BioOrganic Analytical Chemistry Laboratory
Division of Environmental Health Sciences
Wadsworth Center
Department of Health
State of New York

CLIA: 33D0654341

Standard Operating Procedure

Determination of Polychlorinated Biphenyls and Pesticides in Human Serum by Solid Phase Extraction and Gas Chromatography/Selected Ion Monitoring Mass Spectrometry

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Revision Record

Rev	Date	Responsible Person	Description of Change
1	11/15/07	Li Zhang, Buu Tran, Robin Storm and Robert Jansing	Initial Release

The following laboratory staff has read this Manual.
A copy of this page will be distributed to the employee training record file.

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ABBREVIATION LISTING

C18	octadecyl bonded silica gel
CAS	chemical abstract service
CCC	continuing calibration check standard
CCS	calibration check standard
DFTPP	decafluorotriphenylphosphine
EPA	Environmental Protection Agency
GC	gas chromatography
IDA	initial demonstration of accuracy
IDC	initial demonstration of capability
IDP	initial demonstration of precision
IC	internal calibration
IS	internal standard
IUPAC	International Union of Pure and Applied Chemistry
LCL	lower confidence limit
MB	method blank
MDL	method detection limit
MRL	minimum reporting level
MS	matrix spike
MSD	mass selective detector
OCP	organochlorine pesticide
PCB	polychlorinated biphenyl
PDS	primary dilution standard
POP	persistent organic pollutant
PTS	performance test solution
QC	quality control
R	correlation coefficient
R^2	determination coefficient
RB	reagent blank
RF	response factor

RSD	relative standard deviation
RT	retention time
S	standard deviation
SB	solvent blank
SIM	selected ion monitoring
SOP	standard operating procedure
SPE	solid phase extraction
t	Student's statistical test value
TIC	total ion chromatogram
UCL	upper confidence limit

1. SCOPE AND APPLICATION

1.1 Human populations worldwide have been exposed to a broad range of synthetic organic industrial and agricultural chemicals known collectively as persistent organic pollutants (POPs) since the early part of the twentieth century. Polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) are persistent, bioaccumulative POPs that were disseminated in the environment decades before their potential for involvement in adverse health effects in humans was identified.

Measurable levels of PCBs and OCPs were detected in human serum samples obtained in the 1940s and comparable levels of these POPs were detected in serum specimens analyzed in 2002. Peak human serum levels of PCBs and several OCPs occurred in the late 1970s prior to the enactment of legislation banning their production and use. Results from our laboratory indicate that human serum levels are declining, however passive human exposure to PCBs and some OCPs still occurs by inhalation of dust or ingestion of food. It is important from a public health standpoint therefore to determine if serum levels of PCBs and OCPs are decreasing over time.

1.2 The proposed method describes the procedure for the analysis and quantitation of PCB congeners , 4,4'-DDE, 4,4'-DDT and mirex in newborn calf serum by solid phase extraction and gas chromatography with mass selective detection in the selected ion mode (GC/MSD/SIM). The Con2 standard comprising 44 PCB congeners, 4,4'-DDE, 4,4'-DDT and mirex is used to establish the calibration curve while the Calibration

Check Standard (CCS) comprising 21 congeners representing the homologues from tri- to deca-chloro biphenyls is used verify the accuracy of the calibration standard and for the method development

1.3 Polychlorinated biphenyl (PCB) congeners, 4,4'-DDE, 4,4'-DDT and mirex are extracted using the Biotage Rapid-Trace solid phase extraction (SPE) system.

1.4 Minimum Reporting Limit (MRL) - Defined as the lowest concentration of an analyte that can be reported based on the results of the method detection limit study.

1.5 Method Detection Limit (MDL) is defined as the statistically calculated concentration that can be measured with 99% confidence that the reported value is greater than zero. The MDL study for the target analytes can be found in the Table 9.

1.6 This SOP should be followed as written. Sometimes, however, deviations from the SOP are unavoidable. Any deviation for the SOP must be documented and approved by the QC holder.

2. SUMMARY OF METHOD

This work describes the analysis of polychlorinated biphenyls (PCBs) 4,4'-DDE, 4,4'-DDT, and mirex in human serum using gas chromatography / isotope dilution mass spectrometry. Serum specimens are spiked with 8-^{13}C PCB congeners and ^{13}C 4,4'-DDE as internal standards. Newborn calf serum is spiked with a standard mixture containing 44 PCB congeners, 4,4'-DDE, 4,4'-DDT, mirex, and the isotopically labeled internal standards. The serum specimens the spiked new born calf serum(Matrix Spike) are deproteinized with 88% formic acid, and extracted using an automated solid phase extraction (SPE) system equipped with silica-based C18 cartridges. Target analytes are eluted with 15%/85% dichloromethane/hexane (v/v) and lipids are removed by passing extracts through a silica gel/acid silica gel column. The eluate is reduced to 0.1 mL in hexane before a GC/MSD analysis. Extracts are analyzed in SIM mode at ion source temperature of 250^{o}C. Matrix matched calibration at 0.1 0.5,1.0, 2.5, 5.0 and 10 ng/g was used to assess detector linearity.

3. DEFINITIONS

3.1 ANALYSIS BATCH – A set of samples analyzed during a 24-hr period using the same instrumentation, calibration standards and quality control samples. Each analysis batch begins and ends with the analysis of the appropriate Continuing Calibration Check (CCC) Standard.

3.2 CALIBRATION STANDARD – A solution of method analytes prepared from working standard solutions used to calibrate the instrument response with respect to analyte concentration.

3.3 CONTINUING CALIBRATION CHECK STANDARD (CCC) – One of the mid level calibration standards used to verify the acceptability of an existing calibration. A CCC must be analyzed at the beginning and end of each analysis batch.

3.4 CALIBRATION CHECK STANDARD (CCS) – A solution of method analytes prepared from a second stock standard source that is different from the source used to prepare calibration standards. The CCS verifies that the original calibration is acceptable by measuring the measures the accuracy of calibration standard using a second source standard. The CCS need not be qualitatively identical to the Calibration Standard but should contain congeners present in each homologue group and at least one pesticide.

3.5 INTERNAL STANDARD (IS) – A pure compound added to a sample, or standard solution in known amount that is used to measure the relative responses of target analytes. In this study, a ^{13}C PCB mixture, composed of congeners from each homologue group, CL 1 through CL 10, and ^{13}C 4,4'-DDE were used as internal standards.

3.6 MATRIX SPIKE (MS) – An aliquot of new born calf serum spiked with known quantities of target analytes is extracted and analyzed like a sample. The MS is used to calculate recoveries of target analytes and to determine if the sample matrix contributes any bias to the sample results.

3.7 METHOD BLANK (MB) – An aliquot of new born calf serum that is treated the same as a sample. The MB is taken through all the same method procedures and exposed to the same glassware, equipment, preservatives, solvents, reagents, and internal standards as a sample. The MB determines if target analytes or other interferences are present in the laboratory environment, reagents, or equipment.

3.8 METHOD DETECTION LIMIT (MDL) – The statistically calculated minimum concentration of an analyte that can be measured with 99% confidence that the value is greater than zero.

3.9 MINIMUM REPORTING LIMIT (MRL) – The minimum concentration that can be reported for a target analyte. This limit is based on quality control criteria measured in the MDL study and can be no lower than the lowest calibration standard. The MRL is typically 3-5 times the MDL.

3.10 PERFORMANCE TEST – The performance test is composed of IUPAC 58 and IUPAC 186 and is analyzed before each batch to check the reproducibility of the retention time and integrated abundances (peak areas) of the analytes of interest.

3.11 PRIMARY DILUTION STANDARD SOLUTION (PDS) – A solution of method analytes prepared from stock standard solutions (SSS) and diluted as necessary to prepare calibration standards or other necessary analyte solutions.

3.12 REAGENT BLANK (RB) - An aliquot of pure solvent subjected to the same analytical or measurement process as a normal sample. The RB determines if target analytes or other interferences are present in laboratory reagents or glassware.

3.13 SOLVENT BLANK - An aliquot of pure solvent analyzed prior to analysis batch, after analytical standards and after sample extracts showing high levels of target analytes.

3.14 STOCK STANDARD SOLUTION (SSS) – A concentrated solution of target analytes prepared in the laboratory from referenced and certified standards or a concentrated solution of target analytes purchased directly from a referenced and certified source.

4. INTERFERENCES

4.1 Method interferences may be caused by contaminants in solvents, reagents, glassware, and other sample processing apparatus that lead to discrete artifacts or elevated chromatographic baselines. MB and RB must be run to verify that these types of interferences are not present.

4.2 Glassware must be thoroughly cleaned. After use, rinse all glassware with last solvent used, wash with hot water and detergent, rinse with tap water and reagent water, and heat in an oven to dry at 100-200 ^{o}C for a minimum of one hour. Allow glassware to come to room temperature. Rinse three times with nanograde hexane, save last rinse, concentrate to 0.05mL and analyze by GC/MSD to determine if interferences are present.

4.3 Interferences may occur when a sample containing low concentrations of analytes is analyzed following a sample containing high concentrations of analytes. If this type of interference is believed to have occurred, solvent blanks must be run through the system until contamination is eliminated and then the affected sample must be reanalyzed to verify results.

5. SAFETY AND WASTE MANAGEMENT

5.1 PCBs and pesticides 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. Lab coat, protective gloves, and safety glasses are to be worn while working in the biocontainment and chemical hoods. Serum samples will be handled in a biocontainment hood in accordance with NYSDOH Safety Manual (Number 11.2, issued 01/86, and revised 12/04).

5.2 Always follow guidelines listed in material safety data sheets (MSDS) for proper storage, handling, and disposal of solvents, reagents, and standards. MSDSs are located within the laboratory in a labeled, yellow binder.

5.3 Handle solvents in an area with adequate ventilation, such as a chemical fume hood. Flammable solvents should be used away from sources of ignition.

5.4 Solvents are stored in yellow safety storage cabinets. No more than 2 bottles of solvent are allowed to remain on benches overnight in each laboratory room.

5.5 In case of chemical spills, evacuate the area. While wearing a chemical respirator, drop disposable pads over the spill. Place pads inside a chemical fume hood until dry before disposal in the proper waste container. The area should be cleaned according to the Material Safety Data Sheets (MSDS).

5.6 Laboratory trash is segregated from normal trash. All discarded and disposable glass and gloves are placed in separate, plastic lined containers supplied by the Safety Office. When ready for collection, seal the container and label with the designation "Sharps" along with your name, telephone number and room number. The box is left outside the door for collection.

5.7 All disposable glassware being exposed to serum will be disposed of in aluminum stockpots that are clearly marked "Biohazard."

5.7.1 The room number, date and types of biological materials (if known), blood products and body fluids, and others must be listed on the tag attached to each bucket.

5.7.2 Stockpots must not be filled more than three-quarters full. There should be a minimum of ½ inch of water in the bottom.

5.7.3 The top of the autoclave bag must be folded inward and tape must be used to secure the lid.

5.7.4 Radioactive or chemical waste must not be placed in the stockpot.

5.8 Non-disposable glassware will be submerged in 10% Clorox overnight. Liquid solvent waste will be disposed of in red organic-waste containers.

5.9 Dispose of solvent waste in appropriately marked containers. Solvents, except dichloromethane, are disposed of in red 5-gallon waste containers. Yellow tags are filled out completely with no abbreviations and tied to the container. Dichloromethane is collected separately in an appropriately labeled 4L glass solvent bottle. The Safety Office is called to pick up the container.

6 POLLUTION PREVENTION

6.1 Solvent, chemical, reagent, and standard use are minimized whenever possible to reduce the amount of hazardous waste generated.

6.2 All waste is disposed of in appropriately labeled containers for proper handling and future treatment (see section 5.0).

7 COMPETENCY AND TRAINING ASSESSMENT

7.1 Any employee learning a new procedure will go through an initial training period. At the end of the training

period they will be observed and evaluated on the tasks required of them. After being evaluated and proven competent a technician will be observed and evaluated once a year performing the required tasks by either the lab supervisor or another competent technician.

7.2 Methods for evaluating competency may include, but are not limited to:

7.2.1 Direct observation of routine test performance

7.2.2 Use of lab equipment, performance and documentation of instrument use and maintenance

7.2.3 Review of worksheets or QC records

7.2.4 Proficiency tests and internal blind specimens

7.2.5 Written quizzes

7.3 INITIAL TRAINING

7.3.1 New employees will be trained and evaluated in the Biological Organic Analytical Chemistry (BOAC) lab by a previously trained and competent technician.

7.3.2 Training will consist of reading SOPs, direct observation, receipt of samples, preparation with blind samples, analyzing the samples and preparing a report for data review with minimal supervision.

7.3.3 All work will be documented on BioOrganic Analytical Chemistry Laboratory Demonstration of Capability Record (Appendix C). If certain criteria have not been met, the Lab Supervisor will meet with the technician and discuss improvement goals. The new employee would go through the evaluation process again. Once completing the training and showing competency the technician will be allowed to work independently with clinical samples.

7.4 Competency: All employees will be assessed once a year for competency. A competent technician or lab supervisor will observe and evaluate the technician and document these observations on the BioOrganic Analytical Chemistry Laboratory Demonstration of Capability Record (Appendix C).

7.5 Continuing Education : Also a minimum of twelve hours of continuing education must be provided to laboratory staff and is documented. Acceptable forms of continuing education include in-service, professional meetings or industry sponsored training/workshop programs.

7.6 The lab supervisor will keep the Demonstration of Capability Record, a copy of Proficiency Tests that the technician has preformed within the year and Continuing Education records.

8 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

8.1 Most persistent organic pollutants are extremely resistant to degradation in the environment and would not be expected to degrade even if samples are not properly stored. However, a sample may be considered unsuitable for analysis when there is evidence of damage to a sample container, when the sample volume is incorrect, or when documentation accompanying a sample is incomplete. The client sending the sample will then be notified that the sample was rejected. The laboratory will record the reason why the sample was rejected.

8.2 Samples will be received and accessioned in Room D-634 of the Wadsworth Center. The sample submission form should contain detailed information regarding collection of the sample and any relevant observations made by the sample collector. If chain of custody forms are required, they should contain abbreviated sample information and signatures describing the actual custody of the sample from time of collection until receipt by the laboratory. Personnel using proper protective measures will open sample containers only in the biocontainment hood. Samples not processed immediately will be stored in the BOAC laboratory freezer located in the D-level alcove area.

8.2.1 Freezers are monitored 24/7 by in house technical personnel employed by Johnson Controls. Each freezer is required to have a yellow “Equipment Information” card prominently displayed that contains emergency contact information in the event of a malfunction. Johnson Controls also maintains spare freezers that can be used to store specimens until the primary freezer is repaired or replaced.

8.2.1.1 In the event of a freezer malfunction, Johnson Controls will immediately contact the appropriate personnel and arrangements will be made to preserve sample specimens.

8.2.1.2 Document the malfunction on a Nonconformance / Correction Action Report (FORM QS – 11).

8.3 Below is an outline of the sample process.

8.3.1 Sample Collection

8.3.2 Sample Receipt/Tracking

8.3.3 Sample Storage

8.3.4 Sample Preparation

8.3.5 Sample Analysis

8.3.6 Sample Reporting

8.4 Human Serum Collection

8.4.1 The blood is collected in 5-mL or 7-mL red top vacutainers.

8.4.2 A successfully drawn tube should contain less than 1 cm of headspace.

8.4.3 Sample tubes should stay at room temperature at least 1 hour and then spin down the blood cells by centrifugation at 1500 RPM, and take out the top layer, which is the serum.

8.4.4 Care must be taken to insure that samples are kept at refrigerator temperature during storage and shipping. Samples should be kept cool during the whole shipping process. Also, special care should be taken when packing samples to prevent breakage during the shipping process.

8.4.5 Once samples are received at the Wadsworth Center facility, they are logged in, chain of custody documents are generated (if needed), and the samples are brought to the BioOrganic Analytical Chemistry lab to be stored at –20 °C until analysis.

8.4.6 Criteria for unacceptable specimens would be: low volume samples; leaks; broken or cracked tube; headspace requirements; failure to maintain proper temperature; suspected contamination. Reasons for rejection should be recorded. Reports will be generated for all samples received, whether or not analysis is performed.

8.5 Sample Receipt/Tracking Protocol

8.5.1 Each sample, even rejected samples, will be assigned a unique NYSDOH identifier at accession time. This identifier will be recorded on the sample accession form and will be used to track the samples from reception to analysis to final reporting. If the specimen has been assigned an identifier by the collector, this identifier will be recorded on the sample accession form. Immediately notify the client of any and all labeling discrepancies and document the nonconformance using a Nonconformance/Corrective Action Report (FORM QS-11) according to SOP QS-11 'Nonconformance / Corrective Action Procedure'.

9. EQUIPMENT AND SUPPLIES

9.1 SOLID PHASE EXTRACTION (SPE) APPARATUS

9.1.1 SPE extraction and SPE clean-up cartridges used for PCB extraction are described in the Appendix A.

9.1.2 Extraction apparatus: Biotage RapidTrace Automated Workstation (Serial # RT0336N6984) or equivalent. RapidTrace SPE Workstation software installed on a laptop computer with Windows NT is used to control the Workstation.

9.1.3 Extract concentration system – Extracts are concentrated by blowdown with nitrogen using Zymark Turbo Vap LV Concentration Workstation (Serial #TV0332N11869) or equivalent.

9.2 GC/MSD INSTRUMENTATION

9.2.1 Agilent Technologies Gas Chromatograph – Mass Selective Detector (GC/MSD)

9.2.1.1 Gas Chromatograph, model # 6890N (serial # US10633032) - Gas chromatograph capable of reproducibly injecting down to 2 µL aliquots, and performing binary linear gradients at a constant flow rate of helium at 1 mL/min (average velocity of 37 cm/min.). The GC must be equipped for split/splitless injection, autosampler and be capable of temperature programming from 35 to 350^{o}C.

9.2.1.2 Injector, model # 7683B (serial # US63010507) or equivalent,

9.2.1.3 Autosampler, model # N10149 (serial # US62815638) or equivalent,

9.2.1.4 Mass Selective Detector (MSD), model # 5975B (serial # US62723834) or equivalent. The mass spectrometer must be capable of electron ionization at a nominal electron energy of 70 V and of scanning from 35 to 550 amu with a complete scan cycle of 1.5 sec or less. The MSD must be equipped for selected ion monitoring (SIM) mode to acquire the mass of the analytes, and the ion source is capable of operating up to 300 ^{o}C.

9.2.1.5 GC/MSD Data System, MSD ChemStation Software version D.03.06.611 (Agilent Technologies).

9.2.1.6 Capillary column, J&W P/N 121-1232, DB-XLB 30m,x 0.18mm ID, 0.18um film or equivalent.

9.3 REAGENT AND STANDARD PREPARATION EQUIPMENTS

9.3.1 Analytical balance – Ohaus, model # AP250D, serials # 1127211982, capable of weighing from 0.00001 to 10 g.

9.3.2 Sonicator – Branson, model 5510, serial # RNB020497109E.

9.3.3 Vortex – Scientific Industries Inc., model G-560, serial #2-280415.

9.3.4 Glassware – All glassware used in this study must be meticulously cleaned. Wash glassware with detergent and tap water, rinse with tap water, followed by reagent water. Non-volumetric glassware can be heated in an oven at 105 ^{o}C for 24 hr. Volumetric glassware should not be heated above 120 ^{o}C.

9.3.5 Graduated cylinders – Kimble USA, various sizes.

9.3.6 Volumetric flasks – Kimble, USA, Class A, various sizes.

9.3.7 Micro syringes – Hamilton, various sizes.

9.3.8 Beakers - various sizes.

9.3.9 Erlenmeyer flasks - various sizes.

9.3.10 15 mL disposable culture tubes - Kimble Glass Inc., catalog # 73500 16100.

9.3.11 1.5 mL amber screw top autosampler vials - National Scientific Company, catalog # C4000- 1W.

9.3.12 Disposable Pasteur pipettes.

10. REAGENTS AND STANDARDS

10.1 GASES, REAGENTS AND SOLVENTS - Reagent grade or better chemicals should be used. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where such specifications are available.

10.1.1 Nitrogen, Northeast Gas Technologies, Ltd. (grade 5.0) purity 99.999% or equivalent,

10.1.2 Helium, Northeast Gas Technologies, Ltd. (grade 5.0); purity 99.999% or equivalent,

10.1.3 Acetone, Mallinckrodt, purity 99.5 % or equivalent,

10.1.4 Dichloromethane (JT Baker), purity 99.9 % or equivalent,

10.1.5 Hexane (J.T. Baker), purity 99.5 % or equivalent,

10.1.6 Hydrochloric Acid (J.T. Baker), 36.5-38 % or equivalent, ASC reagent

10.1.7 Methanol (JT Baker), ultra high purity, HPLC grade or equivalent,

10.1.8 Reagent Water (Barnstead), HPLC grade or equivalent,

10.1.9 5% methanol/acidic H_2O Solution - In a 1L Pyrex bottle, add 10 mL of MeOH, 190 mL reagent water, and 200 mL of 0.1N HCl, and mix the solution using an ultrasonic bath for 5 minutes. The pH of the solution should be in the range of 1-1.3

10.1.10 15% dichloromethane/hexane solution - In a 1 L Pyrex bottle add 60 mL of dichloromethane and 340 mL of hexane, and mix the solution by an ultrasonic bath for 5 minutes.

All solvents should be prechecked before using

10.2 STANDARD SOLUTIONS

10.2.1 Stock Standard Solution (SSS)

10.2.1.1 Isotopically labeled Internal Standards:Cambridge Isotope Laboratories, Inc. ^{13}C labeled PCBs, 50 ug/ml in nonane,.IUPAC, 28, 52, ,118, ,153, 180, 194, 206, 209, ^{13}C 4, 4'_DDE, 100 ug/ml in nonane

10.2.1.2 Analyte Standard Solution – The PCB congener analytical standard mixture (Con2) is composed of 44 PCB congeners obtained from AccuStandard or Ultra Scientific. These congeners are most often found in the highest concentrations in human serum specimen and their concentrations reflect the extent of human exposure. The concentration of each component in stock standard solution is 100 ug/mL each. 4,4'-DDE, 4,4'-DDTand mirex were obtained from Ultra Scientific, stock standard solution of each is 100ug/ml in methanol (Table 1A).

10.2.1.3 Calibration Check Standard (CCS) – The calibration check standard mixture (AccuStandard, C-CCSREC-R, composed of 21 PCB congeners), was used to develop this method and are included as an example. The concentration of each component in calibration check standard solution is 100 ug/mL each (Table 1B).

10.2.2 Primary Dilution Standard (PDS)

10.2.2.1 IS primary dilution standard - The IS primary dilution standard is prepared by adding 0.5 mL of each of the IS stock standard solution to a 10 mL volumetric flask partially filled with acetone to make a final concentration at 5 µg/mL when filled to volume with acetone. The IS primary dilution standard is stored in 1 mL amber ampoules, and has been shown to be stable for 6 months when stored at 4 °C or less.

10.2.2.2 Analyte primary dilution standard solution

10.2.2.2.1 The analyte primary dilution standards contain all the target analytes of interest listing in the Table 1. The primary dilution standard Con2 is used to establish the calibration curve.

10.2.2.2.2 The analyte primary dilution standard are prepared by adding 1 mL of each of the analyte stock standard solution (SSS) to a 100 mL volumetric flask partially filled with acetone to make a final concentration at 1 µg/mL when filled to the mark with acetone. The analyte primary dilution standard is stored in 1mL amber ampoules, and has been shown to be stable for 6 months when stored at 4 °C or less.

10.3MSD TUNING SOLUTION

10.3.1 MSD tuning solution is used to tune and evaluate the mass spectrometer performance.

10.3.2 MSD tuning solution contains decafluorotriphenylphosphine (DFTPP) at the concentration of 5 ug/mL in dichloromethane (DCM).

10.3.3 MSD tuning solution is prepared by adding 1.25 mL of 100 ug/mL DFTPP stock solution (Ultra Scientific, IST-341-1) to a 25 mL volumetric flask partially filled with dichloromethane to make a final concentration at 5 ug/mL when filled to volume with dichloromethane.

10.3.4 The MSD tuning solution is stored in 1 mL ampoules, and has been shown to be stable for 6 months when stored at 4 °C or less.

10.4 PERFORMANCE TEST SOLUTION (PTS)

10.4.1 The performance test solution (PTS) contains IUPAC 58 and IUPAC 186 at the concentration of 50 ng/mL in hexane.

10.4.2 The PTS solution is prepared by adding 0.1 mL of each of the stock standard solution of IUPAC 58 (Ultra Scientific, RPC-128S) and IUPAC 186 (Ultra Scientific, RPC-116S) at the concentration of 100 ug/mL each to a 200 mL volumetric flask partially filled with hexane to make a final concentration at 50 ng/mL when filled to volume with hexane.

10.4.3 The PTS is stored in 5 mL ampoules, and has been shown to be stable for 6 months when stored at 4 °C or less.

11. GC/MSD PERFORMANCE TEST

11.1DFTPP TUNING

11.1.1 At the beginning of the batch that analytes are to be performed, the GC/MSD system must be calibrated with the tuning solution and procedures prescribed by the manufacturer criteria.

11.1.2 DFTPP is the compound specified by the EPA to be used to verify that mass spectrometer voltages are set correctly [1, 2]. DFTPP tuning allows to adjust mass spectrometer parameters to meet relative abundance criteria defined by EPA methods 625 [3].

11.1.3 Analyze the tuning solution (Section10.3) containing 5 ug/mL DFTPP in DCM using GC/MSD operating conditions shown in the Table 2A.

11.1.4 When the DFTPP is then injected the instrument tuning parameters are stored in DFTPP.U file.

11.1.5 When a target tune is finished, the evaluation of DFTPP tuning is performed from the View menu in the Instrument Control, Manual Tune. The fragmentation pattern in the mass spectrometer should pass the ion ratio criteria specified in the Table 2B.

11.1.6 If one of the listed criteria is not achieved, the analyst must retune the mass spectrometer and repeat the test until all criteria are achieved. The DFTPP key masses and criteria must be achieved before any samples, blanks or standards are analyzed.

11.1.7 Over time the ratios will start to fail. It is necessarily to retune the mass spectrometer.

11.2PERFORMANCE TEST

11.2.1 The performance test is used to test the performance of the GC/MSD after the DFTPP tuning procedure and before each analyze batch.

11.2.2 The performance test is carried out by analyzing 5 replicates of the PTS samples using analysis conditions described in Table 3 for PCB target analytes.

11.2.3 The evaluation of performance test is described in the section 14.1

12. CALIBRATION

12.1 INITIAL CALIBRATION

12.1.1 Selected ion monitoring (SIM) mode is used to acquire the masses of the target analytes and IS. The specific ions characterized for each target analyte were selected to establish a SIM table for all target analytes and IS. The GC/MSD operating conditions for the PCB analysis are summarized in Table 3, and the SIM data of each target analyte constituted Con2 mixture, 4,4'-DDE, 4,4'-DDT and mirex are listed in the Table 4.

12.1.2 Isotopically labeled Internal Standard: PCB 28, 52, 118, 153, 180, 194, 206, 209, 50ug/ml in nonane and ^{13}C-labeled 4,4'-DDE and ^{13}C-labeled mixex 100ug/ml in nonane (Cambridge Isotope Laboratories).

12.1.3 At least five calibration concentrations are required to prepare the initial calibration curve spanning a 100-fold concentration range. Larger concentration ranges require more calibration points. New born calf serum is spiked at 0.1;0.5;1.0;2.5;5.0; 10 ng/g of PCBs 4,4'-DDE, 4, 4'-DDT and mirex. The IS mixture is added to the calibration standard at a concentration of 10 ng/mL. The lowest concentration on the curve must be at or below the MRL, which may depend on system sensitivity.

12.2 CONTINUING CALIBRATION CHECK STANDARD (CCC)

12.2.1 The CCC standards are analyzed at the beginning and at the end of each analysis batch to verify the initial calibration during analysis by injecting a mid-level calibration standard.

12.2.2 The CCC standards are analyzed under the same GC/MSD conditions used in the initial calibration (Table 3).

12.2.3 The evaluation of CCC is described in the section 14.4 of the quality control.

12.3 DATA CALCULATION

The linear calibration curve of each analyte is established by Agilent ChemStation software that is based on integrated abundance (or peak area) - concentration data pair obtained from each of the calibration points. The origin is not included in the curve. The following parameters were calculated:

12.3.1 The response factors (RF_x) of each analyte was given by:

$$RF_x = \frac{A_x \, C_{IS}}{A_{IS} \, C_x} \qquad \text{[Equation 1]}$$

where:

A_x = Integrated abundance (peak area) of the quantitation ions of the analyte,

A_{IS} = Integrated abundance (peak area) of the IS quantitation ions,

C_x = concentration of analyte,

C_{IS} = concentration of internal standard.

12.3.2 Linear Correlation Coefficient - *R*

The linear correlation coefficient between integrated abundance or peak area (*A*) and concentration (*C*) of each analyte is calculated by the following mathematical form:

$$R = \frac{n\sum CA - \left(\sum C\right)\left(\sum A\right)}{\sqrt{n\left(\sum C^2\right) - \left(\sum C\right)^2}\,\sqrt{n\left(\sum A^2\right) - \left(\sum A\right)^2}} \qquad \text{[Equation 2]}$$

where n is the number of calibration points.

12.3.3 Coefficient of Determination - R^2

The coefficient of determination (R^2) of each analyte represents the percentage of the data being the closest to the line of best fit. It is used to measure how well the regression line represents the linear correlation data.

13. AUTOMATED SPE PROCEDURE

The PCB congeners and pesticides in the matrix spike sample are extracted using the Biotage Trace SPE workstation. The sample is allowed to equilibrate to ambient temperature in a biocontainment hood before being spiked with the IS. A specific worksheet (Appendix B) should be filled in for each sample, and the collection tube is labeled with a unique sample identifier

before extraction procedure.

13.1 SAMPLE EXTRACTION

13.1.1 A 1 g sample is dispensed into 15 mL disposable culture tube and spiked with 10 uL of a 100ng/mL IS spiking solution. The tube is capped and allowed to equilibrate for 1 hr. Add 1 g of 88% formic acid, vortex the sample for 10 sec. and add 1 mL methanol, vortex for 10 sec and sonicate for 10 min at room temperature. After diluting with 1 mL of reagent water, the sample is then vortexed again for 10 sec.

13.1.2 Purge the cannula tubes of the RapidTrace workstation using Tlpurge method (Table 5A).

13.1.3 Place C18 cartridge, sample tubes and collection tubes in their position in the RapidTrace workstation. Run Tmethdhx method (Table 5B) for PCB extraction. Concentrate the extract in each collection tube from 12 mL to about 1 mL using a Turbo Vap concentrator. The water bath temperature is set at 40 ^{o}C.

13.2 SAMPLE CLEAN-UP

The co-extracted lipid in the extract above was removed using automated SPE system by the following steps:

13.2.1 Purge the cannula tubes of the RapidTrace workstation using CLPUR704 method (Table 6A).

13.2.2 Place the extracted samples, collection tubes, and clean-up cartridges in their position in the RapidTrace workstation. Run CLEANPCB method (Table 6B) to clean up the sample. The extracts are collected in the culture tubes at the end of the clean up procedure.

13.2.3 Concentrate each extract from 12 mL to less than 1.0 mL using a Turbo Vap concentration workstation. The water bath temperature is set at 40 ^{o}C. Transfer the extract from the collection tube to 0.3 mL GC vial insert and reduce the extract volume with a gentle stream of nitrogen. Rinse the collection tube twice with 0.5 mL of hexane, transfer to the insert and reduce volume to 0.1mL for GC/MSD analysis.

13.3 SAMPLE ANALYSIS

13.3.1 The specimen extracts are analyzed by GC/MSD in the same conditions as described previously in initial calibration (section 12.1.1).

13.3.2 The acceptance criteria for a qualitative identification for each analyte are described in the section 14.7.2

13.3.3 Calculation of analyte concentration - The concentration of each analyte (C_x) in extract is calculated using the response factor (RF_x) determined from Equation 1

$$C_x \quad (ng/g) \quad = \quad \frac{A_x \; m_{IS}}{A_{IS} \; RF_x \; m_x} \qquad \text{[Equation 3]}$$

where:

A_x = Integrated abundance (peak area) of the quantitation ions of the analyte,

A_{IS} = Integrated abundance (peak area) of the IS quantitation ions,

m_{IS} = amount of IS added to each sample (ng),

m_x = amount of sample (g).

14. QUALITY CONTROL

The quality control requirements of this method consist of an initial demonstration of capability, and ongoing quality control requirements that must be met when preparing and analyzing the samples. The acceptance criteria established for each of the quality control requirements in this section allow the analyst to determine if the results of analyses meet the performance characteristics of the method.

14.1 PERFORMANCE TEST

14.1.1 The performance test allows checking the precision of the retention time (RT) and integrated abundances (peak area counts) of IUPAC 58 and IUPAC 186, which are used in this study.

14.1.2 The retention times (RT) and integrated abundances of these compounds are determined from 5 replicate analyses.

14.1.3 The RSD of RT of these compounds should be less than 1%, and the RSD of integrated abundances of these compounds should be less than 10%. The RSD of the integrated abundances for each compound must be less than 10 % of its average value.

14.1.4 If any performance criterion is not met, the analyst should perform routine maintenance procedures such as changing the autosampler syringe and/or injection port liner and seal. Cleaning the mass spectrometer source can also result in higher abundances for performance test compounds. A new performance test must be performed after any routine maintenance.

14.2 INITIAL CALIBRATION

14.2.1 The RSD of all of the response factors should be less than 20%.

14.2.2 The RSD of the IS area counts must be less than 20% of the average value of the initial calibration.

14.2.3 The correlation coefficient of the calibration curve for each analyte must be greater than 0.995 before any analysis of samples can begin.

14.2.4 If these criteria cannot be met a new calibration curve must be established.

14.3 SOLVENT BLANK (SB)

14.3.1 An aliquot of nanograde hexane analyzed prior to the batch analysis after analytical standards and samples containing high levels of target analytes. The SB is used to demonstrate that the analytical system is free of interferents before the analytical sequence is started and to verify that target analytes were not carried over from an analytical standard to a sample,or from a sample containing a high level of target analytes to a sample containing low levels of target analytes.

14.3.2 If any PCBs are found in the solvent blank at a level greater than twice the MDL, a new SB must be analyzed. If any PCBs are still present in the SB, the analyst must check the inlet system including sample vial, syringe, and injector for possible sources of contamination.

14.4 CONTINUING CALIBRATION CHECK (CCC)

14.4.1 A middle level calibration standard is analyzed at the beginning and end of a daily analysis batch to verify the acceptability of an existing calibration.

14.4.2 Determine that the absolute area of the IS quantitation ion has not changed by more than ± 20% from the average area measured during initial calibration.

14.3.3 Calculate the concentration of each analyte in the CCC. The calculated amount for each analyte for medium level CCC must be within ±20% of the true value.

14.3.4 If these conditions are not achieved, then all data for the existing calibration must be considered invalid, and remedial action should be taken, which may require recalibration.

14.5 CALIBRATION CHECK STANDARD (CCS)

14.5.1 A middle level CCS is analyzed after CCC to verify the acceptability of an existing calibration.

14.5.2 Determine that the absolute area of the IS quantitation ion has not changed by more than ± 20% from the average area measured during initial calibration.

14.5.3 Calculate the concentration of each analyte in the CCS. The calculated amount for each analyte for medium level CCS must be within ±20% of the true value.

14.5.4 If these conditions are not achieved, then all data for the existing calibration must be considered invalid and a second CCS must be analyzed. If the second calibration check does not meet the acceptance criterion then routine maintenance such as changing injection port liner and seal, and removal of 6 inches from the injection port end of the analytical column must be performed.

14.6 INITIAL DEMONSTRATION OF CAPABILITY

14.6.1 Initial Demonstration of Low System Background in Method Blank and Reagent Blank

14.6.1.1 Used to identify interference that may be present in the sample matrix. It is processed through the entire procedure. Every analyst must complete an initial demonstration of capability before the analysis of samples can begin.

14.6.1.2 Determine that the absolute area of the IS quantitation ion in MB sample has not changed by more than ± 20% from the average area measured during initial calibration.

14.6.1.3 If any PCBs are found in the method blank (newborn calf serum) or reagent blank (solvent) at greater than twice the MDL, the results obtained for that analyte will not be reported.

14.6.2 Method Detection Limits (MDL)

14.6.2.1 An MDL must be determined before any specimen can be analyzed. An MDL study must be done on an annual basis thereafter and when any major method modifications are instituted.

14.6.2.2 Analyze 8-10 samples that have been spiked with CCS mixture at 0.1 ng/g.

14.6.2.3 Calculate the MDL using the following equation:

$$MDL = S\, t_{(n-1,\ 1-\alpha = 0.99)} \quad \text{[Equation 4]}$$

where:

S: standard deviation of replicate sample

$t_{(n-1,\ 1-\alpha\ =\ 0.99)}$: Student's t value for the 99% confidence level with n-1 degrees of freedom

n: number of replicates

14.6.2.4 The minimum reporting level (MRL) is then determined based on the results of the MDL study. The MRL must be at least 3-5 times the MDL and equal to or greater than the lowest calibration standard.

$$MRL = 3\ MDL \quad \text{[Equation 5]}$$

14.6.3 Initial demonstration of precision (IDP) and accuracy (IDA)

14.6.3.1 Analyze 8-10 samples that have been spiked with CCS at midrange concentration (1.0 ng/mL).

14.6.3.2 The RSD of mean recovery for IS must be less than 20%, and the RSD of the individual recovery for each analyte must be less than 20% of initial value (spiking level).

14.6.3.3 The entire procedure must be repeated if these criteria are not achieved.

14.7 MATRIX SPIKE (MS) / HUMAN SERUM SAMPLE

14.7.1 The matrix spike sample is used to evaluate the effect of the matrix on the recovery of the target analytes. The reference matrix, newborn calf serum is spiked with the target PCBs at a final concentration of 0.5, 1.0, 10ng/g. Recovery pertains to the extraction efficiency of an analytical method within the limits of variability. Recovery to the analyte need not be 100%, but the extent of recovery of an analyte and of the internal standard should be consistent, precise, and reproducible.

14.7.2 Determine that the recovery of internal standards in MS sample must be within 70-130% of the average value of the initial calibration, and the recovery of each target analyte must be within 70-130% of the initial value (spiking level).

14.7.3 Qualitative identification: For both human serum and MS samples, the absolute RT of target analyte must be within ± 0.05 min. of the RT for that analyte in the Initial Calibration, and the ion ratios between target ion and qualified ion must be within ±20% of those established for target

analyte in the SIM table (Table 4).

14.8 INSTRUMENT DETECTION LIMIT (IDL)

14.8.1 Prepare 7 to 10 replicates using standard calibration solutions at a concentration at 2-5 times the noise level.

14.8.2 Analyze these samples under GC/MSD conditions and analyze with the same conditions used during the initial calibration (Section 10.1).

14.8.3 Calculate the IDL using the following equation:

$$IDL = S\, t_{(n-1,\ 1-\alpha = 0.99)} \qquad \text{[Equation 6]}$$

where:

- S: standard deviation of replicate sample
- $t_{(n-1,\ 1-\alpha = 0.99)}$: Student's t value for the 99% confidence level with n-1 degrees of freedom
- n: number of replicates

14.8.4 The lower confidence limit (LCL) and upper confidence limit (UCL) are derived from IDL at 95% confidence level:

$$LCL = 0.64\ IDL \qquad \text{[Equation 7]}$$

$$UCL = 2.2\ IDL \qquad \text{[Equation 8]}$$

14.9 EXTERNAL PROFICIENCY TEST

The laboratory participates in the Arctic Monitoring and Assessment Program that tests proficiency analyzing human serum for PCB congeners, pesticides and pesticide metabolites. The test is administered quarterly by the *Institut national de santé publique, Centre de toxicologie, 945, Ave Wolfe, Quebec Canada, G1V 5B3.*

15. DATA ASSESSMENT

15.1 All analytical batches must meet all quality control criteria described in section.

15.2 These quality control criteria must be documented and archived.

15.3 If any analytical batch contains quality control data that fails any of the criterion, then the relevant data should be reported as "suspect".

16. CORRECTIVE ACTIONS AND CONTINGENCIES FOR OUT OF CONTROL DATA

Corrective actions are different for each quality control criterion. Section 14 (Quality Control) describes all of the necessary quality control and procedures to follow if any data is deemed out of control.

17. REFERENCES

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[3] Agilent Technologies Publication number 05171 (http://www.chem.agilent.com). Using Target Tune to Tune for DFTPP.

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[5] Sjodin A., Jones R.S., Lapeza C.R., Focant, J-F., McGahee, E.E., Patterson, D.G. Semiautomated High-Throughput Extraction and Cleanup Method for the Measurement of Polybrominated Diphenyl Ethers, Polybrominated Biphenyls, and Polychlorinated Biphenyls in Human Serum. Anal. Chem. 2004, 76, 1921-1927.

18. TABLES AND DEMONSTRATION DATA

TABLE 1A
POLYCHLORO BIPHENYLS IN CON2 MIXTURE, 4,4'-DDE, 4,4'-DDT AND ^{13}C INTERNAL

STANDARDS

Symbol	Compound Name	CAS number
IUPAC 28	2,4,4'-Trichlorobiphenyl	7012-37-5
IUPAC 28*	2,4,4'-Trichlorobiphenyl	7012-37-5
IUPAC 44	2,2',3,5'-Tetrachlorobiphenyl	41464-39-5
IUPAC 52	2,2',5,5'-Tetrachlorobiphenyl	35693-99-3
IUPAC 52*	2,2',5,5'-Tetrachlorobiphenyl	35693-99-3
IUPAC 66	2,3',4,4'-Tetrachlorobiphenyl	32598-10-0
IUPAC 74	2,4,4',5-Tetrachlorobiphenyl	32690-93-0
IUPAC 77	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3
IUPAC 99	2,2,4,4',5-Pentachlorobiphenyl	38380-01-7
IUPAC 101	2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2
IUPAC 105	2,3,3',4,4'-Pentachlorobiphenyl	32598-14-4
IUPAC 110	2,3,3',4',6-Pentachlorobiphenyl	38380-03-9
IUPAC 118	2,3',4,4',5-Pentachlorobiphenyl	31508-00-6
IUPAC 118*	2,3',4,4',5-Pentachlorobiphenyl	31508-00-6
IUPAC 126	3,3',4,4',5-Pentachlorobiphenyl	57465-28-8
IUPAC 128	2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3
IUPAC 138	2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2
IUPAC 146	2,2',3,4',5,5'-Hexachlorobiphenyl	51908-16-8
IUPAC 153	2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1
IUPAC 153*	2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1
IUPAC 156	2,3,3',4,4',5-Hexachlorobiphenyl	38380-08-4
IUPAC 167	2,3',4,4',5,5'-Hexachlorobiphenyl	52663-72-6
IUPAC 170	2,2',3,3',4,4',5-Heptachlorobiphenyl	35065-30-6
IUPAC 172	2,2',3,3',4,5,5'-Heptachlorobiphenyl	52663-74-8
IUPAC 177	2,2',3,3',4',5,6-Heptachlorobiphenyl	52663-70-4
IUPAC 178	2,2',3,3',5,5',6-Heptachlorobiphenyl	52663-67-9
IUPAC 180	2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3
IUPAC 180*	2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3
IUPAC 183	2,2',3,4,4',5',6-Heptachlorobiphenyl	52663-69-1
IUPAC 187	2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0
IUPAC 194	2,2',3,3',4,4',5,5'-Octachlorobiphenyl	35694-08-7
IUPAC 194*	2,2',3,3',4,4',5,5'-Octachlorobiphenyl	35694-08-7
IUPAC 195	2,2',3,3',4,4',5,6-Octachlorobiphenyl	52663-78-2
IUPAC 199	2,2',3,3',4,5,5',6'-Octachlorobiphenyl	52663-75-9
IUPAC 201	2,2',3,3',4,5',6,6'-Octachlorobiphenyl	40186-71-8
IUPAC 203	2,2',3,4,4',5,5',6-Octachlorobiphenyl	52663-76-0
IUPAC 206	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	40186-72-9
IUPAC 206*	2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl	40186-72-9
IUPAC 209	2,2',3,3',4,4',5,5',6,6'-Decachlorbiphenyl	2051-24-3
IUPAC 209*	2,2',3,3',4,4',5,5',6,6'-Decachlorbiphenyl	2051-24-3
IUPAC 49	2,2',4,5'-Tetrachlorobiphenyl	41464-40-8
IUPAC 87	2,2',3,4,5'-Pentachlorobiphenyl	38380-02-8

IUPAC 81	3,4,4',5-Tetrachlorobiphenyl	70362-50-4
IUPAC 151	2,2',3,5,5',6-Hexachlorobiphenyl	52663-63-5
IUPAC 149	2,2',3,4',5',6-Hexachlorobiphenyl	38380-04-0
IUPAC 123	2',3,4,4',5-Pentachlorobiphenyl	65510-44-3
IUPAC 114	2,3,4,4',5-Pentachlorobiphenyl	74472-37-0
IUPAC 158	2,3,3',4,4',6-Hexachlorobiphenyl	74472-42-7
IUPAC 157	2,3,3',4,4',5'-Hexachlorobiphenyl	69782-90-7
IUPAC 169	3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6
IUPAC 196	2,2',3,3',4,4',5,6'-Octachlorobiphenyl	42740-50-1
IUPAC 189	2,3,3',4,4',5,5'-Heptachlorobiphenyl	39635-31-9
4,4'-DDE	1,1'-(Dichloroethenylidene)bis(4-chlorobenzene)	72-55-9
4,4'-DDE*	1,1'-(Dichloroethenylidene)bis(4-chlorobenzene)	72-55-9
Mirex	1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachloro-octohydro-1,3,4-metheno-1H-cyclobuta[cd}pentalene	2385-85-5
Mirex*	1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachloro-octohydro-1,3,4-metheno-1H-cyclobuta[cd}pentalene	2385-85-5
4,4'-DDT	1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene dichlordiphenyldichlorethylene	50-29-3

* $^{13}C_{12}$ Internal Standard

TABLE 1B
POLYCHLORO BIPHENYLS IN CCS MIXTURE

Symbol	Compound Name	CAS number
IUPAC 8	2,4'-Dichlorobiphenyl	34883-43-7
IUPAC 18	2,2',5-Trichlorobiphenyl	37680-65-2
IUPAC 28	2,4,4'-Trichlorobiphenyl	7012-37-5
IUPAC 44	2,2',3,5'-Tetrachlorobiphenyl	41464-39-5
IUPAC 52	2,2',5,5'-Tetrachlorobiphenyl	35693-99-3
IUPAC 66	2,3',4,4'-Tetrachlorobiphenyl	32598-10-0
IUPAC 77	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3
IUPAC 101	2,2',4,5,5'-Pentachlorobiphenyl	37680-73-2
IUPAC 105	2,3,3',4,4'-Pentachlorobiphenyl	32598-14-4
IUPAC 118	2,3',4,4',5-Pentachlorobiphenyl	31508-00-6
IUPAC 126	3,3',4,4',5-Pentachlorobiphenyl	57465-28-8
IUPAC 128	2,2',3,3',4,4'-Hexachlorobiphenyl	38380-07-3
IUPAC 138	2,2',3,4,4',5'-Hexachlorobiphenyl	35065-28-2
IUPAC 153	2,2',4,4',5,5'-Hexachlorobiphenyl	35065-27-1
IUPAC 170	2,2',3,3',4,4',5-Heptachlorobiphenyl	35065-30-6
IUPAC 180	2,2',3,4,4',5,5'-Heptachlorobiphenyl	35065-29-3
IUPAC 187	2,2',3,4',5,5',6-Heptachlorobiphenyl	52663-68-0
IUPAC 195	2,2',3,3',4,4',5,6-Octachlorobiphenyl	52663-78-2
IUPAC 201	2,2',3,3',4,56,6'-Octachlorobiphenyl	40186-71-8

IUPAC 206	2,2',3,3',4,4',5,5',6-nonachlorobiphenyl	40186-72-9
IUPAC 209	2,2',3,3',4,4',5,5',6,6'-Decachlorbiphenyl	2051-24-3

TABLE 2A

GC/MSD OPERATING CONDITIONS FOR DFTPP TUNING

1. GC OPERATING CONDITIONS

- Injection volume: 1 uL
- Inlet temperature: 250 ^{o}C
- Temperature program:

Oven Ramp	Rate ^{o}C/min.	Final Temperature ^{o}C	Holding Time minutes	Run time minutes
Initial	0	90	2	2
Ramp 1	20	300	2	14.5

2. MSD OEPRATING CONDITIONS

- Acquisition Mode: SCAN
- Electron energy: 70 V (nominal)
- Mass spectrometer source temperature: 300 ^{o}C
- Mass spectrometer quadruple temperature: 150 ^{o}C
- Mass Range: 35-550 amu
- Scan time: At least 5 scans/peak but not exceed 7 seconds/scan.

TABLE 2B

DFTPP KEY MASSES AND ABUNDANCE CRITERIA

Target Mass	Relative to Mass	Lower Limit %	Upper Limit %
51	198	20	60
68	69	0	3
69	198	0	100
70	69	0	2
127	198	40	60
197	198	0	1
198	442	40	100
199	198	5	9
275	198	10	40
365	198	1	100
441	443	1	100

443	442	17	23

TABLE 3

GC/MSD OPERATING CONDITIONS FOR PCB/PESTICIDE ANALYSIS

1. GC OPERATING CONDITIONS

- Injection volume: 2 uL
- Inlet temperature: 250 ^{o}C
- Temperature program:

Oven Ramp	Rate ^{o}C/min.	Final Temperature ^{o}C	Holding Time minutes	Run time minutes
Initial	0	90	1	1
Ramp 1	30	150	0	3
Ramp 2	2	244	0	50
Ramp 3	5	280	0	57.2
Ramp 4	50	300	2	59.6

2. MSD CONDITIONS

- Acquisition Mode: SIM
- Electron energy: 70 V (nominal)
- Mass spectrometer source temperature: 300 ^{o}C
- Mass spectrometer Quadruple temperature: 150 ^{o}C
- Mass Range: 35-550 amu
- Scan time: Select a dwell time that yields 15-20 cycles across a peak for each analyte [4].

TABLE 4
TARGET ION AND QUALIFICATION ION MASSES

Analyte	Target ion	Qual ion (relative ratio)	Qual ion (relative ratio)	Target ion	Qual ion (relative ratio)
IUPAC 28*	257.9	255.9 (94.5)		268.1	269.9 (91.0)
IUPAC 52*	291.9	289.9 (79.7)		303.9	301.9 (78.3)
IUPAC 49	219.9	222 (71.5)	291.9 (115.4)		
IUPAC 44	291.9	289.9 (75.5)	219.8 (96.0)		
IUPAC 74	291.8	289.9 (47.8)	293.9 (78.7)		
IUPAC 66	291.8	289.9 (77.5)	293.8 (48.9)		
IUPAC 101	325.8	327.8 (64.9)	323.9 (59.1)		
IUPAC 99	325.8	323.8 (62.4)	327.8 (59.0)		
IUPAC 87	325.9	327.9 (66.7)	255.9 (68.0)		
4,4'-DDE*	246	248 (63.0)		258	260 (65.0)
IUPAC 110	325.8	323.8 (62.8	327.8 (63.4)		
IUPAC 81	291.9	289.9 (80.8)	293.9 (51.2)		
IUPAC 151	359.9	289.9 (73.6)	361.9 (80.1)		
IUPAC 77	291.8	289.9 (71.0)	293.9 (47.1)		
IUPAC 149	359.9	361.9 (82.0)	289.9 (68.0)		
IUPAC 123	325.9	327.9 (65.2)	323.9 (64.2)		
IUPAC 118*	325.9	327.9 (65.2)		337.9	339.9 (62.8)
IUPAC 146+114**	359.8	361.8 (82.0)		325.9	323.9 (64.2)
IUPAC 153*	359.8	361.8 (82.5)		371.9	373.9 (79.9)
IUPAC 105	325.8	323.8 (63.8)	327.8 (62.0)		
IUPAC 138+4,4'-DDT**	359.8	361.8 (77.4)		235	237 (64.3)
IUPAC 178+158**	393.8	395.8 (96.1)		359.9	361.9 (80.1)
IUPAC 187	393.8	395.8 (96.4)	397.8 (52.0)		
IUPAC 183	393.7	395.7 (96.3)	397.8 (51.6)		
IUPAC 126	325.8	323.8 (58.2)	327.8 (56.4)		
IUPAC 128	359.8	289.8 (79.2)	361.8 (83.9)		
IUPAC 167	359.8	357.8 (50.8)	361.8 (78.1)		
IUPAC 177	393.7	395.7 (96.9)	397.7 (52.6)		
IUPAC 201	429.7	427.7 (89.7)	431.6 (63.3)		
IUPAC 156	359.8	357.8 (45.1)	361.8 (75.5)		
IUPAC 172+157**	393.8	395.8 (95.3)		359.9	361.9 (77.1)
IUPAC 180*	393.7	395.8 996.0)		405.9	407.9 (92.1)
IUPAC 170	393.8	395.7 (98.7)	397.7 (58.5)		
IUPAC 199	429.7	427.7 (87.8)	431.7 (67.4)		
IUPAC 169+(196&203)**	362	363.9 (56.4		429.9	427.8 (95.5)
IUPAC 189+195**	393.9	395.9 (99.0)		429.8	427.8 (87.5)
IUPAC 194*	429.7	427.6 (71.9)		439.9	441.9 (112)

Mirex	271.70	273.90(94.60)	269.80(57.1)		
IUPAC 206*	463.8	461.7 (72.9)		475.9	473.9 (85.1)
IUPAC 209*	497.7	499.7 (82.9)		509.8	511.8 (80.8)

The analytes are classified following the order of the retention time
Relative ion ratio between qualified ion and target ion
* Native and IS coelute, choose two ions for each
** Coelute, choose two ions for each analyte

TABLE 5A

AUTOMATED EXTRACTION PROCEDURE –
TLPURGE PURGE METHOD

Step	Description	Flow Rate, ml/min	Output
1	Purge Cannula with 4 mL of Dichloromethane	30	Canula
2	Purge Cannula with 4 mL of Dichloromethane	30	Canula
3	Purge Cannula with 4 mL of Methanol	30	Canula
4	Purge Cannula with 4 mL of Methanol	30	Canula
5	Purge Cannula with 4 mL of 5% MeOH/Acidic H_2O	30	Canula
6	Purge Cannula with 4 mL of 5% MeOH/Acidic H_2O	30	Canula
7	Purge Cannula with 4 mL of H_2O	30	Canula
8	Purge Cannula with 4 mL of H_2O	30	Canula
9	Purge Cannula with 4 mL of 15% Dichloromethane /Hexane	30	Canula
10	Purge Cannula with 4 mL of 15% Dichloromethane /Hexane	30	Canula
11	Purge Cannula with 4 mL of H_2O	30	Canula

TABLE 5B
AUTOMATED EXTRACTION PROCEDURE –
TMETHDHX PCBS EXTRACTION METHOD

Step	Description	Flow Rate, ml/min	Output
1	Condition Cartridge with 3 mL of Methanol	5	Organic solvents waste
2	Condition Cartridge with 3 mL of 5% MeOH/Acidic H_2O	5	Organic solvents waste
3	Condition Cartridge with 3 mL of Dichloromethane	5	Organic solvents waste
4	Condition Cartridge with 3 mL of Methanol	5	Organic solvents waste
5	Condition Cartridge with 3 mL of 5% MeOH/Acidic H_2O	5	Organic solvents waste
6	Load 5mL of Sample onto Cartridge (*)	0.38	Organic solvents waste
7	Rinse Cartridge with 1.5 mL of 5% MeOH/Acidic H_2O	5	Organic solvents waste
8	Dry Cartridge with Nitrogen gas for 40 minutes		
9	Purge Cannula with 3 mL of 15% Dichloromethane /Hexane	5	Cannula
10	Collect Fraction 1 with 12mL of 15% Dichloromethane /Hexane	0.38	Fract 1
11	Purge Cannula with 3 mL of 15% Dichloromethane /Hexane	5	Cannula
12	Purge Cannula with 3 mL of water	5	Cannula

(*) A sample volume larger than the actual sample size is entered to ensure that whole sample is used.

TABLE 6A
AUTOMATED CLEAN-UP PROCEDURE –
CLPUR704 PURGE METHOD

Step	Description	Flow Rate, ml/min	Output
1	Purge Cannula with 6 mL of Hexane	30	Cannula
2	Purge Cannula with 6 mL of Hexane	30	Cannula
3	Purge Cannula with 6 mL of Methanol	30	Cannula
4	Purge Cannula with 6 mL of Methanol	30	Cannula
5	Purge Cannula with 6 mL of Reagent water	30	Cannula
6	Purge Cannula with 6 mL of Reagent water	30	Cannula

TABLE 6B
AUTOMATED CLEAN-UP PROCEDURE -
CLEANPCB CLEAN-UP METHOD

Step	Description	Flow Rate, ml/min	Output
1	Condition Cartridge with 5 mL of Hexane	5	Organic solvents waste
2	Condition Cartridge with 5 mL of Hexane	5	Organic solvents waste
3	Load 2 mL of Sample onto Cartridge (*)	0.38	Organic solvents waste
4	Purge Cannula with 3 mL of Hexane	5	Cannula
5	Collect Fraction 1 with 12 mL of Hexane	0.38	Fract 1
6	Purge Cannula with 3 mL of Methanol	5	Cannula
7	Purge Cannula with 3 mL of Reagent Water	5	Cannula

(*) A sample volume larger than the actual sample size is entered to ensure that whole sample is used.

TABLE 7
INSTRUMENT DETECTION LIMIT (IDL) (*)

Analyte	Mean	SD	RSD%	IDL	LCL	UCL
IUPAC 8	0.48	0.025	5	0.07	0.05	0.16
IUPAC 18	0.51	0.033	6	0.10	0.06	0.21
IUPAC 28	0.45	0.027	6	0.08	0.05	0.18
IUPAC 52	0.53	0.018	3	0.05	0.03	0.12
IUPAC 44	0.47	0.031	7	0.09	0.06	0.20
IUPAC 66	0.42	0.030	7	0.09	0.06	0.20
IUPAC 101	0.56	0.029	5	0.09	0.06	0.19
IUPAC 77	0.36	0.023	6	0.07	0.04	0.15
IUPAC 118	0.50	0.041	8	0.12	0.08	0.27
IUPAC 153	0.55	0.037	7	0.11	0.07	0.24
IUPAC 105	0.45	0.037	8	0.11	0.07	0.24
IUPAC 138	0.57	0.043	8	0.13	0.08	0.29
IUPAC 187	0.61	0.035	6	0.11	0.07	0.23
IUPAC 126	0.38	0.039	10	0.12	0.08	0.26
IUPAC 128	0.55	0.048	9	0.14	0.09	0.32
IUPAC 201	0.56	0.047	8	0.14	0.09	0.31
IUPAC 180	0.56	0.051	9	0.15	0.10	0.33
IUPAC 170	0.53	0.049	9	0.15	0.09	0.32
IUPAC 195	0.58	0.034	6	0.10	0.06	0.22
IUPAC 206	0.58	0.047	8	0.14	0.09	0.31
IUPAC 209	0.58	0.036	6	0.11	0.07	0.24

(*) determined from GC/MSD analysis of 0.5 ng/mL of CCS mixture in hexane (n=8)

TABLE 8
CALIBRATION CURVE FOR PCBs/ PESTICIDES IN NEW BORN CAIF SERUM*
ng/g

Analyte	0.1	0.5	1	2.5	5	10	R
IUPAC 28	0.13	0.56	0.82	2.02	3.67	7.71	0.9993
IUPAC 52	0.12	0.54	0.93	2.33	4.34	9.21	0.9994
IUPAC 49	0.09	0.41	0.85	1.87	3.58	7.79	0.9989
IUPAC 44	0.10	0.48	0.79	1.99	3.74	7.83	0.9996
IUPAC 74	0.14	0.62	1.18	2.89	5.67	12.37	0.9990
IUPAC 66	0.13	0.64	1.18	3.01	5.81	12.50	0.9993
IUPAC 101	0.12	0.49	0.84	2.06	3.97	8.21	0.9998
IUPAC 99	0.10	0.45	0.82	1.97	4.03	8.33	0.9998
IUPAC 87	0.08	0.38	0.66	1.61	3.21	6.54	0.9999
4,4'-DDE	0.28	0.72	1.21	2.66	5.05	10.09	0.9999
IUPAC 110	0.13	0.54	0.96	2.40	4.83	9.52	1.0000
IUPAC 81	0.11	0.44	0.98	2.48	4.81	10.53	0.9991
IUPAC 151	0.09	0.43	0.81	2.02	3.99	8.01	1.0000
IUPAC 77	0.10	0.47	1.00	2.56	4.92	10.50	0.9995
IUPAC 149	0.12	0.53	0.98	2.51	4.89	9.77	1.0000
IUPAC 123	0.09	0.41	0.81	1.95	4.00	8.25	0.9998
IUPAC 118	0.11	0.48	0.89	2.19	4.38	9.16	0.9997
IUPAC 146	0.09	0.41	0.82	1.98	4.02	8.18	0.9999
IUPAC 114	0.09	0.43	0.84	1.97	4.17	8.68	0.9996
IUPAC 153	0.11	0.49	0.93	2.27	4.48	9.13	0.9999
IUPAC 105	0.09	0.43	0.82	2.05	4.13	8.52	0.9999
IUPAC 138	0.09	0.41	0.78	1.93	3.77	7.67	0.9999
4,4'-DDT	0.06	0.24	0.51	1.35	2.54	5.22	0.9998
IUPAC 178	0.11	0.58	1.05	2.55	5.23	10.47	1.0000
IUPAC 158	0.10	0.50	0.98	2.45	4.93	9.99	1.0000
IUPAC 187	0.13	0.63	1.17	2.87	5.87	11.85	0.9999
IUPAC 183	0.14	0.62	1.13	2.79	5.77	11.52	0.9999
IUPAC 126	0.07	0.29	0.60	1.46	2.94	6.17	0.9997
IUPAC 128	0.06	0.33	0.62	1.57	3.08	6.15	1.0000
IUPAC 167	0.07	0.40	0.78	1.96	3.88	8.01	0.9999
IUPAC 177	0.10	0.47	0.88	2.28	4.53	9.08	1.0000
IUPAC 201	0.36	1.73	3.09	8.15	15.65	32.14	0.9999
IUPAC 156	0.06	0.33	0.66	1.72	3.42	6.89	1.0000
IUPAC 172	0.04	0.21	0.40	0.99	1.94	4.03	0.9998
IUPAC 157	0.17	0.75	1.28	3.28	6.26	12.19	0.9998
IUPAC 180	0.11	0.48	0.89	2.18	4.43	9.06	0.9999

IUPAC 170	0.08	0.37	0.70	1.75	3.57	7.16	0.9996
IUPAC 199	0.20	0.94	1.67	4.34	8.08	16.96	0.9996
IUPAC 169	0.05	0.25	0.48	1.12	2.30	4.69	0.9999
IUPAC 196 and 203	0.22	0.85	1.71	4.26	8.75	17.70	0.9999
IUPAC 189	0.08	0.34	0.64	1.60	3.24	6.65	0.9999
IUPAC 195	0.15	0.74	1.28	3.20	6.11	12.85	0.9996
IUPAC 194	0.12	0.56	0.99	2.47	4.65	9.68	0.9997
IUPAC 206	0.09	0.41	0.86	1.99	3.87	7.96	0.9998
IUPAC 209	0.09	0.44	0.90	2.18	4.35	8.98	0.9999

(*) New born calf serum spiked at 0.1, 0.5, 1.0,2.5,5.0,10 ng/g Con2 mixture and 4,4'-DDE and 4,4'-DDT
Used the analyte concentration and the ratio of the analyte response to IS response to calculate the coefficient

TABLE 9
METHOD DETECTION LIMIT (MDL) (*) AND
MINIMUM REPORTING LEVEL (MRL)**

Analyte	Mean	SD	RSD%	MDL nn/g	MRL ng/g
IUPAC 18	0.11	0.014	13	0.04	0.2
IUPAC 28	0.11	0.008	7	0.02	0.1
IUPAC 52	0.12	0.009	8	0.03	0.15
IUPAC 44	0.11	0.011	11	0.03	0.15
IUPAC 66	0.14	0.006	4	0.02	0.10
IUPAC 101	0.10	0.006	6	0.02	0.10
IUPAC 77	0.14	0.003	2	0.01	0.05
IUPAC 118	0.12	0.006	5	0.02	0.10
IUPAC 153	0.11	0.009	9	0.03	0.15
IUPAC 105	0.11	0.007	6	0.02	0.10
IUPAC 138	0.11	0.004	4	0.01	0.05
IUPAC 187	0.10	0.007	7	0.02	0.10
IUPAC 126	0.12	0.005	4	0.01	0.05
IUPAC 128	0.11	0.008	7	0.02	0.10
IUPAC 201	0.10	0.006	6	0.02	0.10
IUPAC 180	0.11	0.005	5	0.02	0.10
IUPAC 170	0.13	0.003	3	0.01	0.05
IUPAC 195	0.11	0.004	3	0.01	0.05
IUPAC 206	0.11	0.006	5	0.02	0.10
IUPAC 209	0.11	0.007	6	0.02	0.10
4,4'-DDE	0.17	0.009	5	0.02	0.10
Mirex				0.02	0.10

(*) determined from matrix spike at 0.1 ng/g CCS mixture and 4,4'-DDE (n=9), Mirex=3, based on GC/ECD analyses.

(**) Calculated from Equation 5

TABLE 10
METHOD VALIDATION – PRECISION AND ACCURACY
OF MATRIX SPIKE (*)

Analyte	Max Recovery%	Min Recovery%	Mean Recovery%	SD	RSD%
IUPAC 28	120	94	107	9.72	9
IUPAC 52	115	86	101	10.65	11
IUPAC 49	104	93	99	4.76	5
IUPAC 44	109	88	101	9.14	9
IUPAC 74	98	82	91	6.45	7
IUPAC 66	98	84	93	6.63	7
IUPAC 101	117	86	101	10.82	11
IUPAC 99	97	85	92	4.88	5
IUPAC 87	101	86	95	5.91	6
4,4'-DDE	113	93	105	7.85	7
IUPAC 110	107	90	100	7.59	8
IUPAC 81	99	80	89	6.77	8
IUPAC 151	97	85	92	4.72	5
IUPAC 77	102	80	90	8.78	10
IUPAC 149	100	87	94	5.16	5
IUPAC 123	98	83	90	6.11	7
IUPAC 118	103	86	95	6.71	7
IUPAC 146	97	84	91	5.32	6
IUPAC 114	96	80	89	5.64	6
IUPAC 153	103	84	95	6.97	7
IUPAC 105	95	83	91	5.71	6
IUPAC 138	100	86	94	6.12	7
4,4'-DDT	107	76	94	13.07	14
Mirex	79	71	75	0.39	5
IUPAC 178	97	84	90	5.38	6
IUPAC 158	97	83	89	5.57	6
IUPAC 187	99	83	91	6.59	7
IUPAC 183	97	83	91	5.58	6
IUPAC 126	96	80	88	6.01	7
IUPAC 128	101	82	92	7.17	8
IUPAC 167	98	80	90	7.13	8
IUPAC 177	98	85	91	5.57	6

IUPAC 201	95	85	89	3.66	4
IUPAC 156	98	83	89	6.28	7
IUPAC 172	99	83	93	6.84	7
IUPAC 157	95	81	87	5.54	6
IUPAC 180	100	82	92	6.94	8
IUPAC 170	100	82	93	7.87	8
IUPAC 199	95	86	90	3.87	4
IUPAC 169	105	88	94	5.71	6
IUPAC 196 and 203	94	80	88	5.75	7
IUPAC 189	101	81	91	7.33	8
IUPAC 195	108	84	95	8.85	9
IUPAC 194	105	86	97	6.68	7
IUPAC 206	103	86	95	6.74	7
IUPAC 209	99	81	92	7.71	8

(*) New born calf serum spiked at 1 ng/g Con2 mixture and 4,4'-DDE and 4,4'-DDT (n=6), Mirex , spiked at 10ng/mL n=3

FIGURE 1

TOTAL ION CHROMATOGRAM IN SIM MODE OF CON2 AND PESTICIDES AND IS EXTRACTED FROM NEW BORN CALF SERUM

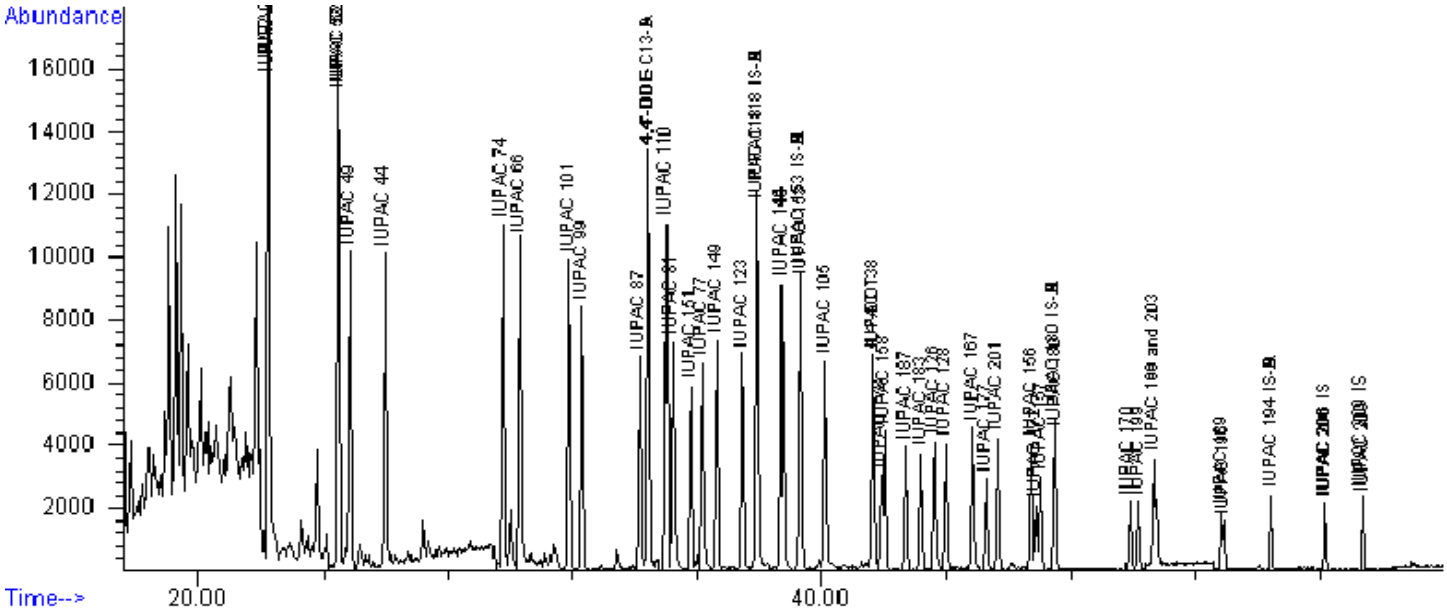
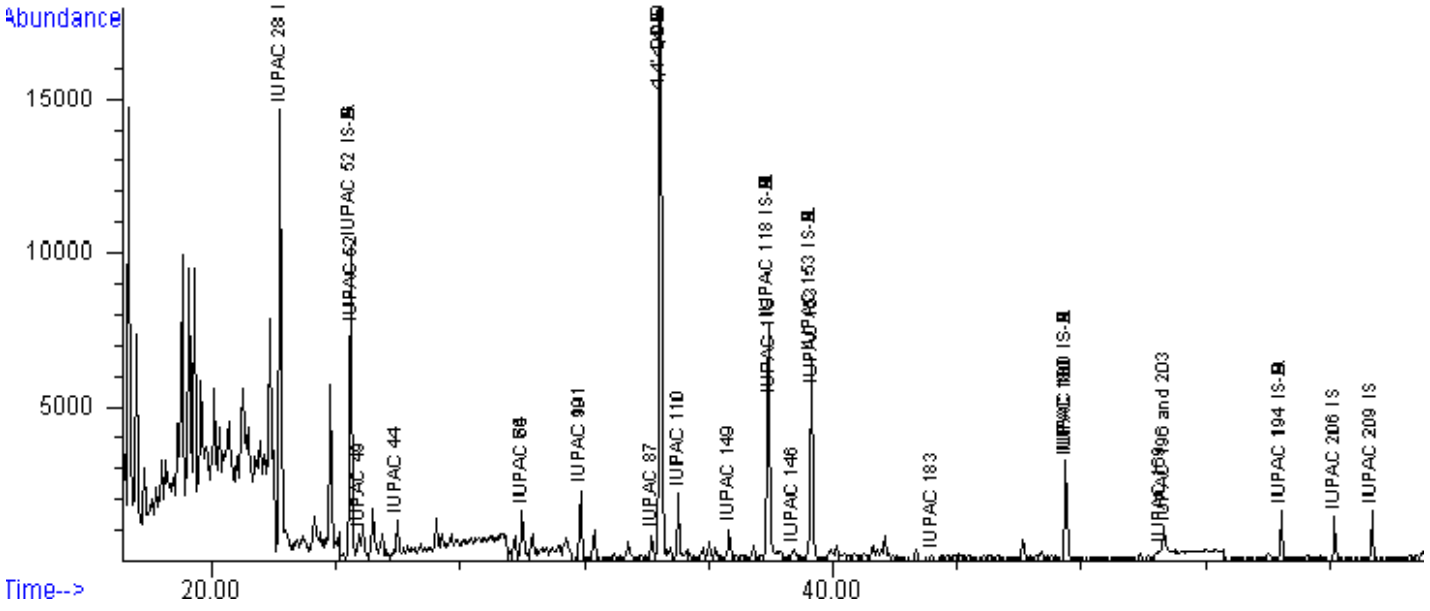


FIGURE 2

TIC IN SIM MODE OF EXTRACTED HUMAN SERUM SPECIMEN



APPENDIX A

SPE CARTRIDGE PREPARATION

Two types of SPE cartridges have been used for SPE extraction and clean-up PCB [5].

Reagent and Supplies

- SPE polypropylene cartridge (Phenomenex), catalog #AHO-7001

- Polyethylene frits (Phenomenex), catalog #AHO-7007

- C18 sorbent (Phenomenex; 50 um; 65 Å), catalog #04G-4348

- Silica gel (Mallinckrod; 100-200 Mesh; 60 Å), catalog #6447

- Sulfuric acid (Mallinckrodt), catalog #2468

Procedure

A. C18 cartridge (Extraction column)

Pack 1.3 g of C18 sorbent in a polypropylene tube. Two polyethylene frits were placed at the bottom and at the top of the cartridge (Figure A.1).

B. Silica gel cartridge (Clean-up column)

1. Washing of the silica gel

a) Weigh 200g of silica gel into a Pyrex heavy wall filter flask,
b) Add dichloromethane to cover the silica gel powder and mix well to form a slurry,
c) Place the flask into a vacuum oven maintained at room temperature to evaporate the DCM over the weekend.

2. Sulfuric acid treatment silica gel

a) Weigh 100 g of washed silica gel out into an amber round bottom bottle
b) Add 50 g or 28 ml of sulfuric acid (51% concentration) to the bottle,
c) Cap the bottle with a Teflon cap and place it on the roller overnight to form a homogenous mixture.

3. Silica Gel Cartridge Preparation

A clean up cartridge is made by packing two layer sorbents in a polypropylene tube. The first layer (lower layer) contains 1 g of sulfuric acid treatment silica gel obtained from the section 2 above, and the second one (upper layer) contains 0.1 g of silica gel. A polyethylene frit is placed between layers (Figure A.2).

Figure A.1: Extraction column

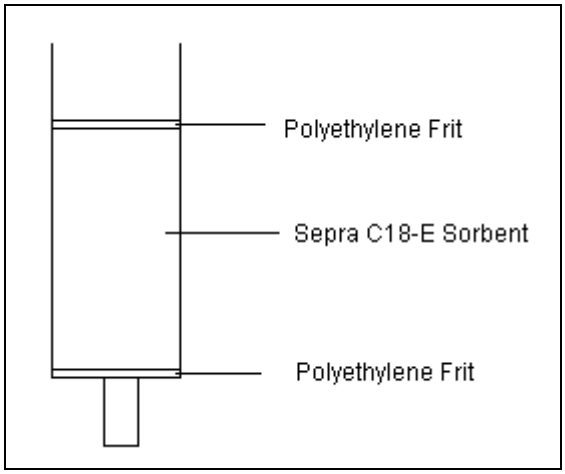
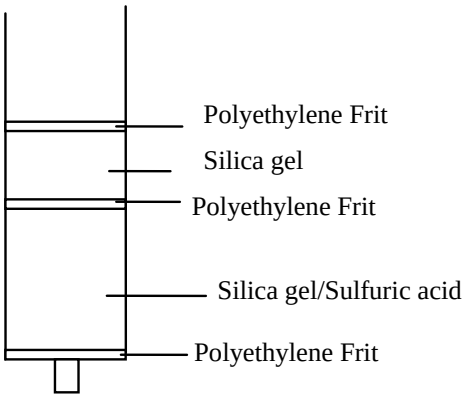


Figure A.2: Clean up column



Appendix B
BioOrganic Analytical Chemistry

Solid Phase Extraction

Batch Number: _____ **Sample ID# _____**

Sample Type: Serum sample **Project:_____**

	Initials	Date
A. Sample Preparation and Extraction		
1. Sample weight_____g.	_____	_____
Balance:___AND EK-200G____		
	Witness	
2. Spike:_____uL $^{13}C_{12}$ PCB/DDE Mix @100ng/mL (9-16-10)	_____	_____
Let sit for 1 hour		
3. Sample Preparation		
a) Add 1g 88% Formic Acid, b) Add 1ml Methanol, vortex 10 seconds c) Sonicate for 10 min. @ room temp d) Add 1g DI water, vortex 10 seconds	_____	_____
4. Solid Phase Extraction using the Rapid Trace Automated System:		
a) Column: Strata C18 b) Purge lines method: **tlpurge.spe** c) Extraction method: **tmethdhx.spe** d) Concentrate to 1 mL	_____	_____

B. Sample Cleanup

a) Column: Silicagel:Sulfuric Acid/silicagel
b.) Purge lines method: **clpur704.spe**
b) Cleanup method: **cleanpcb.spe** _____ _____

C. Concentrate to _____ _____ _____

D. Final Concentration

PCB and Pesticides
Conc. to _____uL
Date_____
Initials_____

D. Notes:

Hexane_____
DCM _____
Methanol_____
Water_____
Formic Acid _____

Sepra C-18 _____
Silica Gel _____
Acidified Silica Gel
_____

PCB Analyzed by_____
Date_____
Reviewed By_____
Date_____

APPENDIX C – BioOrganic Analytical Chemistry Laboratory Demonstration of Capability Record

Employee:_____ Evaluated by:_____

(__) Initial Training Assessment (__) Annual Competency Assessment

Sample Receiving and Accessioning

Task	Not Trained	Training in Progress	Trained / Competent	Employee Initials / Date	Evaluator Initials / Date	* Method of Assessment
Knowledge of procedure						
Follows safety precautions						
Follows SOP						
Recognizes acceptable specimens						

Accession and prepares paperwork						
Sample Storage						

Sample Preparation and Extraction

Task	Not Trained	Training in Progress	Trained / Competent	Employee Initials / Date	Evaluator Initials / Date	* Method of Assessment
Knowledge of procedure						
Follows SOP						
Syringe and balance quality control						
Prepares worksheet						
Prepares reagents						
Spike Samples						
Extraction procedure						
Concentration techniques						
Proper cleanup						

Instrumentation

Task	Not Trained	Training in Progress	Trained / Competent	Employee Initials / Date	Evaluator Initials / Date	* Method of Assessment
Follows SOP						
Able to perform routine maintenance						
Check calibration						
Check instrument for contamination						
Set up sample sequence and analytical run						
Ability to troubleshoot						

Data Acquisition and Processing

Task	Not Trained	Training in Progress	Trained / Competent	Employee Initials / Date	Evaluator Initials / Date	* Method of Assessment
Knowledge of procedure						
Follows SOP						
Quantitative and review integration						
Prepare preliminary report for data review						

Safety

Task	Not Trained	Training in Progress	Trained / Competent	Employee Initials / Date	Evaluator Initials / Date	* Method of Assessment
Adheres to the Wadsworth Center safety policies						

Continuing Education

Task	Not Trained	Training in Progress	Trained / Competent	Employee Initials / Date	Evaluator Initials / Date	* Method of Assessment
Has completed required annual training for: **Confidentiality**						
Blood borne pathogens						
Hazard communications						
Other:						
Has completed the minimum requirement of 12 hours of continuing education						

***KEY: Method of Assessment**

DO = Direct Observation
PT = Proficiency Test Performance
W = Written Quiz
Doc = Documentation Review

Action Recommended

Training ( ) Completed ( ) Continue Training

Comments/Developmental Plan:_____
_____
_____
_____

Competency ( ) Satisfactory ( ) Unsatisfactory

Comments/Developmental Plan:_____
_____
_____
_____

EmployeeName_____Signature_____ Date_____

Trainer/Observer Name_____Signature_____ Date_____

Supervisor/Director Name_____Signature_____ Date_____