Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

Wadsworth Center

New York State Department of Health

Analyte: PCB congeners (comprehensive), PBDE congeners (comprehensive) and organochlorine pesticides (OCPs)

Matrix: Serum (applicable to plasma and whole blood); applicable to fat with some modifications to extraction

Method: High Resolution Gas Chromatography-High Resolution Mass spectrometry (HRGC/HRMS); High Resolution Gas Chromatography-Low Resolution Mass Spectrometry (LRGC/MS) and Electron Capture Detection (GC/ECD)

Method No:

Performed by: Organic Chemical Biomonitoring Laboratory, Wadsworth Center, New York State Department of Health

Contact: Dr. K. Kannan, kkannan@wadsworth.org

Information for users: The biomonitoring laboratory at Wadsworth Center periodically refines these laboratory methods as new technologies become available. 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.

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

1. History:

   Polybrominated diphenyl ethers (PBDEs) are used in large quantities as flame-retardant additives in polymers, especially in the manufacture of a great variety of electrical appliances, including televisions, computers, building materials and textiles. Polychlorinated biphenyls (PCBs) are legacy environmental pollutants. Organochlorine pesticides (OCPs) including DDT, HCH isomers, chlordanes and hexachlorobenzene are persistent and bioaccumulative compounds. PBDEs have a structural similarity to other environmental chemicals with known toxic effects, such as polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs) and dioxins indicating that PCBs and PBDEs can be harmful to health.

2. Summary:

   A 2 g-serum sample is spiked with internal standard ^{13}C isotopic analytes (^{13}C-PBDEs, ^{13}C-PCBs and ^{13}C-OCPs – details of the internal standards are given in 7) at the beginning of the procedure at a pre-determined concentration (10 pg each for PBDEs, 10 pg each for PCBs and OCPs and 10 pg for CB30 and 204) and prepared. Two g of the serum sample/fat sample is homogenized with 20 g of anhydrous sodium sulfate (pre-baked at 450ºC). Transfer 10 ml of 15% dichloromethane in hexane to the sample and shake for 30 minutes. Transfer the solvent to a round bottom flask and extract the sample 3 times with 10 ml of 15% dichloromethane in hexane by shaking. Pool all the solvents and rotary-evaporate to 5 ml. An aliquot (4 ml) is treated with sulfuric acid (3 ml) and further cleaned using the automated Rapid Trace instrument. The clean-up column is then packed with silicagel/sulfuric acid silicagel and readied. The Rapid Trace is purged using a pre-determined method. The concentrated sample is then transferred to a Rapid Trace tube and placed in the proper slots on the Rapid Trace. The instrument then runs a pre-determined clean-up method. The elution fractions are then combined and blown down to a final volume of 50 µl. The extract is then run on a HRGC-HRMS to determine target analyte levels.

   Target analytes are:
   PCBs – as many congeners as possible: importantly, #8, 18, 28, 44, 49, 52, 66, 74, 87, 93, 99, 101, 105, 110, 114, 118, 126, 128, 130, 137, 138, 146, 149, 151, 153, 156, 157, 167, 170, 172, 177, 178, 180, 183, 187, 189, 194, 195, 196, 201, 206, 209.

   PBDEs - Tri-to deca-BDE (#28, 47, 49, 66, 85, 99, 100, 153, 154, 183, 196, 197, 203, 207, 209)

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

OCPs – Hexachlorobenzene (HCB), alpha-HCH, gamma-HCH, beta-HCH, delta-HCH, Oxychlordane, T-nonachlor, T-chlordane, C-nonachlor, C-chlordane, p,p'-DDT, o,p'-DDT, p,p'-DDE, o,p'-DDE, o,p'-DDD, and p,p'-DDD, Dieldrin, Dechlorane, Toxaphene (Parlar 26 & 50)

3. Sample Tracking:

   Each sample contains a unique identifier specific for the sample. This identifier will be used to track the samples from reception to analysis to final reporting.

4. Equipment:
   - Biological Hood
   - Chemical Hood
   - NANOpure Diamond (Barnstead)
   - Ultrasonic Cleaner (BRANSON 2510)
   - Scales (OHAUS/METTLER TOLEDO AG245)
   - Pipettors (Eppendorf Research)
   - Oven (PRICISION/SYBRON)
   - Corning Hot Plates (PC-400D)
   - Vortex
   - Orbital shaker (Eberbach Corp.)
   - Centrifuge (Eppendorf 5804)
   - RapidTrace Automated SPE Extraction Instrument
   - TurboVap LV Evaporator (Zymark)
   - Multivap 113 Nitrogen Evaporator (OA-SYS)
   - Rotary evaporator (BUCHI R-200 with B-490 heating bath)
   - Agilent 6890N GC/ECD
   - Thermo-Finnigan HRGC-HRMS MAT95XT or DFS

5. Supplies:
   - Sodium Sulfate, Anhydrous, Granular Power (Na_2SO_4)
   - Pestle and mortar
   - Acetone
   - n-Hexane 95%
   - Dichloromethane (DCM)
   - Methanol (MeOH)
   - Conical Polypropylene (PP) tubes (15 and 50 ml)
   - Round Bottom Centrifuge Tubes 35mL (29 X 100mm)

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

- Empty SPE Tubes, 3cc polypropylene with 3cc, 20u polyethylene frits (Phenomenex)
- Sepra C18-E (50um, 65Å) bulk packing sorbent 100g (Phenomenex)
- Silicagel (Sigma-Aldrich, Grade 644, 150Å, 100-200 mesh)
- Sulfuric Acid (H_2SO_4), AR®(ACS) 96-98% (Mallinckrodt Chemicals)
- Silicagel/Sulfuric Acid (2:1 w/w)
- 5-3/4" and 9" disposable glass transfer pasteur pipets
- Small Latex Droppers 1mL
- Finntips (Finntip-20, -250 Universal, and -1000 Ext)
- Disposable Culture Tubes (16 X 100 mm)
- Disposable Aluminium Dish
- 15 mL Screw top tube with cap
- Target DP Amber ID 2 ml vial, cap, and septa
- 150 µL or 50 uL glass inserts with standard polyspring
- Stainless steel spatula
- Purple nitrile gloves
- 50 and 100 hole vial rack

6. Data Handling:

   The software (Xcalibur) is interfaced with the HRGC-HRMS and capable of collecting, recording and storing chromatographic data. A computer, which is dedicated to the instrument, is used to store and perform analysis on the data.

7. Reagents and Standards:

   Solvents: Ultra-high purity Methanol (MeOH), Hexane (lot certified to be free of contaminants), HPLC water, dichloromethane (DCM), Acetone, 88% Formic Acid, 0.1N hydrochloric Acid (HCl), concentrated sulfuric acid.

   Standards: ^{13}C-labeled analyte specific isotopic internal standards
   Mass labeled PBDE standard
   MBDE-MXFS; Wellington Laboratories, Guelph, Ontario, Canada

MBDE-MXFS Isomer #	Concentration (ng/ml)
28	2000
47	2000

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

100	2000
99	2000
154	2000
153	2000
183	2000

Running Stock#1 (100 ppb) = > 1.2ml + 22.8ml isooctane
Running Stock#2 (10 ppb) = > 1ml (stock#1) + 9 ml isooctane

Mass-labeled PCB standard:
EC-4058 (Cambridge Isotope Laboratories, [CIL] Andover, MA) - Surrogate Stock Solution (5 ug/ml; 5ppm) 3ml

7 compounds; PCBs #: 28, 52,101, 138, 153, 180, 209
Running Stock#1 (2 ppm) => 3ml + 4.5ml isooctane
Running Stock#2 (200ppb) => 1ml (stock#1) + 9ml isooctane

Mass-labeled OCP standards
4,4'-DDT($^{13}C_{12}$) 100ug/ml in Nonane, 1.2ml (CIL) Cat. Number: CLM-1281-S
4,4'-DDE($^{13}C_{12}$) 100ug/ml in Nonane, 1.2ml (CIL) Cat. Number: CLM-1281-S
Beta-BHC($^{13}C_6$) 50ug/ml in Nonane, 1.2ml X2 (CIL) Cat. Number: CLM-3623-S

Native standards for all compounds were prepared for instrumental analysis to generate external calibration curves. The native standards consisted of the following compounds.

BDE-MXF Native PBDEs Stock Solution (2000 ng/ml; 2ppm) 1.2ml X 2 for Calibration; contained the native PBDE congeners 28, 47, 66, 100, 97, 85, 154, 153, and 183, from Wellington Laboratories.
BDE-196, -197, -203, -207, -209 was purchased from AccuStandard, New Haven, CT.

PCB calibration solution was C-CCSEC-R PCB Calibration Check Solution (100 ug/mL in Acetone; 100 ppm), from AccuStandard. This contained the following congeners: BZ#
8,18,28,44,52,66,77,101,105,118,126,128,138,153,170,180,187,195,201,206,209

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

The OCP mix was Z-014C-R Pesticides Mix#2 (2.0 mg/mL in Toluene; 2000 ppm) from AccuStandard. This mixture contained the following compounds: Aldrin, α-BHC, β-BHC, γ-BHC, δ-BHC, α-chlordane(c-chlor), γ-chlordane (t-chlor), 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, Dieldrin. We also prepared a target OCP mixture that contained the following compounds: HCB, Oxychlordane, o,p'-DDE, c-chlor, t-nonachlor, p,p'-DDE, o,p'-DDD, c-nonachlor, o,p'-DDT

8. Sample Preparation- Extraction:

    1. The analyst will prepare a specific worksheet/or describe in lab notebook for each sample to be analyzed
    2. The samples will be removed from the freezer and placed within the biological safety hood to allow for thawing
    3. The analyst will then weigh 2 g of the sample and transfer this onto sodium sulfate (20 g) placed in pestle and mortar
    4. The samples are to be handled in such a way that the unique label for each sample should be accompanied in all procedures with all equipment/tools. The mortar will be labeled with the unique sample identifier
    5. Grind the sample in pestle and mortar until it becomes a dry, freely flowing powdery material.
    6. Transfer the dry powdery, homogenized sample into a 50 ml polypropylene (PP) tube using a solvent rinsed stainless steel spatula. Make sure that the sample is not lost by spill. Label the PP tube with unique sample identification code.
    7. The sample is spiked with mass-labeled standards and surrogate: 10 pg for each PBDE congeners, 10 pg PCB congeners, 10 pg OCP congeners, and 10 pg for unlabeled CB30 and 204.
    8. Add 10 ml of 15% dichloromethane in hexane to the 50mL PP tube or 35mL Centrifuge Tube.
    9. Shake the PP tube for 30 minutes in an orbital shaker for 30 min.
    10. Centrifuge the PP tube at 4000 rpm for 5 min.
    11. Transfer the solvent to a round bottom flask (100 ml) using solvent cleaned Pasteur pipettes.
    12. Add another 10 ml of 15% dichloromethane in hexane to the PP tube containing residual sample (this is second extraction) and shake for 30 minutes as above.
    13. Centrifuge the PP tube and transfer the solvent to the round bottom flask containing the first extract.
    14. Repeat the extraction 3 times.

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

15. Combine all three extracts (30 ml total) and concentrate using a rotary evaporator set at 40ºC.
16. Transfer the extract to the 15mL PP tube to cleanup.
17. Evaporate to 5 ml and aliquot 4 ml for PBDEs, PCBs and OCPs analysis; 1 ml is set aside for lipid analysis.
18. To the 4 ml of the extract, add 3 ml of concentrated sulfuric acid.
19. Treatment with HPLC water (Milli-Q water) to remove sulfur.
20. Remove the supernatant (organic layer) to a glass tube for cleanup using Rapid Trace.

9. Sample Clean Up:

1. The analyst will then pack a two layered clean up column with one frit on bottom, ~1.0g of silicagel/sulfuric acid (2:1 w/w), a frit added, ~0.1g silicagel, and a frit placed on top (Refer to figure 1)
2. The Rapid Trace extraction instrument is purged using method ENDOCL1 (Refer to Appendix B)
3. Three Rapid Trace tubes are labeled with the unique sample identifier
4. The sample is transferred from the concentration conical tube to one of the Rapid Trace tubes
5. The Rapid Trace tubes are loaded onto the extraction tray
6. The prepared clean up columns are placed into the turret on the Rapid Trace extraction instrument
7. The sample is cleaned up using the method ENDOCL1.
8. The elution fractions, 15% dichloromethane in hexane (6ml x2 each), are combined into one conical tube labeled with the unique sample identifier
9. The conical tube placed into the TurboVap and concentrated to a final volume of 50ul.
10. The sample is transferred to an amber vial with remarked 50ul level at 150ul glass insert with the unique sample identifier capped and stored at -20ºC until analysis

10. Sample Analysis:

1. Sample is analyzed using a Thermo-Finnigan HRGC-HRMS (MAT95XT or DFS) and an Agilent GC/ECD.

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

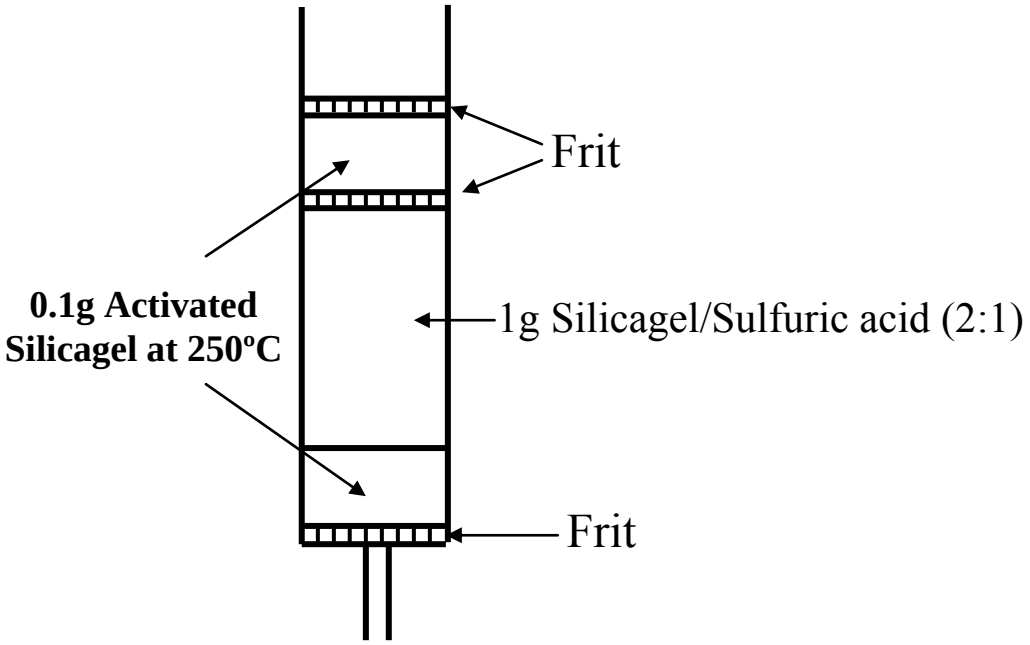

Figure 1 Clean Up Column

11. % Lipid Determination (if and when needed)

1. Take one round bottom flask per sample and label each with the perspective sample number. Add a few boiling chips and weigh the flask to acquire the initial weight of the flask and chips. Record this information on the sample sheets.
2. Weigh out 0.5g of serum, in the Biocontainment Hood, using an ANDEK200g balance with printer, which can measure weights out to two decimal places; i.e. (0.00g).
3. Add equal amount of MeOH to the sample to denature the protein. (Biocontainment Hood)
4. Extract serum with 15ml of 50% ether/hexane in three aliquots of 5ml. (Chemical Hood)
5. Evaporate extracted solvent to dryness in a dessicator.
6. An ANDHR-60 balance is used in weighing the sample in lipid determination. The balance can measure out to four decimal places; i.e. (0.0000g).

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

Weight of the flask + lipid + chip – Weight of the flask + chip = weight of lipid

28.6959g - 28.6934g = 0.0025g

is the weight of the lipid in the sample

Then, divide the weight of lipid in the sample by the starting weight, multiply by 100; this will give the percent lipid in the sample.

$$X = \frac{0.0025g}{0.5g} (100) = 0.50\%$$

12. Gas Chromatographic-Mass spectrometric Analysis:

The Thermo GC is typically fitted with 30-m x 0.25-mm internal diameter fused silica capillary column with a 0.25-µm film thickness bonded phase. DB-5 (5% phenyl-, 95% methyl-polysiloxane) or equivalent is suitable column phases. Suggested GC and inlet conditions are as follows (other columns and/or conditions may be specified in project plans):

Injection port temperature	270 °C
Detector temperature	300 °C
Initial oven temperature	100 °C
Initial hold	1 min
Ramp 1 rate	8 °C /min to 160°C (hold 1 min)
Ramp 2 rate	2.1 °C /min to 260° (hold 1 min)
Ramp 3 rate	10 °C /min to 300°C (hold 1 min)
Carrier gas flow	1 mL/min (helium)

The Agilent GC/ECD is connected with DB-5ms (8m x 0.25mm x 0.1um).

Injection port temperature	240 °C
Detector temperature	300 °C
Initial oven temperature	100 °C
Initial hold	1 min

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

Ramp 1 rate	6 °C /min to 190°C (hold 2 min)
Ramp 2 rate	6 °C /min to 240° (hold 1 min)
Ramp 3 rate	5 °C /min to 300°C (hold 5.67 min)
Carrier gas flow	2 mL/min (helium)
Makeup (N_2)	

12.1. Mass Spectrometer

A Thermo Finnigan Trace GC Ultra gas chromatograph / MAT95XP high resolution mass spectrometer (HRGC-HRMS) is used for the identification and quantification of all target compounds except PBDE-196, -197, -203, -207, -209, for which GC with electron capture detection (ECD) is used. Measurements are carried out at a resolution of R >9000-10000. PBDE congeners are monitored using the two most abundant masses of the ion clusters [M+2] (m/z 405.8026 and 407.8026) for tri-BDE, [M+4], (m/z 485.7106 and 483.7126) for tetra-BDE, [M-2Br], (m/z 403.7865 and 405.7845) for penta-BDE, [M-2Br] (m/z 481.6970 and 483.6950) for hexa-BDE, and [M-2Br], (m/z 561.6055 and 563.6035) for hepta-BDE. A eight-point calibration curve (0.1 - 20 ng/mL) is prepared every time when samples were measured. The analysis of 2,2',3,3',4,4',5,6'-octabromodiphenyl ether (BDE-196), 2,2',3,3',4,4',6,6'-octabromodiphenyl ether (BDE-197), 2,2',3,4,4',5,5',6-octabromodiphenyl ether (BDE-203), 2,2',3,3',4,4',5,6,6'-nonabromodiphenyl ether (BDE-207) and 2,2',3,4,4',5,5',6-octabromodiphenyl ether (BDE-203), and decabromodiphenyl ether (BDE-209) is performed using an Agilent Technologies 6890N gas chromatograph / electron capture detector (GC/ECD). Quantification of PBDE congeners is based on isotope-dilution method. An external calibration curve is prepared for each congener, to determine the recoveries of target compounds in samples.

The quantification and confirmation ions used in the analysis of selected PCB and PBDE congeners are based on most intense ions of the molecular ion cluster. If possible, ion groups should be selected so that no more than 20 ions are monitored in a single group. It should be noted that as the number of ions scanned per group increases and the individual dwell time decreases, sensitivity will also decrease. Each ion in a group (and each ion between groups) should have identical dwell times to ensure that correct ion ratios are preserved.

Prior to sample analysis, the elution order of the analytes of interest must be determined and/or verified. This is performed by analyzing the analytes individually or in combination with analytes having known pre-determined retention orders. The elution order and retention times will be verified with each set of samples by analyzing a mixed congener standard that contains all target congeners. Quantification of dioxin-like congeners PCB77, 81, 123, and 126 may be affected by mass fragment contributions from congeners PCB110, 87, 149, and 129 when

Reviewed by: _____

Date reviewed: _____

analyzed on a DB-5 column and may therefore result in unreliable results. PCB77, 81, 123, and 126 can therefore not be reliably analyzed on a DB- 5 column without pre-separation such as a Carbon Column Chromatography from the other congeners. PCB118 coelutes with PCB106 (same level of chlorination) on the DB-5 column and those are reported as coeluters (PCB106 is a minor congener relative to PCB118 in Aroclor and environmental samples).

Quantification of OCPs is based on external calibration standard, because labeled congeners are not available for all OCPs.

12.2. GC/MS Mass Calibration (Tuning) (If applicable)

Prior to the analysis of analytical standards and/or samples the mass spectrometer must be tuned. This procedure utilizes perfluorotributlyamine (PFTBA) to maximize the sensitivity of the instrument. The technical acceptance criteria for tuning the mass spectrometer are as follows: Mass 69 100%, Base peak, Mass 219 40-65% of mass 69, Mass 502 4-12% of mass 69

12.3. Calibration and Standardization

Demonstration of a linear initial calibration is required prior to the analysis of samples. The GC/MS or HRGC/HRMS must be calibrated to determine the retention time of each target congener and to determine the instrument sensitivity and linearity of the response of the target compounds. If the response factor (RF) for any compound is constant (less than 25% RSD) over the calibration range, an averaged response factor may be used for that compound; otherwise, the complete calibration curve for that compound shall be used over the calibrated range.

12.4. Identification of Target Compounds

When using SIM mode in GC/MS or HRGC/HRMS techniques, an analyst competent in the interpretation of mass spectra identifies the compounds in a sample by comparing the ratio of the target ion and the confirmation ion with that provided in this SOP. Comparison is also made to other reference standards such as the instrument control check (ICC) sample, and/or other project specific samples. Two criteria must be satisfied to verify the identifications of target compounds:
1. Elution of the sample analyte within GC retention time window
2. Correspondence of the sample analyte and calibration standard ion ratios

GC Retention Time. The sample component retention time (RT) should be within ± 0.1 min (6 sec) of the standard component.

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

Mass Spectra. For each target analyte, the corresponding ions listed in Attachment 5 must be present and the relative intensities must agree to within ±20 percent.

Signal to Noise Ratio. A quantifiable analyte peak should exhibit a signal to noise ratio of approximately 3:1 or greater.

12.5. Quantification of Target Compounds

Target PCB, PBDE congeners and pesticide (OCP) compounds are quantified by the internal standard method, using the average response factor from the initial calibration or response factors from the single-point calibration, unless otherwise specified in the project protocols. The average response factor (RF) from the initial calibration is used to calculate the concentration in the sample for the fully calibrated analytes. The response factors from the single-point calibration are used to quantify the other target analytes. Secondary ion quantification is allowed only when there are sample interferences with the primary ion. If secondary ion quantification is performed, the reason must be documented in the case narrative.

The equation for calculating the concentration of chlorinated pesticides, PCB congeners and homologues in a sample is as follows:

$Ca = ((Aa/RFi)\ XD)/Va$

Where,
Ca = Concentration target analyte
Aa = Area quantification ion for target analyte
RFi = Average RF for analyte determined from initial calibration or single-point calibration
D = Dilution factor if applicable
Va = Sample size
Sample size may refer to sample volume or sample wet/lipid weight. The project protocol will specify reporting criteria.

13. Quality Control

Glassware: All Solvents should be high purity and should be tested for contamination. All glassware and tools that come in contact with samples should be rinsed with acetone and hexane (see Appendix B)

Procedural blank: Procedural blank contains 1-2 ml water in lieu of serum/plasma sample. All solvents and reagents used in the analysis will be analyzed. Any contamination in procedural

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

blank should be reported. One procedural blank is analyzed for every 20 samples. If the levels of contaminants in procedural blanks exceed the detection limits, data should be flagged or the batch should be repeated.

Matrix spike and matrix spike duplicate (MS and MSD): For every 20 samples a matrix spike and a matrix spike duplicate is analyzed. The recoveries of matrix spike should be 100±30%. The RPD of repeated analysis should be <20%. Any deviation from the results should be flagged or if samples are available, analysis should be repeated.

^{13}C-labelled internal standards: The recoveries of labeled internal standards of PCBs, PBDEs, and OCPs should be between 100 ± 30%. Any deviation from the results should be flagged or if samples are available, analysis should be repeated.

Duplicate analysis: For every 30-40 samples, duplicate analysis of samples is needed. A randomly selected sample is analyzed in triplicate and precision is calculated. The precision should be >90%. Any deviation from the results should be flagged or if samples are available, analysis should be repeated.

Continuing calibration curve: A mid-point calibration curve should be injected after every 10 sample to check instrumental stability and response. If the responses vary by 50%, the batch should be reinjected with a new calibration curve.

14. Safety:

PCBs, PBDEs and OCPs should be treated as potential health hazards and exposure to these compounds should be reduced to the lowest possible level. The laboratory is responsible for maintaining a current awareness file of current regulations regarding the safe handling of chemicals used in this method. All personnel handling serum will be encouraged to receive the hepatitis B vaccine. Serum samples will be handled in a biocontainment hood in accordance with NYSDOH Safety Manual (Number II.11, issued 12/90, and revised 9/92). All disposable glassware being exposed to serum will be disposed of in aluminum stockpots that are clearly marked "Biohazard." Non-disposable glassware will be submerged in Clorox overnight. Liquid waste will be disposed of in red organic-waste containers.

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

Cleanup:

All disposable glassware being exposed to blood or serum should be disposed of in aluminum stockpots that are clearly marked "Infectious Waste." After soaking overnight in 50% Clorox solution. Non-disposable glassware should be in a 50% Clorox solution overnight. Liquid organic waste should be disposed of in organic-waste containers.

All manipulations of the sample before extractions will be carried out in the biocontainment hood. Lab coat and latex gloves are to be worn and the blower on while working in the biocontainment hood. In addition to gloves and lab coat, safety glasses should be worn while working in the chemical fume hood.

15. Documentation:

The laboratory will maintain a hardbound notebook where any modifications to the analytical system will be recorded. Modifications such as changing columns, inlet liners, any routine maintenance performed, and any malfunction that occurs in the analytical system will be noted. The notebook will also have recorded the location of all samples and sample extracts. The Rapid Trace automated extraction system will have a hardbound book to show any maintenance and sample location during extraction.

Data files (the electronic data) transfer to other computers for data reduction. All chromatography data are archived including: ChemStation raw data files, processed data files, and associated calibration and integration files.

REFERENCES

United States Environmental Protection Agency (U.S. EPA), 1999. Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS, Method 1668, Revision A, Office of Water, U.S. Environmental Protection Agency, Washington, DC.

United States Environmental Protection Agency (U.S. EPA), 1985. Test Methods for Determination of Pesticides and PCBs in Water and Soil/Sediment by Gas Chromatography/Mass Spectroscopy, Method 680, Physical and Chemical Methods Branch, Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio.

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

Appendix A (If applicable)

Sample Preparation and Extraction

1. Sample weight (1g) into a 15mL test tube.
2. Sample spiked with 10ul of ^{13}C-labeled surrogate standards at 10ng/mL and wait 1hr
3. Sample Preparation:
    a) 88% Formic acid is then added to the sample in a 1:1 ratio (1g)
    b) The sample was then vortexed (10s) and sonicated with no heat (10min)
    c) HPLC H_2O was added to the sample at a 1:1 ratio (2g) and vortexed (10s)
    d) The sample is transferred into the Rapid Trace tube
4. SPE using the Rapid Trace Automated System
    a) Column packed with 1.3g of C18-Sepra Sorbent
5. Instrument extraction method (ENDOEXT1 method)
6. Concentrate to 1mL

<ENDOEXT1 Method> (If applicable)

Condition	MeOH	3mL	3mL/min
Condition	5%MeOH	3mL	3mL/min
Condition	DCM	3mL	3mL/min
Condition	5%MeOH	3mL	3mL/min
Load	Sample	5mL	0.40mL/min
Rinse	5%MeOH	1mL	3mL/min
Dry	N_2	Time = 20min	
Dry	N_2	Time = 20min	
Purge-cannula	DCM	3mL	3mL/min
Collect1	30%DCM/Hex	6mL	0.40mL/min
Collect2	30%DCM/Hex	6mL	0.40mL/min
Purge-Cannula	DCM	3mL	3mL/min
Purge-Cannula	H_2O	3mL	3mL/min

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

Appendix B

<ENDOCL1 Method>

SPE Packing: 0.1g Silica gel (SG) + 1g SG/Sulfuric Acid (2:1) + 0.1g SG

Condition1	Hexane	5ml	5ml/min
Condition2	15%DCM/Hex	5ml	5ml/min
Load	Sample	2.5ml	0.38ml/min
Purge	15%DCM/Hex	3ml	5ml/min
Collect1	15%DCM/Hex	6ml	0.38ml/min
Collect2	15%DCM/Hex	6ml	0.38ml/min
Purge	15%DCM/Hex	3ml	5ml/min

Appendix C

Glassware Cleaning

1. Wash glassware in hot soapy water using brushes that are specific for BOAC use
2. Thoroughly rinse glassware with copious amounts of hot water
3. Rinse glassware again with copious amounts of organic-free water
4. Oven dry glassware @ 105°C for at least 1.5 hours
5. Remove glassware from oven and allow too cool to room temperature
6. After cooling, rinse glassware 3x with nano-grade hexane (pre-checked lot), saving third rinse. (Note: use tongs to hold glassware while rinsing)
7. Concentrate combined rinses to 1ml, then analyze by GC
8. If there are any contaminant is detected, the glassware is to be re-rinsed and re-checked before using. If a contaminant still appears, then the glassware is placed in the washtub to start from the beginning

Vial and Insert Cleaning

Cleaning glass insert:

1. Remove plastic feet from inserts
2. Place vials or inserts into BOD bottle or flask
3. Add acetone until all inserts are completely filled
4. Soak for at least 15 minutes
5. Discard acetone and add hexane

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

6. Soak for at least 15 minutes
7. Discard hexane and let vials dry in crystallizing dish covered with foil
8. When solvent is completely gone put into 105°C oven, uncovered, overnight
9. When completely cooled, feet can be put back on insert vials and both vials are stored in foil-covered beakers

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

Appendix D

Method Detection Limits (MDL) for PCB, PBDEs, and OCP congeners

PCBs

IUPAC No.	MDL (ng/g, wet weight)
PCB18	<0.0032
PCB28	<0.0032
PCB44	<0.0032
PCB49	<0.0032
PCB52	<0.0032
PCB66	<0.0032
PCB74	<0.0032
PCB87	<0.0032
PCB99	<0.0032
PCB101	<0.0032
PCB118	<0.0032
PCB128	<0.0032
PCB138	<0.0032
PCB146	<0.0032
PCB149	<0.0032
PCB151	<0.0032
PCB153	<0.0032
PCB156	<0.0032
PCB157	<0.0032
PCB167	<0.0032
PCB170	<0.0032
PCB172	<0.0032
PCB177	<0.0032
PCB178	<0.0032
PCB180	<0.0032
PCB183	<0.0032
PCB187	<0.0032
PCB189	<0.0032
PCB194	<0.0032
PCB195	<0.0032
PCB196	<0.0032
PCB201	<0.0032
PCB206	<0.0032
PCB209	<0.0032

PBDEs

IUPAC No.	MDL (ng/g, wet weight)
PBDE28	<0.0050
PBDE47	<0.0050
PBDE49	<0.0050
PBDE66	<0.0050
PBDE85	<0.0050
PBDE99	<0.0050
PBDE100	<0.0050
PBDE153	<0.0050
PBDE154	<0.0050
PBDE183	<0.0050
PBDE197	<0.0050
PBDE203	<0.0050
PBDE196	<0.0050
PBDE207	<0.0050
PBDE209	<0.0050

OCPs

IUPAC No.	MDL (ng/g, wet weight)
HCB	<0.0085
α-HCH	<0.0100
β-HCH	<0.0098
g-HCH	<0.0100
δ-HCH	<0.0100
Oxychlordane	<0.0460
Trans-nona	<0.0150
Trans-chlordane	<0.0086
Cis-chlordane	<0.0273
Cis-nona	<0.0452
p,p'-DDT	<0.0391
o,p'-DDT	<0.0132
p,p'-DDE	<0.0100
o,p'-DDE	<0.0100
o,p'-DDD	<0.0100
p,p'-DDD	<0.0100

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

Supplies

Ordering: **Krackler Scientific (Contract)**
P.O. Box 1849
Tel: (518) 462-4281

#207-C4000-2W-PK Target DP Amber ID Vials 100/pk $17.74/pk
#207-C4012-530-PK 150µL Standard Polyspring Glass Inserts 100/pk $46.26/pk
#207-C4000-53G Target DP Caps and Septa 100/pk $25.25
#6-73500-16100 Disposable Culture Tubes 16x100mm 1000/CS $54.93
#343-V349-1000 300 Series Amber Boston Round Bottom Flask 12/CS $55.57
11-0128-05 Formic Acid, 2.5L, 88% $64.97
#11-3891-05 Sodium Sulfate, Anhydrous, Granular Powder 2.5kg (4/CS) $378.20
#3-352096-CS 15mL PP Tubes (500/CS) $86.47
#3-352070-CS 50mL PP Tubes (500/CS) $97.81
#4-9590-3-PK Universal range pH Strips (100/PK) $18.00

Ordering: **Phenomenex (Contract)**
411 Madrid Ave
Torrance, CA 90501
Tel: (310) 212-0555

# AH0-7001 Empty SPE Tubes, 3cc (50/Box)	$ 32.00
# AH0-7007 Frits for 3cc SPE Tubes, 20u (100/Bag)	$27.25
# 04G-4348 Sepra Bulk C18 (100g)	$190.00

Ordering: **Sigma-Aldrich**
PO Box 14508, St.Louis, MO 63178
Tel: 800-325-3010

#236829-1KG Silicagel, 100-200mesh, Grade644, Pore 150Å $229.50

Silica Gel/Sulfuric Acid Procedure (2:1 w/w)

1. Washing the Silica Gel

Reviewed by: _____

Date reviewed: _____

Analysis of Polychlorinated Biphenyls (PCBs), Polybrominated Diphenyl Ether (PBDE) and Organochlorine Pesticides (OCPs) in Serum

a) 200 g of Silica gel is weighed into a Pyrex-Plus Heavy Wall Filter Flask. dichloromethane (DCM) is added to cover the weighed out powder and a slurry is made and washed around
b) The apparatus is placed into a vacuum oven with no heat to evaporate the DCM off the Silica gel over the weekend

2. Silica Gel/ Sulfuric Acid (2:1 w/w)

a) 100 g of washed Silica gel is weighed out into a Amber Boston Round Bottom Bottle
b) 50 g (or ~28ml) of Sulfuric Acid is added to the bottle
c) The bottle is capped with a Teflon cap or tape and placed on the roller overnight to give a homogenous mixture.

Acid Water Procedure

1. 190 mL of HPLC water is placed into a Pyrex bottle
2. 10 mL of MeOH is added
3. 200 mL of 0.1N HCl is added
4. Mix well
5. The solvent should test at a pH of 1-1.3

30% DCM/Hex Procedure

1. In a Pyrex bottle add 180mL of dichloromethane
2. Add 420 mL of Hexane
3. Mix well

15% DCM/Hex Procedure

1. In a Pyrex bottle add 850mL of dichloromethane
2. Add 150 mL of Hexane
3. Mix well

Reviewed by: _____

Date reviewed: _____