measurement	--	**-6.1**	--	**-0.8**	--	**-7.3**	--	**-6.9**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**OcCDF**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	3.51	3.20	3.51	2.76	3.51	3.11	3.51	3.34
Replicate 2	2.58	2.84	2.58	2.77	2.58	2.99	2.58	3.30
Replicate 3	3.72	3.00	3.72	3.14	3.72	3.02	3.72	3.39
Mean	3.27	3.01	3.27	2.89	3.27	3.04	3.27	3.34
% difference from initial measurement	--	**-7.9**	--	**-11.6**	--	**-7.1**	--	**2.1**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	1.43	1.35	1.43	1.13	1.43	1.05	1.43	1.23
Replicate 2	1.23	1.24	1.23	1.12	1.23	1.10	1.23	1.23
Replicate 3	1.26	1.19	1.26	1.19	1.26	1.19	1.26	1.26
Mean	1.30	1.26	1.30	1.15	1.30	1.11	1.30	1.24
% difference from initial measurement	--	**-3.4**	--	**-11.9**	--	**-14.7**	--	**-5.2**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**PCB81**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	9.63	10.16	9.63	8.44	9.63	10.27	9.63	10.78
Replicate 2	12.49	10.01	12.49	8.02	12.49	10.22	12.49	10.76
Replicate 3	10.04	10.19	10.04	8.42	10.04	10.56	10.04	10.59
Mean	10.72	10.12	10.72	8.29	10.72	10.35	10.72	10.71
% difference from initial measurement	--	**-5.5**	--	**-22.6**	--	**-3.4**	--	**-0.1**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	4.54	3.42	4.54	3.32	4.54	4.25	4.54	3.93
Replicate 2	4.53	3.86	4.53	4.11	4.53	4.18	4.53	4.07
Replicate 3	3.49	3.76	3.49	3.92	3.49	4.20	3.49	4.15
Mean	4.19	3.68	4.19	3.78	4.19	4.21	4.19	4.05
% difference from initial measurement	--	**-12.2**	--	**-9.7**	--	**0.4**	--	**-3.4**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**PCB126**

Quality material 1 (PTP1)

	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	24.90	28.70	24.90	24.25	24.90	28.79	24.90	30.42
Replicate 2	33.11	27.95	33.11	38.81	33.11	28.19	33.11	29.82
Replicate 3	27.96	29.07	27.96	28.68	27.96	27.98	27.96	30.78
Mean	28.65	28.57	28.65	30.58	28.65	28.32	28.65	30.34
% difference from initial measurement	--	**-0.3**	--	**6.7**	--	**-1.2**	--	**5.9**

Quality material 2 (PTP3)

	Initial measurement	Three freeze-thaw cycles	Initial measurement	Bench-top stability	Initial measurement	Processed sample stability	Initial measurement	Long-term stability
Replicate 1	12.42	14.40	12.42		12.42	10.92	12.42	11.96
Replicate 2	12.24	10.70	12.24	11.93	12.24	10.64	12.24	11.94
Replicate 3	10.15	10.90	10.15	11.20	10.15	10.86	10.15	11.85
Mean	11.60	12.00	11.60	11.57	11.60	10.81	11.60	11.92
% difference from initial measurement	--	**3.4**	--	**-0.3**	--	**-6.9**	--	**2.7**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions
Describe condition: Serum frozen (-70C) and thawed three times before beginning sample preparation
Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)
Describe condition: Serum left at room temperature overnight before beginning sample preparation
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: Prepared extract stored at room temperature for 24 hours before instrumental analysis
Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis
Describe condition: Sample stored between April/May 2015 and August 2017 (2 years and 4 months) before analysis

All stability sample results should be within ±15% of nominal concentration

Method name:	Flame retardant metabolites in urine
Method #:	6501
Matrix:	Serum
Units:	fg/g of serum
Analyte:	**PCB169**

Quality material 1 (PTP1)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	26.21	27.53	26.21	23.52	26.21	28.56	26.21	29.10
Replicate 2	33.89	27.06	33.89	25.17	33.89	27.95	33.89	28.25
Replicate 3	27.00	27.14	27.00	28.45	27.00	28.86	27.00	28.87
Mean	29.03	27.24	29.03	25.71	29.03	28.46	29.03	28.74
% difference from initial measurement	--	**-6.2**	--	**-11.4**	--	**-2.0**	--	**-1.0**

Quality material 2 (PTP3)								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	12.48	10.97	12.48	11.72	12.48	10.71	12.48	11.23
Replicate 2	12.41	10.62	12.41	10.91	12.41	10.76	12.41	11.17
Replicate 3	10.24	10.54	10.24	11.02	10.24	10.98	10.24	11.48
Mean	11.71	10.71	11.71	11.22	11.71	10.82	11.71	11.29
% difference from initial measurement	--	**-8.6**	--	**-4.2**	--	**-7.6**	--	**-3.6**

Appendix B: Typical accurate masses, target isotopic ratios, 13C-labeled standards used, selected ion monitoring window (SIM) and lock and calibration masses used for high resolution isotope dilution measurements.

Appendix B. 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)