Routine Analysis of 101 Polychlorinated Biphenyl Congeners in Human Serum by Parallel Dual-Column Gas Chromatography with Electron Capture Detection

Anthony P. DeCaprio[1], Alice M. Tarbell[2], Amelie Bott[1], Dana L. Wagemaker[1], Randy L. Williams[1], and Colleen M. O'Hehir[1]

[1]SPH Analytical Laboratory, School of Public Health, The University at Albany, State University of New York, One University Place, Rensselaer, New York, 12144 and [2]First Environment Research Projects/Akwesasne Task Force on the Environment, Raquette Point Road, Hogansburg, New York, 13655

Abstract

Polychlorinated biphenyl (PCB) exposure has been linked to a variety of toxic effects in animal experiments and in certain human case reports and epidemiologic studies. A total of 209 individual PCB congeners are possible, based on chlorination level and ring substitution pattern. Commercial PCBs are a complex mixture of congeners, and over 75 of these have been reported to be present in human tissues at widely varying levels. Because the biologic activity of individual PCBs is a function of extent and pattern of chlorine substitution, "congener-specific" PCB analysis of human tissue has gained increasing importance in assessing possible links between PCB exposure and toxic effects. A high-sensitivity analytical method using dual-column gas chromatography (GC) with electron capture detection (ECD) for determining 101 PCB congeners (83 individual, 18 as pairs/triplets) plus 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'*-DDE), hexachlorobenzene (HCB), and mirex, in human serum is described. Separation is performed concurrently on parallel-configured DB-5 and Apiezon-L capillary columns. The current method is a modification of previously reported dual-column GC–ECD systems with improvements to the extraction and analytical protocols and the implementation of a comprehensive QA/QC program. The method employs two surrogate standards (PCBs IUPAC 125 and 192) and internal standard (IUPAC 104)-based quantitation, in addition to per-batch check standards and method blanks. Although optimized for serum, the method is applicable to all human, experimental animal, and environmental biota samples. The accuracy, precision, and reliability of the method were assessed using a variety of QA/QC endpoints. Finally, the use of the method in determining level and prevalence of PCB congeners in a cohort of adult Native-American individuals with historical environmental PCB exposure is reported.

Introduction

Polychlorinated biphenyls (PCBs) are widely dispersed, lipophilic environmental contaminants that in many cases are bioaccumulated, resulting in prolonged residence time in various biota and human tissues (1–3). Although banned from commercial production in the U.S. in 1977, significant environmental sources of PCBs (either from disposal sites or from existing equipment and products) still exist. With current analytical capabilities, PCBs can be detected in blood from virtually all people, including those with no known specific exposure to these contaminants. However, recent work has demonstrated a general trend toward decreasing blood PCB levels, probably reflecting both decreasing exposure and net loss from body stores (4). PCB exposure has been linked to chloracne and to reproductive, neurodevelopmental, and respiratory effects in humans, whereas animal studies have demonstrated a wide range of effects on immunological, endocrine, neurochemical, and neurobehavioral endpoints (2,5–7). In addition, PCBs are carcinogenic in rodent bioassays and are classified as probable human carcinogens by both the U.S. Environmental Protection Agency and the International Agency for Research on Cancer (2).

It is clear that the biologic activity of individual PCBs is a function of the extent and pattern of chlorine substitution. As a broad generalization, highly chlorinated congeners (i.e., those with five or more chlorine atoms) are more persistent and less subject to biotransformation than those with fewer chlorine atoms (5). More important, the presence or absence of chlorines in the ortho positions (i.e., ring positions 2 and 6) appears to be a major determinant of toxic effect. Those congeners lacking multiple ortho chlorine substitution generally bind to the aryl hydrocarbon hydroxylase (Ah) receptor and induce pleiotropic responses associated with such binding. In contrast, di- and higher-ortho-substituted PCBs, which cannot assume a planar configuration, may be associated with a host of neurologic, developmental, and immunologic endpoints not seen with coplanar derivatives (6,8,9).

Until the mid-to-late 1980s, PCBs in biologic or environmental media were usually determined by packed column gas chromatography with electron capture detection (GC–ECD)

and reported as total PCB values in "Aroclor equivalents" (3). Although measurement of total PCBs in this manner was useful as a screening tool, the technique can be highly inaccurate in cases where weathering, dechlorination, and metabolic processes have altered the original congener profile of the PCB mixture (6). As research into the toxicology and environmental distribution of PCBs has evolved, high-sensitivity, congener-specific analyses have been more widely reported (4,6,10–16) and are now accepted as the gold standard for PCB analysis. Of the 209 possible PCB congeners, up to 150 are present at significant levels in at least one commercial PCB mixture, and over 75 have been detected in human and animal tissues and various biota (6,13,17,18). Congeners not normally present in commercial PCB mixtures can also be formed and distributed into the environment as a result of biological and physical dechlorination processes.

A substantial database now exists on levels of individual PCB congeners in human milk, adipose tissue, and blood. For example, significant human data are available for several non-ortho-substituted congeners (PCBs IUPAC 77, 126, and 169) that are present at very low levels in commercial PCB mixtures (and human tissues) but that are potent Ah receptor agonists (19). Quantitation of these analytes requires large sample sizes (50–100 g of blood) and/or very highly sensitive instrumentation. Additional data have been reported on a set of approximately 20 higher chlorinated, mono- and higher-ortho substituted congeners that are persistent in tissue because of their high lipophilicity and/or low rate of metabolic conversion and excretion (20). Some of these also exhibit Ah receptor agonist activity, although at potencies less than those of the coplanar PCBs (5). Many of the congeners in this second group can be readily quantitated in adult human serum samples of 1–5 g by single column capillary GC–ECD, using a second column for confirmation when necessary.

Other lower chlorinated and/or mono- and higher-ortho substituted congeners have been reported by various investigators in human tissues at levels and prevalences much lower than the major species discussed earlier (13,14). Within this group, the specific congeners that have been detected in individuals vary widely. Interest in the detection and quantitation of these "sporadic" or low level congeners has increased in recent years along with the recognition that certain PCB-related toxicities may be mediated by mechanisms other than Ah receptor binding (6,7). Most of these congeners are not expected to exhibit significant receptor affinity. In addition, their presence may reflect interindividual differences in route of exposure (e.g., ingestion vs. inhalation), recentness of exposure, and biotransformation rates. These factors may suggest their potential use as PCB exposure biomarkers and were among those considered in the recent publication of a comprehensive list of 81 PCB congeners (as singlets or pairs) recommended for routine monitoring in human and environmental studies (6).

A number of GC–ECD based analytical methods, with varying capabilities for individual congener quantitation, are currently available for PCBs in human tissues (1,14,15,21–26). In the present report, we describe sample extraction and high-sensitivity dual-column GC–ECD analytical methods for determining 101 PCB congeners (83 individual, 18 as pairs/triplets) plus 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'*-DDE), hexachlorobenzene (HCB), and mirex, in human serum. The current method is a modification of previously reported dual-column systems developed by other investigators (15,25,27–29), with improvements to both the extraction and analytical protocols and the implementation of a comprehensive quality assurance/quality control (QA/QC) program. Although optimized for serum, the method is applicable to essentially any human, experimental animal, or environmental biota samples. The accuracy, precision, and reliability of the method are assessed using a variety of QA/QC endpoints. Finally, we report on the use of the method in determining level and prevalence of serum PCB congeners in a cohort of adult Native-American individuals with historical environmental PCB exposure.

Methods

Chemicals and glassware

All solvents, including methanol (HPLC grade, J.T. Baker, Phillipsburg, NJ), hexane (Nanograde, J.T. Baker), diethylether (Burdick & Jackson Inc., Muskegon, MI), and acetone (Burdick & Jackson) were purchased by lot and analyzed prior to receipt to verify purity. For the purity check, 500 mL of solvent were reduced in volume (see below) to 0.5 mL, followed by GC–ECD analysis. Fully activated Florisil® (magnesium silicate) was purchased by lot in 1-kg jars (U.S. Silica, Inc., Berkeley Springs, WV). Deactivation was performed by dropwise addition with continuous shaking of distilled/deionized water to a final concentration of 4%, followed by rolling for at least 4 h. Calibration was performed on a freshly prepared Florisil extraction column by addition of 1 mL of 20-ng/mL mixed Aroclor calibration standard to the column and elution with hexane. Six 10-mL fractions were collected and individually analyzed by GC–ECD to determine the volume necessary to elute all congeners. This value was indelibly marked on the bottle, and the label was signed and dated by the technician performing the calibration. Anhydrous sodium sulfate (Mallinckrodt, Inc., Paris, KY) was hexane-washed for 24 h, placed in a 100°C oven for 24 h, and then stored in a hexane-washed glass container with a PTFE lid. Newborn calf serum was obtained in lots from Gibco, Inc. (Grand Island, NY). All reusable glassware (sample tubes, concentrator bottles, flasks, chromatography columns) was soap and water washed, dried, and subjected to heating at 450°C for 4 h in a muffle oven to minimize residual contamination. Disposable pipets and other glassware were also muffled prior to use. PTFE stoppers were cleaned after each use by Soxhlet extraction in hexane.

Blood collection and accessioning

The data reported in this paper were generated in support of the University at Albany's Superfund Basic Research Program (SBRP) in collaboration with the Akwesasne Mohawk Nation in northern New York state. Blood was collected by trained Mohawk staff at a satellite laboratory located within the Akwesasne reservation or at study participant's homes. Approximately 20 mL of blood was collected from each adult participant

by venipuncture into two 10-mL red-top (no additive) Vacutainer® tubes. Blood was allowed to clot at room temperature for 20 min and was centrifuged at 800 × *g* for 15 min. Aliquots (5 mL) of serum were transferred using hexane-washed glass pipets into hexane-washed PTFE-capped glass vials. The vials were stored at –20°C at the satellite laboratory. This collection protocol provided sufficient sample for both a primary extraction and a reserve sample to be used for repeat or duplicate analysis. Batches of samples were periodically transferred on ice from the satellite to the analytical laboratory (located at the University at Albany East Campus) for storage at –20°C and eventual processing. Samples were received with unique six-digit identification numbers but no other identifying information. Each sample was accompanied by a documented chain-of-custody (COC) form. Upon receipt of samples, laboratory staff input the sample information into a computerized database to create a sample-analysis sheet (SAS) for internal laboratory use. The SAS was used to track the sample throughout laboratory processing and final disposal.

Serum extraction

Universal safety precautions were observed for all human samples processed in the laboratory. Each extraction batch consisted of five samples (unknown and/or QC) and a method blank. Human serum (5 g) was weighed into a clean glass 50-mL centrifuge tube. The method blank was an empty centrifuge tube carried through the entire extraction process. Methanol (7.5 mL) was added, along with the two surrogate standards (IUPAC 125 and 192; 5 µL of a 1 ng/µL solution of each in acetone), and the tube was capped with a PTFE stopper and shaken vigorously by wrist-action shaker for 2 min. Fifteen milliliters of 1:1 (v/v) diethylether/hexane was added and the tube was shaken again. The tubes were kept at room temperature for 15 min followed by centrifugation for 2 min (1000 × *g*). The upper layer was pipetted into a 50-mL volumetric flask (100-mL flask for the method blank). Centrifugation was necessary to eliminate problems associated with formation of emulsions in the extracted samples. The diethylether/hexane extraction was repeated two more times, each upper layer being transferred to its respective volumetric flask each time, and the volume of the combined extracts was adjusted to 50 mL (100 mL for the blank) with diethylether/hexane. One milliliter of combined extract (2 mL for blanks) was removed and placed in a tared weighing tin for gravimetric determination of total fat content. The remaining extract was transferred to a 600-mL evaporator bottle with 1.5-mL graduated stem (Labconco, Inc., Kansas City, MO) and reduced in volume (N_2 pressure 2 psi, 65°C, vortex speed 70%, ~20 min) on an N_2 evaporator unit (RapidVap, Labconco, Inc.) to approximately 1 mL.

Sample cleanup

Removal of polar lipids and other interferents from extracts of human serum was accomplished via adsorption onto Florisil. Concentrated sample extracts were transferred to a 1 × 15-cm glass column containing 10 g of calibrated, 4% deactivated Florisil overlaid with 2 g anhydrous sodium sulfate. The column was eluted with 65 mL of hexane and a predetermined amount (based on previous Florisil calibration) of eluate was collected and transferred to a clean evaporator bottle. The eluate was reduced in volume to 0.5 mL, transferred to a hexane-washed glass GC vial, and capped with a PTFE-lined silicone rubber GC vial cap following addition of internal standard (IUPAC 104; 1 µL of a 1 ng/µL solution in hexane).

Instrumentation and analysis

High-resolution, congener-specific analysis of up to 83 individual PCB congeners and 18 congeners as pairs or triplets (total of 101 congeners), in addition to HCB, *p,p'*-DDE, and mirex, was performed by simultaneous, parallel dual-column (splitless injection) GC separation with dual ECD. Instrumentation consisted of two Hewlett-Packard 5890 GCs equipped with dual ^{63}Ni ECDs, Hewlett-Packard model 7673 autosamplers, Hewlett-Packard Model 3396 series II integrators, and analog/digital (A/D) conversion boards (Shimadzu Scientific Instruments, Inc., Columbia, MD) for automated data transfer to an acquisition PC. The integrators were employed only to control the GC operating parameters and run start; data collection and peak integration were performed via A/D boards and dedicated chromatography software. The columns employed were a Hewlett-Packard Ultra II 5% phenylmethyloctadecylsilyl bonded (DB-5) fused silica (25 m, 0.33-µm film, 0.25-mm i.d.) capillary column and a fused silica Apiezon L (30 m, 0.25-µm film, 0.25-mm i.d.) column. Helium carrier gas flow was approximately 2 mL/min with a linear flow rate of 30 cm/s. The column temperature was programmed as follows: 100°C hold for 2 min, 10°C/min to 160°C, 1°C/min to 190°C, and 2°C/min to 270°C, with a 10-min hold until the end of run. Columns were linked to the injector via a "Y" connection and 5-m plain glass capillary guard column. Injector and detector temperatures were maintained at 250°C and 330°C, respectively. Make-up gas was N_2 at flow rates of 45 and 60 mL/min for the DB-5 and Apiezon column detectors, respectively.

Each analysis batch consisted of a sequence of a hexane blank, congener check standard, QC (Aroclor) check standard, method blank, and a maximum of five analytical (unknown plus QC serum spike) samples. Each analysis run contained from one to three analysis batches. The QC and congener check standards were prepared by pipeting 0.5 mL of working standard solution (see below) into separate vials and adding 1 µL of internal standard solution to each. Three microliters of each sample was injected into the GC per run, with the injected sample split equally to each column.

Calibration and QC standards

Aroclors 1221, 1016, 1254, and 1260 for use in standards were originally obtained as neat liquids from Monsanto Chemical Corp. (St. Louis, MO). They are currently available as solutions in hexane from AccuStandard, Inc. (New Haven, CT). HCB, *p,p'*-DDE, mirex, and 3,4,3',4'-tetrachlorobiphenyl (IUPAC 77) were also obtained as neat materials or standard solutions from AccuStandard, as were five individual mixtures of non-coeluting PCB congeners (i.e., "Frame" mixes 1–5) and a standard solution of 32 selected congeners (i.e., "food and human tissue" mixture). A mixture of 3,4,5,2',6'-pentachlorobiphenyl (IUPAC 125) and 2,3,4,5,6,3',5'-heptachlorobiphenyl (IUPAC 192) in acetone (1 µg/mL each) for use as a surrogate

Table I. Method Detection Limits and Analyte Composition of Standards

Analyte†	MDL‡	Standard* calibration§	Standard* QC check"	Standard* congener check#	Analyte	MDL	Standard calibration	Standard QC check	Standard congener check
1	0.15	8.20	9.13	–	83	0.02	0.14	0.10	–
3	0.04	4.27	4.27	–	97	0.02	0.71	0.73	–
4+2	0.02	6.98	6.90	–	87	0.02	1.36	1.25	4.00
10	0.02	0.17	0.16	–	*p,p'*-DDE+85	0.02	–**	–**	–**
7	0.02	0.45	0.43	–	136	0.03	0.69	0.56	–
9	0.02	0.62	0.60	–	110	0.02	2.83	2.43	–
6	0.02	1.43	1.42	–	77	0.02	2.50**	2.50**	4.00
8	0.02	4.30	4.25	4.00	151	0.02	1.22	1.06	–
HCB	0.02	–**	–**	–	144	0.02	0.22	0.18	–
19	0.03	0.34	0.32	–	147+109	0.03	0.18	0.27	–
13	0.02	0.86	0.89	–	123+149	0.02	2.94	2.47	–
18	0.02	2.74	2.62	–	118	0.02	2.01	3.34	4.00
15	0.03	1.13	0.96	–	134	0.01	0.24	0.20	–
17	0.03	1.08	1.03	–	114	0.02	0.09	0.20	4.00
24+27	0.02	0.21	0.23	–	146	0.02	0.56	0.47	–
32+16	0.04	1.77	1.74	–	153	0.02	3.27	3.14	4.00
29	0.01	0.04	0.04	–	132	0.02	1.56	1.02	–
26	0.03	0.47	0.48	–	105	0.02	0.68	1.96	4.00
25	0.01	0.25	0.27	–	141	0.02	1.02	0.95	–
31	0.02	2.49	2.42	–	179	0.01	0.64	0.60	4.00
28	0.02	2.56	2.48	4.00	137	0.02	0.12	0.14	–
33	0.02	0.89	0.85	–	176	0.01	0.18	0.17	–
53	0.02	0.42	0.38	–	130	0.01	0.21	0.17	–
51	0.05	0.13	0.13	–	164+163+138	0.02	3.95	3.86	4.00
22	0.04	1.01	1.03	–	158	0.01	0.36	0.35	4.00
45	0.04	0.37	0.35	–	129	0.02	0.11	0.10	–
46	0.02	0.15	0.14	–	187	0.02	1.47	1.38	4.00
52	0.02	2.61	1.48	4.00	183	0.01	0.40	0.40	4.00
49	0.03	1.00	0.82	4.00	128	0.02	0.47	0.51	4.00
47+59	0.02	0.40	0.38	–	185	0.02	0.15	0.15	–
44	0.02	1.77	1.33	4.00	174	0.01	1.38	1.34	–
42	0.01	0.39	0.38	–	177	0.01	0.76	0.72	–
71	0.02	0.30	0.32	–	171	0.02	0.46	0.39	–
64	0.02	0.49	0.49	–	156	0.02	0.36	0.49	4.00
40	0.02	0.24	0.36	–	201	0.02	0.23	0.29	–
67	0.02	0.02	0.05	–	172	0.02	0.24	0.22	–
63	0.01	0.02	0.05	–	180	0.02	2.51	2.51	4.00
74	0.02	0.32	0.81	4.00	200	0.02	0.11	0.11	–
70	0.02	1.05	2.01	4.00	mirex	0.02	–**	–**	–
66	0.02	0.36	1.11	4.00	170	0.02	0.97	0.88	4.00
95	0.02	2.06	1.13	–	190	0.02	0.19	0.17	–
91	0.03	0.27	0.19	–	199	0.01	0.57	0.57	–
56	0.02	0.13	0.44	–	203	0.02	0.29	0.30	–
92	0.02	0.56	0.33	–	196	0.01	0.24	0.23	–
84	0.02	0.67	0.49	–	195	0.02	0.26	0.26	–
90+101	0.02	3.54	2.70	4.00	194	0.02	0.52	0.53	–
99	0.02	1.05	1.25	4.00	206	0.02	0.17	0.17	–

* PCB level expressed as weight percent of total PCB in standard.
† PCB IUPAC# or pesticide.
‡ Method detection limit (ppb) for analyte based on a 5 g serum sample.
§ Prepared from AccuStandard, Inc. Aroclor solutions.
" Prepared from Monsanto Chemical Corp. neat Aroclors.
Prepared from AccuStandard, Inc. "food and human tissue" standard solution.
**IUPAC 77, *p,p'*-DDE, HCB, and mirex added to calibration and QC check standards; *p,p'*-DDE added to congener check standard (see under Methods).

standard and a solution of 2,4,6,2',6'-pentachlorobiphenyl (IUPAC 104) in hexane (5 µg/mL) for use as an internal standard (IS) were custom prepared by AccuStandard, Inc.

A 1:1:1:1 mixture of Aroclors 1221, 1016, 1254, and 1260 (20 µg/mL of each in hexane; obtained from AccuStandard, Inc.), fortified with HCB, *p,p'*-DDE, and mirex at 1 µg/mL each, in addition to 3,4,3',4'-tetrachlorobiphenyl (IUPAC 77) at 2 µg/mL, was employed as the stock calibration standard solution. Peak identity and individual congener levels in a mixed Aroclor standard were originally determined by literature-based composition values, GC–ECD, and GC–MS–FTIR (30–32). They were confirmed and recalculated for the present study using "Frame" mixes 1–5 as primary standards (22). The mixed Aroclor standard includes essentially all of the PCB congeners originally distributed in the environment plus certain non-Aroclor congeners formed during microbial dehalogenation of more highly chlorinated PCBs (Table I). The stock standard solution was diluted in hexane to create a series of working solutions at 20, 40, 50, 100, 200, 300, and 400 ng/mL of each Aroclor. Seven-point calibration curves, bracketing the range of typical sample concentrations, were generated at biweekly intervals. An IS-based average response factor (RF) and relative standard deviation (RSD) value for each calibrated peak was automatically calculated from the curve data and updated by the chromatography software. Each calibrated peak must have ≤ 20% RSD in order for the calibration to pass.

The QC check standard solution was a mixed Aroclor standard prepared as above but using Aroclors originally obtained from Monsanto Chemical Corp. Primarily because of differences in the composition of the Aroclor 1254 from this source (33), the individual congener levels in this standard vary slightly from the calibration mixture (Table I), making it appropriate for use as a QC check material. This standard was also used to prepare the matrix spiking solution for method detection limit (MDL) determinations, routine QC spike samples, and method recovery studies. For these purposes, the stock solution in hexane was diluted 100-fold in acetone to yield a working solution at 200 ng/mL of each Aroclor. The congener check standard consisted of the AccuStandard 32-congener "food and human tissue" mixture diluted in hexane to 1 ng/mL of each congener, with *p,p'*-DDE added to 100 ng/mL (Table I).

Data collection and processing

Raw GC–ECD peak data were sent, following A/D conversion, to a Pentium PC for storage. Integration, calibration, editing, and reprocessing of individual sample data and production of final chromatograms were carried out using chromatography software (Class VP Chromatography Data System, Version 4.2, Shimadzu Scientific Instruments, Inc., Columbia, MD). Data for each batch of analyzed samples were initially processed by the software and then reviewed by technical staff for appropriate integration and identification of each peak (Figure 1). Manual editing and reprocessing of selected peaks in the initial chromatogram were carried out if required to correct any deficiencies in the initial integration parameters.

Edited data were exported into an Excel spreadsheet for compilation of individual column data and then into a Lotus 1-2-3 spreadsheet via a custom macro designed to validate and combine the individual data sets to produce a final report. The macro included a provision to confirm the presence of peaks for a given congener (or coeluting congeners) on both columns as a condition of reporting a data value. The decision paradigm used by the software was as follows: (1) a peak containing a specific congener must appear on both columns within the expected retention time window (Table II), if not, it is assumed that the signal arose from an interfering compound and the data are censored; (2) individual congeners that are resolved on DB-5 but not on Apiezon L are reported using the results from DB-5; (3) individual congeners that are resolved on Apiezon L but not on DB-5 are reported using the results from Apiezon L; (4) individual congeners that are resolved on both columns are reported using results from the column previously determined to exhibit the better resolution and reproducibility for the particular congener; and (5) congeners that are not resolved on either column are reported as pairs or triplets. Congeners that were quantitated using DB-5 data were confirmed on Apiezon and vice versa (Table II). Every congener reported in a pair or triplet using data from one column was confirmed as being present in the expected peak(s) from the second column. The software also automatically performed a cross-column comparison check for selected major congeners/pairs that eluted cleanly on both columns (i.e., IUPAC 52, 74, 90+101, 99, 118, 177, 187, 194, 199). Between-column variation for these congeners was assessed for QC purposes as a relative difference parameter (mean of column congener levels divided by the sum of both column values × 100%) (26). Finally, the software produced a combined report containing values for total PCB (sum of individual congener levels), individual congener concentrations, and IS areas for every sample (QC, blank, and unknown) within the analytical batch. Recoveries for both surrogates were also reported for each sample; sample data were not routinely corrected for recovery. Current MDLs for each congener were also provided on the report.

Step 1: Lab Technician

Activity	Acceptance criteria	Action(s) on fail
Review IS areas	Within control limits	Re-extract samples
Review surrogate recoveries	Within 60-130%	Flag for further review
Review QC check standard	Eight major congeners and ≥80% of rest within 15% RSD of expected value	Check peak integration; re-extract samples if still unacceptable
Review method blank	<1.25 ppb total PCB	Flag for further review; re-extract samples
Review column comparison data	≤15% difference between columns	Check peak integration; flag for further review
Review/edit chromatograms	Proper peak assignment, integration, and baseline	Manually edit as necessary
Print out data report, assemble paper file, copy electronic data files to QC and backup computers	—	—

Step 2: QC Manager

Activity	Acceptance criteria	Action(s) on fail
Review paper file for: - completed database sheets - COC forms - batch file printout - QC check standard report - individual column reports - combined column (final) report - chromatograms	Present in file	Return to technician
Re-check IS areas	Within control limits	Return to technician
Review QC check standard	Eight major congeners must pass	Return to technician
Review final report for expected congeners	IUPAC 118, 138, 153, 180 should be non-zero in adult samples; DDE should be non-zero in all samples	Return to technician
Review final report for unusually high levels of uncommon congeners	No specific criteria	Flag for further review
Copy IS, surrogate, blank, duplicate, and QC spike data into cumulative QC data files	—	—

Step 3: Lab Manager

Activity	Acceptance criteria	Action(s) on fail
Overall review of final report for unexpectedly high/low individual congener values	No specific criteria	Return to technician for re-editing and/or correction
Review flagged results from Steps 1 and 2	No specific criteria	Return to technician for re-editing, correction, or re-extraction
Review cumulative QC files on weekly basis	No unusual results or trends	Identify and correct problem

Figure 1. Summary of the multistep QA/QC program employed in the present study.

QA/QC program

The QA/QC program in use in the laboratory was developed in consultation with outside scientists in the field of PCB analysis and according to recommendations in relevant publications (21,23,24,34–38). The laboratory maintains a comprehensive and ongoing program to ensure the validity of reported data as a necessary requirement for accreditation under the NYSDOH Clinical Laboratory Evaluation Program. The QA/QC program included provisions for surrogate standards, instrument detection limit and MDL determinations, routine QC check standard verification, matrix spike (QC) samples, method blanks, trip blanks, field blanks, duplicates, and interim and final data reviews (Figure 1).

MDLs were generated for each instrument on a yearly basis or following modifications in methodology or instrumentation that may have resulted in altered quantitation. MDLs for all individual congeners and pairs/triplets were generated by means of a calibration curve-based method using newborn calf serum spiked with mixed Aroclor standard (39). For each calibrated peak, spike levels were decreased until the peak was just detected and confirmed by the chromatography software. The standard deviation determined from seven replicate samples at this spike level was multiplied by three to yield the MDL (40). Because of the large (~400-fold) range of individual congener concentrations in the standard, at least eight separate spike levels were required to determine valid MDLs for all congeners.

QC limits to IS areas for each column were set as two (warning limit) and three (control limit) SDs from the mean of 14 replicate injections of Aroclor standard in hexane. Statistical control was assured by inputting all IS data into cumulative QA/QC spreadsheets and plotting control charts of IS areas. Samples for which the IS areas exceeded the control limits were rejected and re-extracted. If the QC check standard IS area exceeded control

Table II. Relative Retention Times and Response Factors for PCB Congeners and Pesticides in Dual-Column Method

DB-5						Apiezon					
Peak #*	Analyte†	RRT‡	RSD§	RRF"	RSD§	Peak #*	Analyte†	RRT‡	RSD§	RRF"	RSD§
1	**1**	**0.4318**	**0.014**	**0.0072**	**16.6**	1	1	0.4087	0.021	0.0071	7.0
2	2	0.4928	0.035	0.0047	84.4	**2**	**4+2**	**0.4914**	**0.015**	**0.0044**	**11.5**
3	3	0.4999	0.015	0.0048	17.0	**3**	**10**	**0.4986**	**0.034**	**0.0667**	**17.6**
4	4+10	0.5404	0.013	0.0147	13.4	**4**	**3**	**0.5130**	**0.054**	**0.0030**	**1.9**
5	7+9	0.5908	0.007	0.0665	12.0	**5**	**9**	**0.5814**	**0.012**	**0.0466**	**8.2**
6	**6**	**0.6139**	**0.012**	**0.0422**	**13.5**	**6**	**7**	**0.5906**	**0.012**	**0.0512**	**7.1**
7	8+*5*	0.6271	0.012	0.0476	17.7	7	6	0.5968	0.020	0.0314	16.2
8	**HCB**	**0.6442**	**0.009**	**0.1775**	**9.0**	8	*5*+19	0.6073	0.005	0.0347	17.6
9	**19**	**0.6741**	**0.012**	**0.0556**	**13.3**	**9**	**8**	**0.6191**	**0.026**	**0.0497**	**12.5**
10	**13**	**0.7209**	**0.029**	**0.0172**	**20.0**	10	18	0.6969	0.015	0.0582	12.5
11	**18**	**0.7288**	**0.007**	**0.0556**	**17.3**	**11**	**17**	**0.7184**	**0.012**	**0.0715**	**10.1**
12	15+17	0.7341	0.008	0.0541	16.3	12	27	0.7239	0.013	0.0775	7.6
13	**24+27**	**0.7558**	**0.009**	**0.0918**	**20.0**	13	HCB+16+24	0.7284	0.012	0.1199	8.5
14	**32+16**	**0.7778**	**0.010**	**0.0777**	**18.8**	14	13+32	0.7643	0.009	0.0461	8.8
15	**29**	**0.8129**	**0.020**	**0.1165**	**20.7**	**15**	**15**	**0.7903**	**0.051**	**0.0179**	**17.8**
16	**26**	**0.8261**	**0.009**	**0.0822**	**13.2**	**16**	**53**	**0.8413**	**0.010**	**0.0595**	**9.6**
17	**25**	**0.8331**	**0.008**	**0.1005**	**13.0**	17	29+26	0.8633	0.009	0.0700	7.4
18	31	0.8490	0.008	0.0644	17.5	18	45+25	0.8722	0.009	0.0562	9.2
19	**28**	**0.8525**	**0.008**	**0.0879**	**14.7**	19	46	0.8785	0.011	0.1070	4.6
20	33+53	0.8795	0.007	0.1124	14.4	20	51	0.8836	0.008	0.2065	9.7
21	**51**	**0.8955**	**0.010**	**0.0790**	**13.1**	**21**	**31**	**0.8966**	**0.009**	**0.0777**	**11.6**
22	**22**	**0.9013**	**0.007**	**0.0890**	**13.4**	**22**	**33**	**0.9131**	**0.020**	**0.0952**	**11.5**
23	**45**	**0.9157**	**0.009**	**0.0964**	**11.7**	23	28	0.9146	0.015	0.0925	13.2
24	**46**	**0.9388**	**0.007**	**0.0930**	**12.2**	24	22	0.9382	0.008	0.0971	6.6
25	**52**	**0.9522**	**0.008**	**0.0753**	**19.0**	25	52	0.9824	0.007	0.0795	13.9
26	**49**	**0.9650**	**0.006**	**0.1110**	**15.4**	**26**	**104(IS)**	**1.0000**	–	–	–
27	47+*75*+*48*	0.9757	0.018	0.1003	14.4	27	*48*+49	1.0110	0.007	0.1044	13.5
28	**104(IS)**	**1.0000**	–	–	–	28	44	1.0265	0.006	0.0915	11.7
29	**44**	**1.0137**	**0.008**	**0.0899**	**20.7**	**29**	**47+59**	**1.0435**	**0.009**	**0.1266**	**6.6**
30	59+*37*+42	1.0241	0.010	0.0913	11.9	**30**	**42**	**1.0589**	**0.006**	**0.1193**	**6.6**
31	71	1.0479	0.009	0.0757	10.3	31	*75*+40+*41*	1.0726	0.010	0.0439	5.8
32	*41*+64	1.0511	0.008	0.0987	12.4	**32**	**71**	**1.0802**	**0.006**	**0.1193**	**4.0**
33	**40**	**1.0759**	**0.011**	**0.1132**	**5.3**	**33**	**64**	**1.1034**	**0.011**	**0.1365**	**7.3**
34	**67**	**1.0941**	**0.018**	**0.1555**	**41.5**	34	37	1.1557	0.016	0.0302	9.9
35	**63**	**1.1119**	**0.017**	**0.1502**	**23.9**	**35**	**95**	**1.1707**	**0.008**	**0.1142**	**14.7**
36	**74**	**1.1238**	**0.006**	**0.1026**	**10.9**	36	84+91	1.2238	0.009	0.1047	11.5
37	**70**	**1.1358**	**0.008**	**0.0978**	**15.4**	37	67	1.2301	0.052	0.1159	17.8
38	66+95	1.1492	0.006	0.1056	16.5	38	63	1.2514	0.011	0.2545	11.8
39	**91**	**1.1694**	**0.006**	**0.1217**	**10.7**	39	70	1.2681	0.010	0.0837	10.9
40	56+*60*	1.2004	0.009	0.1121	13.2	40	74	1.2771	0.010	0.1150	8.5
41	**92**	**1.2054**	**0.008**	**0.0993**	**14.3**	**41**	**66**	**1.2916**	**0.010**	**0.0909**	**9.1**
42	**84**	**1.2161**	**0.003**	**0.1016**	**13.3**	**42**	**56**	**1.3183**	**0.020**	**0.0779**	**14.3**
43	**90+101**	**1.2236**	**0.005**	**0.0747**	**18.8**	43	92	1.3265	0.012	0.0909	8.0
44	**99**	**1.2404**	**0.004**	**0.0812**	**15.6**	44	*60*+136	1.3397	0.008	0.0794	10.2
45	**83**	**1.2758**	**0.006**	**0.1033**	**9.0**	45	90+101	1.3618	0.011	0.0744	14.7
46	**97**	**1.2927**	**0.008**	**0.0987**	**14.0**	46	83	1.3773	0.010	0.1093	10.4
47	**125 (SURR)**	**1.3005**	**0.008**	**0.1179**	**16.3**	47	99	1.3981	0.008	0.0754	11.3
48	**87**	**1.3095**	**0.006**	**0.0980**	**15.0**	48	125(SURR)	1.4097	0.009	0.1139	12.1
49	***p,p'*-DDE+85**	**1.3207**	**0.006**	**0.0708**	**13.4**	49	97	1.4153	0.007	0.1005	11.8
50	**136**	**1.3318**	**0.007**	**0.0925**	**14.4**	50	87+*p,p'*-DDE	1.4260	0.007	0.0986	9.5
51	110+*77*	1.3419	0.006	0.0672	17.1	51	85	1.4668	0.008	0.0520	7.8
52	*82*	1.3792	0.011	0.0756	11.9	**52**	**110**	**1.4727**	**0.011**	**0.0894**	**13.1**

* Bold indicates peak data used for quantitation, all other peaks used for confirmation.
† PCB IUPAC# or pesticide; italics indicates congener identified in standard but not currently quantitated; SURR - surrogate, IS - internal standard.
‡ Mean relative (to IUPAC 104) retention time based upon data from seven-point calibration curve.
§ Relative standard deviation (%) based upon data from seven-point calibration curve.
" Mean relative response factor based upon data from seven-point calibration curve, calculated as $(Area_{congener}/Amount_{congener}) \times (Amount_{IS}/Area_{IS})$.

Table II. (continued) Relative Retention Times and Response Factors for PCB Congeners and Pesticides in Dual-Column Method

DB-5						Apiezon					
Peak #*	Analyte†	RRT‡	RSD§	RRF"	RSD§	Peak #*	Analyte†	RRT‡	RSD§	RRF"	RSD§
53	**151**	**1.3827**	**0.007**	**0.0916**	**16.4**	53	*82*	1.4821	0.009	0.1419	6.8
54	*135*+144	1.3973	0.006	0.0947	13.3	54	*135*+151	1.5040	0.008	0.0795	12.2
55	**147+109**	**1.4123**	**0.007**	**0.0896**	**15.2**	**55**	**144**	**1.5389**	**0.012**	**0.1349**	**10.1**
56	**123+149**	**1.4225**	**0.007**	**0.0899**	**19.3**	56	149+134	1.5573	0.008	0.0828	15.4
57	118	1.4283	0.007	0.0862	16.5	57	147	1.5618	0.015	0.3257	17.0
58	**134**	**1.4565**	**0.004**	**0.1018**	**11.6**	**58**	**77**	**1.5884**	**0.017**	**0.0423**	**6.1**
59	**114**	**1.4643**	**0.014**	**0.1309**	**26.8**	59	132	1.6290	0.007	0.0820	11.2
60	**146**	**1.4849**	**0.008**	**0.0944**	**12.2**	60	179	1.6470	0.011	0.0853	9.4
61	**153**	**1.5048**	**0.007**	**0.0845**	**20.0**	61	109+123	1.6606	0.012	0.1151	10.1
62	**132**	**1.5120**	**0.005**	**0.0935**	**18.4**	**62**	**118**	**1.6891**	**0.012**	**0.0815**	**11.9**
63	105	1.5153	0.010	0.0910	10.3	**63**	**176**	**1.7020**	**0.012**	**0.1445**	**6.7**
64	**141**	**1.5465**	**0.006**	**0.1015**	**18.0**	64	114	1.7175	0.009	0.0668	18.9
65	**179**	**1.5515**	**0.008**	**0.0853**	**12.6**	65	146	1.7329	0.012	0.0865	7.9
66	**137**	**1.5695**	**0.008**	**0.1295**	**7.7**	**66**	**105**	**1.7579**	**0.011**	**0.1025**	**7.7**
67	176+130	1.5775	0.008	0.1078	12.1	67	153+141	1.7717	0.009	0.0898	14.0
68	**164+163+138**	**1.5964**	**0.008**	**0.0840**	**18.9**	**68**	**130**	**1.8015**	**0.017**	**0.0977**	**3.3**
69	158	1.6044	0.010	0.1431	10.6	69	164+137	1.8158	0.016	0.1238	6.3
70	129+178	1.6255	0.010	0.1197	14.8	**70**	**129**	**1.8312**	**0.010**	**0.1388**	**6.2**
71	**187**	**1.6541**	**0.008**	**0.0864**	**17.5**	71	138+*178*+163	1.8428	0.011	0.1062	12.6
72	**183**	**1.6707**	**0.007**	**0.1789**	**15.9**	**72**	**158**	**1.8696**	**0.012**	**0.1210**	**6.9**
73	128+*167*	1.6877	0.008	0.1046	14.5	73	187	1.8993	0.014	0.0851	13.6
74	**185**	**1.7054**	**0.010**	**0.1377**	**14.3**	**74**	**128**	**1.9168**	**0.014**	**0.1182**	**7.5**
75	**174**	**1.7318**	**0.012**	**0.0877**	**16.7**	75	174+183+185	1.9378	0.013	0.1013	12.9
76	**177**	**1.7501**	**0.010**	**0.0896**	**15.2**	76	177	1.9741	0.014	0.0844	9.8
77	**171**	**1.7653**	**0.017**	**0.0964**	**16.5**	77	171+201	2.0138	0.013	0.0453	8.4
78	**156**	**1.7678**	**0.014**	**0.0967**	**11.3**	78	*167*+200	2.0649	0.016	0.0542	6.8
79	**201**	**1.7881**	**0.014**	**0.0761**	**17.1**	79	156+mirex	2.1383	0.014	0.0974	12.2
80	172+192(SURR)	1.8055	0.010	0.1159	20.0	**80**	**172**	**2.1429**	**0.008**	**0.0732**	**9.2**
81	**180**	**1.8264**	**0.011**	**0.0856**	**19.6**	**81**	**192(SURR)**	**2.1708**	**0.016**	**0.1201**	**10.7**
82	*193*	1.8352	0.007	0.0946	8.2	82	*193*+180	2.1866	0.015	0.0849	10.6
83	**200**	**1.8654**	**0.017**	**0.0680**	**27.7**	**83**	**170**	**2.2599**	**0.016**	**0.1005**	**10.5**
84	**mirex**	**1.8952**	**0.010**	**0.1137**	**20.7**	84	199	2.2707	0.016	0.0837	8.0
85	170+190	1.9246	0.011	0.1090	17.1	**85**	**190**	**2.3027**	**0.020**	**0.1072**	**8.7**
86	**199**	**1.9544**	**0.011**	**0.0898**	**14.6**	**86**	**196**	**2.3117**	**0.014**	**0.1004**	**10.1**
87	203+196	1.9721	0.013	0.1118	16.7	**87**	**203**	**2.3315**	**0.017**	**0.0966**	**11.4**
88	**195**	**2.0710**	**0.008**	**0.0938**	**16.3**	88	195	2.4102	0.016	0.0707	6.9
89	**194**	**2.1435**	**0.013**	**0.0949**	**16.3**	89	194	2.6048	0.018	0.0749	6.3
90	**206**	**2.2948**	**0.016**	**0.0906**	**9.1**	90	206	2.7376	0.019	0.0648	4.5

* Bold indicates peak data used for quantitation, all other peaks used for confirmation.
† PCB IUPAC# or pesticide; italics indicates congener identified in standard but not currently quantitated; SURR - surrogate, IS - internal standard.
‡ Mean relative (to IUPAC 104) retention time based upon data from seven-point calibration curve.
§ Relative standard deviation (%) based upon data from seven-point calibration curve.
" Mean relative response factor based upon data from seven-point calibration curve, calculated as $(Area_{congener}/Amount_{congener}) \times (Amount_{IS}/Area_{IS})$.

limits, the entire analytical batch was rejected. Recovery data for each surrogate (IUPAC 125 and 192) were also input into cumulative spreadsheets and monitored by QC charts. As discussed above, the QC check standards were mixed Aroclors prepared from an independent source and calibrated against the AccuStandard mixed Aroclor preparation. For a batch to pass QC, the levels of several major congeners (IUPAC 4+2, 18, 74, 99, 118, 138, 153, and 180) and 80% of the remaining congeners must have been within ± 15% of the expected value for each column. In addition, acceptable samples must have had a ≤ 15% mean difference between selected congener levels determined using the DB-5 versus Apiezon column areas. Data from method blanks were similarly compiled for QC purposes. Total PCB in method blanks was typically < 0.75 ppb; data from sample batches where the blank exceeded 1.25 ppb were subject to additional review to assess possible contamination.

Internal proficiency QC samples (spikes) were analyzed at a rate of one sample per every second batch of extracted samples. Newborn calf serum was spiked with mixed Aroclor standard at nominal levels of 5 and 10 ppb for low and high level spikes, respectively. Aroclor QC check standard working solution in acetone (200 ng each Aroclor/mL) was added dropwise to serum

(1 or 0.5 mL/100 mL serum) and stirred overnight at room temperature. Five mL aliquots of serum were placed into hexane-washed vials, PTFE capped, and frozen at –20°C. Unspiked calf serum was employed as a "blank" QC sample. These three individual levels of QC samples were blindly selected by technicians for inclusion in extracted batches. Recoveries of each congener and of total PCBs were calculated for each sample and results were summarized in cumulative QC spreadsheets for regular review.

Duplicate samples, consisting of serum from a single individual split into two tubes (each with a unique identification number) at the collection laboratory, were analyzed at a 5% rate. Laboratory staff were blinded with respect to all samples; codes were broken only after the results were reported. In addition, one empty sample tube per month was provided (following piercing with a fresh syringe/needle) to the analytical laboratory for analysis as a field blank. These blanks were analyzed by adding 7.5 mL of methanol to the tube and then transferring the sample into a glass centrifuge tube for processing as described. Interlaboratory QC was performed by participation in the ATSDR-funded serum QA program organized by the Michigan State Health Department and by exchange of samples with two other PCB laboratories for independent comparison.

Raw sample data underwent several levels of QC review prior to being released in final form (Figure 1). Each technician verified IS areas as acceptable for each sample as the initial check, followed by verification of QC check standard acceptability, manual editing of data if necessary, data export to spreadsheets, and final processing via Lotus macro software. Hard copies of chromatograms, individual and combined column data reports, QC check standard reports, batch sample reports, and COC forms were included in each sample batch file. Electronic files of original and edited chromatograms and data reports were transferred to a backup PC and archived by tape or ZIP drive. The batch files were then reviewed by a QC manager, who confirmed (by checklist) the acceptability of IS and QC check standards in addition to the presence of several expected congeners in human serum samples. The QC manager then signed off on the file and passed it on to the lab manager for review. The lab manager performed a final overall review for unexpected or unusual results and approved the data for release, and also regularly reviewed trends in IS areas, surrogate and QC sample recoveries, and blank (method and field) data to identify any developing problems. Each step of the QA/QC process was signed off by the individual staff person. This multicomponent review directly involved the technical staff in the QA/QC program and insured a high level of confidence in the quality of reported data.

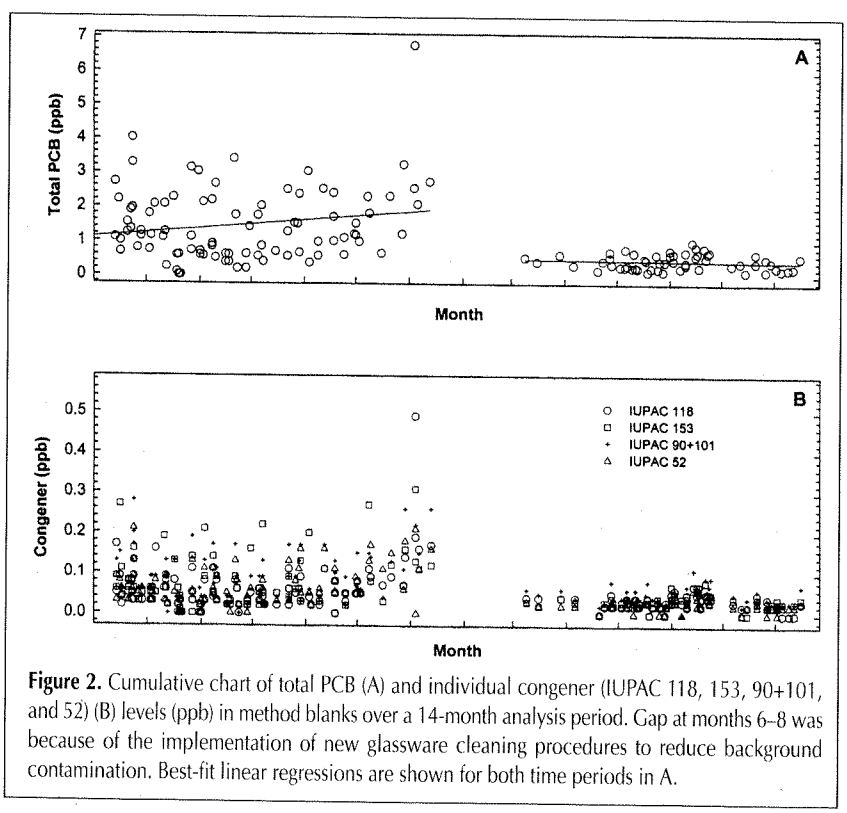

Figure 2. Cumulative chart of total PCB (A) and individual congener (IUPAC 118, 153, 90+101, and 52) (B) levels (ppb) in method blanks over a 14-month analysis period. Gap at months 6–8 was because of the implementation of new glassware cleaning procedures to reduce background contamination. Best-fit linear regressions are shown for both time periods in A.

Results and Discussion

The routine ultra-trace analysis of over 100 PCB congeners in human serum described here is made possible by a number of factors, including improvements to extraction throughput and efficiency, highly reproducible peak retention times, the use of dual quantitation/confirmation columns, and the availability of advanced chromatography software that allows automated integration combined with manual re-editing of difficult peak separations where necessary. Minimization of background laboratory contamination as reflected by low method blank values contributes to the high level of sensitivity required for these analyses. The production of consistent and reliable data from such a complex protocol requires thorough characterization and a clear understanding of the strengths and weaknesses of the analytical system and major attention to QA/QC at each stage of the process. These issues are addressed for the present system below.

Sample extraction and processing

Several modifications to previously published PCB analytical methods were explored in order to increase sample extraction efficiency and throughput and to decrease sample volume requirements. Earlier versions of the present method employed a 10-g sample requirement with concentration of extracts to a final 1 mL volume using Kuderna-Danish (K-D) apparatus (41). The present study examined the use of a semi-automated N_2 blowdown procedure to increase precision and reduce overall technician labor and time needed for sample concentration. Using the RapidVap apparatus, concentration of a batch of six hexane extracts (~60 mL each) to 0.5–1.0 mL required approximately 20–30 min. Comparative studies were performed to assess individual congener recoveries using each sample concentration method. Recovery of individual congeners from hexane spiked with mixed Aroclor standard at 200 ppb and concentrated to 0.5 mL ranged

from 80 to 100% using the RapidVap concentrator (data not shown). Recovery did not appear dependent on PCB chlorination level. Individual congener recoveries using K-D evaporators were generally 10–20% lower than with the N_2 blowdown technique. Routine concentration to 0.5 mL by N_2 blowdown allowed a twofold reduction of the serum sample requirement, to 5 mL. Reproducible semi-automated concentration of extracts to < 0.5 mL using the RapidVap technique was not successful because of variable recoveries.

Minimization of laboratory background PCB levels was found to be essential to high sensitivity analyses. Per-batch method blanks were analyzed to assess overall contamination over a 14-month period (Figure 2). Initially, routine glassware cleaning involved soap and water wash followed by acetone and hexane rinsing of reusable glassware, in addition to use of hexane-rinsed disposable glass pipets for sample transfers. During the first month of analysis, total PCB levels in method blanks were in the 1.00–1.25 ppb range (Figure 2A). However, a gradual increase to an unacceptably high ~2 ppb was observed over the following six-month period. No particular congener was found to be responsible for the increase (Figure 2B); the overall pattern appeared to be similar to that of the Aroclor standard. At that point, analyses were stopped and studies undertaken to determine the source of contamination. Laboratory air and surface samples were analyzed but not found to contribute significantly to background. The contamination was ultimately traced to glassware carryover, despite the solvent wash protocol. Treatment of reusable glassware after soap-and-water wash by heating to 450°C for 4 h in a glass kiln was found to be partially effective in reducing contamination. Ultimately, kiln heating of both reusable glassware and disposable pipets, in addition to Soxhlet extraction of PTFE stoppers, was necessary to reach minimize contamination levels. With this protocol, method blank contamination levels were reduced to 0.5–0.8 ppb total PCB and have been stable at this level to the present time (Figure 2A).

Calibration and chromatography

With the recent commercial availability of pure individual PCB congeners and various congener calibration mixtures, Aroclor-based calibration methods for PCB analysis are being abandoned in favor of congener-based techniques (21,22). Whereas the latter have clear advantages, the routine preparation of a calibration standard with 101 individual congeners is a daunting and expensive task and prone to technical errors. The commonly used alternative of using a limited congener mixture as primary calibrant combined with RFs for the remaining congeners introduces additional errors and inaccuracies and imposes a requirement for regular redetermination of these values (3). Aroclor-based methods can suffer from limitations imposed by differences in congener composition between batches and the lack of comprehensive data on individual congener levels in these commercial products (21). Consequently, investigators using Aroclor-based calibration must thoroughly characterize their materials using independent primary standards (as was done in the current study). Despite these disadvantages, other workers have recognized that Aroclor-based standards can provide a range of calibration levels for individual congeners that, in many cases, are more appropriate for biological samples than those provided by calibration mixtures with equal congener levels (3,22).

The present analytical system is a refinement of earlier published versions employing Aroclor-based calibration (42,43). The rationale behind retaining this method was to provide data consistency with previous studies of the Native American cohort investigated in this project which employed Aroclor-based calibration (41). Prior to beginning analyses, the mixed Aroclor standard was completely recharacterized with respect to peak identities and individual congener contents using the commercial "Frame" mixes as primary calibrants (18,22). In addition, to facilitate an eventual switch to a congener-based standard, we have routinely included such a mixture as a QC sample in our analytical batches.

Table I presents the individual congener compositions (as weight percent of total PCBs) of the calibration, QC check, and "congener check" standards used in the present method. As described previously, the calibration and QC check solutions were prepared from Aroclors obtained from different sources (i.e., AccuStandard vs. Monsanto), and differ in their compositions. Most of the variations are minor, but significant differences are seen for several important congeners, including IUPAC 52, 74, 70, 90+101, 95, 118, and 105. These can be traced to differences in the composition of the Aroclors 1254 from each source, an issue that has recently been discussed in depth (33). These differences facilitate the use of one mixture as the calibrant and the other as the QC check standard. Although the extreme dynamic range (> 400-fold) of congener levels in these materials mimics that seen in actual human samples, it does complicate the routine determination of MDLs, as many dilution levels are required to generate valid limits for all congeners. The congener check standard is prepared by dilution of a commercially available "food and human tissue" congener mixture (AccuStandard), with addition of *p,p'*-DDE. Although this solution contains 32 individual congeners at equal levels, the present method is calibrated for only 25 of them. Values for individual congeners in this check standard have routinely been within 10–15% of expected, indicating the overall robustness of our Aroclor-based calibration, at least for these congeners.

Table II lists the elution order, retention times and RFs (both relative to IS), and selected calibration peaks for each column. Peak retention times were found to be highly reproducible for both columns, with RSD values in the 0.01–0.05% range. This allowed for the use of narrow retention time windows (0.09–0.20 min), which minimized false peak detection by the automated integration procedure. RSDs for RFs were generally < 15%, with a small number of congeners exhibiting higher variance (e.g., IUPAC 2 and 67 on DB-5). RFs were generated as average values from seven-point calibration curves, rather than by linear regression or some other data fitting procedure. Consequently, the RSD values reflect both ECD drift and the degree of non-linearity of the calibration curve. Linearity was routinely checked for all congeners in the present method, with r^2 values typically ≥ 0.98 over the calibration range used. Overall RF variance was slightly higher for DB-5 than for Apiezon (16.27 vs. 10.14% RSD, respectively).

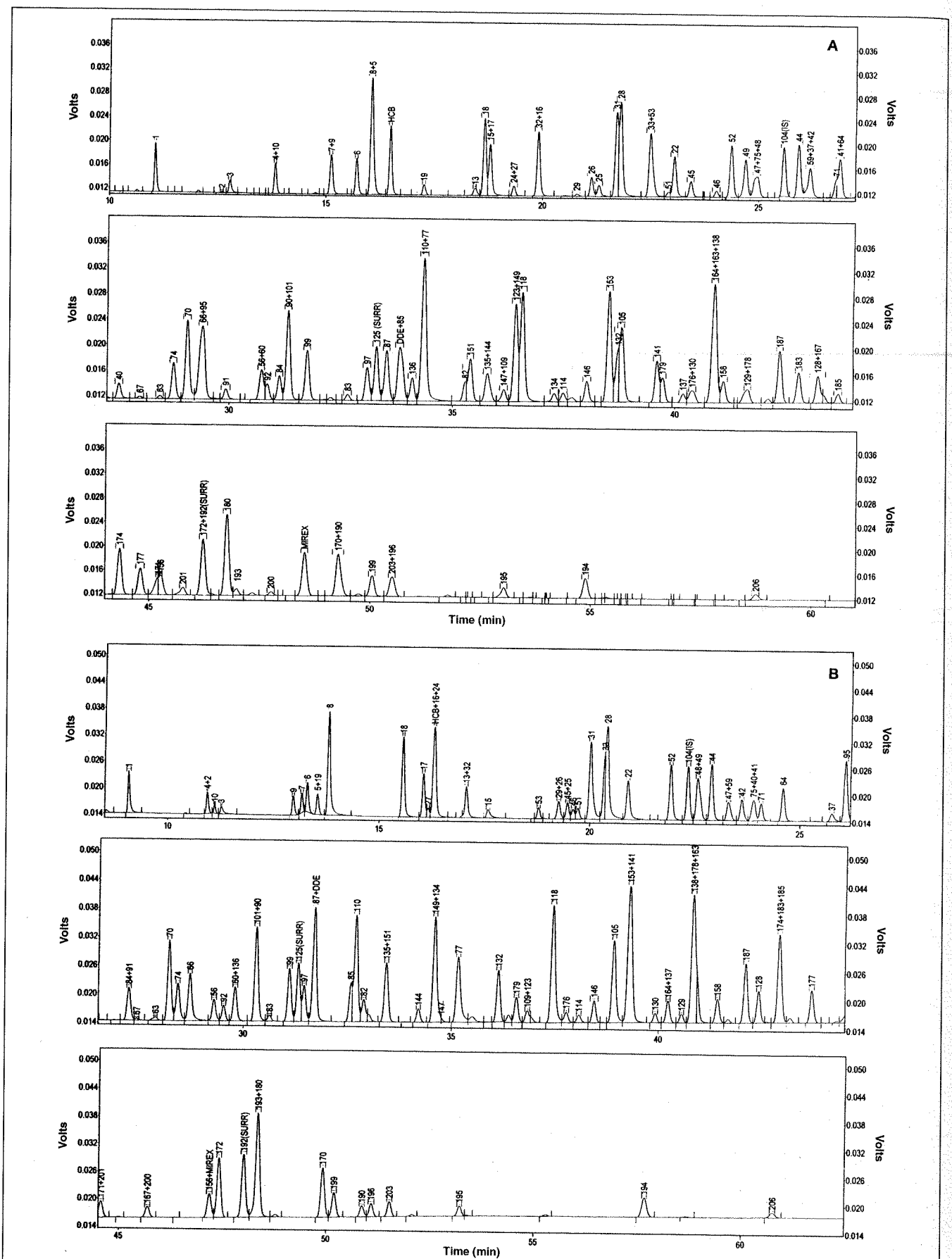

Figure 3. GC–ECD chromatogram of typical mixed Aroclor QC check standard on DB-5 (A) and Apiezon-L (B) columns. Peaks are labeled by IUPAC number. Positions of internal standard (IS; IUPAC 104) and surrogate standards (SURR; IUPAC 125 and 192) are also shown.

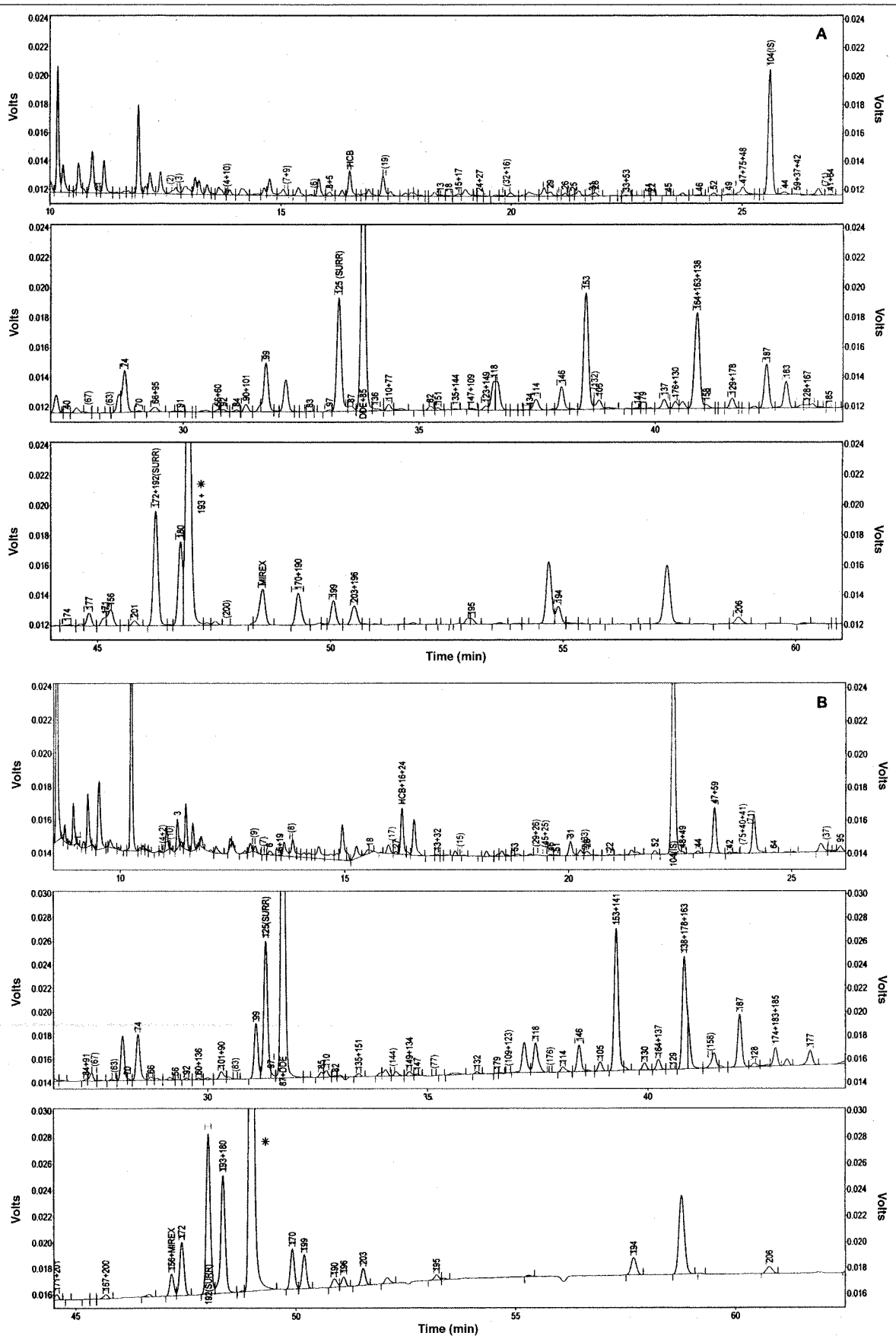

Figure 4. GC–ECD chromatogram of typical human serum sample (total PCB 6.91 ppb) on DB-5 (A) and Apiezon-L (B) columns. Peaks are labeled by IUPAC number. Position of unidentified contaminant peak is shown by "*" on each chromatogram. Analytes shown in parentheses were not detected in this sample by the chromatography software.

Selection of surrogate (recovery) standards and IS was based upon several criteria. PCB congeners were preferred over other halogenated aromatic derivatives. Clean elution and baseline resolution on both columns was sought, along with minimal reported presence in both commercial Aroclors and human and environmental samples (6,14,17). After screening several dozen congeners, only IUPAC 104 (2,2',4,6,6' penta-CB) was found to fulfill all criteria and was therefore selected as the IS (Figures 3A and 3B). IUPAC 125 (3,4,5,2',6' penta-CB) and 192 (2,3,3',4,5,5',6 hepta-CB) were chosen as surrogate standards based on clean elution on DB-5 and Apiezon, respectively (Figures 3A and 3B), along with satisfaction of the other selection criteria. Chromatograms generated from a number of samples analyzed without addition of IS and surrogates were also examined to confirm the absence of peaks at the expected RTs of the selected congeners.

The chromatographic separation of a total of 112 PCB congeners (as singlets, pairs, or triplets), HCB, *p,p'*-DDE, and mirex achievable over a 60-min GC run time with the present system is illustrated for a typical Aroclor check standard on DB-5 and Apiezon in Figures 3A and 3B, respectively. Although 112 congeners are identified on the chromatograms, 11 of these (IUPAC 5, 37, 41, 48, 60, 75, 82, 135, 167, 178, and 193) are not currently quantitated because of inadequate separation, lack of a suitable confirmation peak, and/or interferences. Of the 101 quantitated PCBs, 83 are reported individually, as are HCB and mirex. The majority of these are resolved at baseline or near baseline separation on one column. Twenty congeners (IUPAC 1, 3, 6, 18, 22, 44, 63, 64, 70, 74, 83, 99, 114, 146, 177, 187, 194, 195, 199, and 206) are resolved at baseline or near baseline on both columns. Several incompletely resolved peaks (IUPAC 18/15+17, 31/28, 56+60/92, 123+149/118, 141/179 on DB-5; 45+25/46/51, 110/82 on Apiezon) and shoulders present on larger peaks (IUPAC 71/41+64, 82/151, 132/105, 171/156 on DB-5; 17/27/HCB+16+24, 33/28, 85/110, 149+134/147 on Apiezon) are integrated by a vertical split to baseline. Following processing by the chromatography software, each of these peaks is re-edited manually if necessary to ensure proper and consistent integration between samples. Fifteen remaining congeners and *p,p'*-DDE are reported as pairs, and three congeners as a triplet. Peaks corresponding to an additional 18 congeners (IUPAC 34, 35, 93, 103, 115, 119, 124, 131, 154, 173, 175, 189, 191, 202, 205, 207, 208, and 209) have been identified in the mixed Aroclor standard at low levels on either or both columns. It is expected that future refinements to the method will allow for quantitation of these and the 11 other congeners discussed above, for a total overall capability of 130 congeners. Finally, algorithms are being developed and validated that will allow for determination of individual levels of coeluting congeners based upon a subtractive process using data from the second column.

Chromatograms of a typical human serum extract (total PCB 6.91 ppb) on DB-5 and Apiezon are shown in Figures 4A and 4B, respectively. Increased baseline noise and the presence of extraneous peaks as compared to the Aroclor standard, particular in the first one-third of the separation, are apparent. A contaminant coeluting with IUPAC 193 on DB-5 but present as a unique peak on Apiezon (asterisks in Figures 4A and 4B) is occasionally encountered in serum samples, precluding routine quantitation of this congener. Nevertheless, a total of 47 congeners, in addition to HCB, *p,p'*-DDE, and mirex, were confirmed and reported in this sample following QC review. The major analytes included IUPAC 74, 99, 118, 138 (with 163 and 164), 153, 180, 187, 199, *p,p'*-DDE (with 85), and mirex.

The use of dual column quantitation/confirmation and the presence of coeluting congeners reported as pairs or triplets introduces the potential for inaccuracies in the analytical system. In theory, only those coeluting congeners with exactly the same RFs can be accurately reported as a single summed concentration, rather than as a range of possible concentrations based on the differences in RFs. The extent of bias introduced by these "multicomponent peaks" has been examined in previous studies of Aroclor mixture composition (44). In the present study, the level of uncertainty introduced by this factor can be evaluated using the data in Table III, which presents RFs for individual congeners reported as pairs or triplets. Most of the differences between RFs average ≤ 15%, which should be acceptable for the ultra-trace analysis required in human serum PCB determination. While more substantial differences are encountered for the

Table III. Comparison of Response Factors for Congeners Reported as Pairs/Triplets

IUPAC #*	RF1†	RF2†	RF3†	%Δ‡
4+2	834	204	–	121.3
24+27	3894	3469	–	11.5
32+16	3518	3273	–	7.2
47+59	6812	7953	–	15.4
90+101	3238	3277	–	1.2
***p,p'*-DDE**+85	4489	3393	–	27.8
123+**149**	2873	2635	–	8.6
147+109	3066	2455	–	22.1
164+163+**138**	2629	2519	2566	4.3

* Bold indicates predominant congener in human serum based upon published data.
† Mean response factor (amount/area) for each congener in pair/triplet determined from calibration curve of "Frame" mixes 1–5.
‡ Mean percent difference between RFs calculated as (difference between highest and lowest RFs/mean of RFs) × 100%.

Table IV. Comparison of Analytical Data for Selected Major Congeners Resolved on Both DB-5 and Apiezon

	Standards*		Serum samples†	
IUPAC #‡	level§	A/D"	level§	A/D
74(D)	4.37	–0.7	0.15	–10.9
99(D)	6.96	–0.2	0.14	–7.0
118(A)#	18.02	–0.2	0.21	–2.1
187(D)	7.73	0.4	0.11	–3.8
199(D)	3.09	0.6	0.07	3.8

* Data based on 72 QC check standards.
† Data based on 192 adult human serum samples.
‡ PCB congener and primary quantitation column (D - DB-5, A - Apiezon).
§ Mean congener level (ppb) determined from primary quantitation column.
" Percent difference between column values calculated as difference between Apiezon and DB-5 values/sum of Apiezon and DB-5 values × 100% (26).
~50% resolution from 123+149 on DB-5.

pairs of 147+109, *p,p'*-DDE+85, and, in particular, 4+2, other factors may limit their significance to overall data interpretation. The first pair is rarely reported (see below) and thus of lesser concern. Based on current and previous data, *p,p'*-DDE is typically the dominant chlorinated analyte in this system, and is likely to be present at > 100-fold higher serum levels than IUPAC 85. IUPAC 4 is present at levels 10- to 20-fold higher in Aroclor mixtures than IUPAC 2 (18), and a similar dominance might be expected in cases where they are both present in tissue samples. Consequently, only minor error is likely in the quantitation of congener pairs and triplets as performed in the current method.

Most dual-column PCB analytical systems employ one column for quantitation alone and the other for confirmation alone (11,15,23–25,28,29,38). However, there is no a priori reason why each column cannot be used for both purposes, thus maximizing the amount of useful data obtained from the overall system. If this is done, care must be taken to insure that peak confirmation is as unambiguous as possible and that appropriate criteria are in place for selecting, for each congener, which column will be used for which function (26). Common approaches to the processing of PCB congener data derived from dual-column systems include utilization of either the average of the column data or the lower of the two values as the reported result (3). Different criteria were employed in the present study, where data from the column yielding the better separation and peak shape, and/or the better reproducibility, were selected for the reported result. This procedure is less arbitrary and appears to yield more consistent reporting over the entire data set.

Table V. Summary of QC Sample Data for Major Congeners

		Spike level*					
		low			high		
	blank		RSD	%		RSD	%
Analyte†	ppb‡	ppb‡	(%)§	recov"	ppb‡	(%)§	recov"
1	nd	0.35	24	59	0.74	23	58
4+2	nd	0.25	24	61	0.52	17	60
8	nd	0.16	28	69	0.34	24	70
HCB	nd	0.04	21	99	0.06	16	79
18	nd	0.13	35	82	0.22	55	68
32+16	nd	0.11	29	103	0.18	17	79
31	nd	0.11	10	89	0.24	19	87
28	nd	0.12	26	86	0.21	34	70
52	nd	0.11	22	124	0.17	16	92
49	nd	0.04	24	96	0.08	15	84
44	nd	0.10	35	124	0.15	22	85
95	nd	0.08	21	113	0.14	16	90
90 + 101	0.04 ± 0.02	0.20	23	94#	0.33	15	85#
99	nd	0.10	24	120	0.17	17	92
87	nd	0.13	22	200	0.17	19	124
p,p'-DDE+85	0.53 ± 0.05	0.63	11	77#	0.75	16	85#
110	0.05 ± 0.02	0.21	25	100#	0.32	17	82#
77	nd	0.16	17	82	0.31	22	75
123+149	nd	0.17	20	110	0.28	14	86
118	0.03 ± 0.02	0.22	17	95#	0.37	17	85#
153	0.03 ± 0.02	0.23	19	105#	0.38	14	92#
132	nd	0.07	22	100	0.10	17	71
164+163+138	0.02 ± 0.03	0.27	17	109#	0.44	13	89#
187	nd	0.10	17	111	0.15	15	81
174	nd	0.08	16	99	0.14	15	78
180	nd	0.17	8	112	0.29	17	88
mirex	nd	0.09	17	117	0.15	23	92
170	nd	0.06	51	98	0.11	45	87
199	nd	0.04	16	115	0.06	12	82
total PCB	0.17 ± 0.03	5.42	17	95	9.84	16	83

* Mixed Aroclor standard in hexane spiked into newborn calf serum at levels of 0 (blank), 5.68 (low), or 11.79 (high) ppb total PCB.
† PCB IUPAC# or pesticide.
‡ Mean concentration of analyte (ppb; sample values <MDL set to zero) determined in 17 QC samples at each level; nd - mean is <MDL.
§ Percent relative standard deviation for measured concentration.
" Mean percent recovery based upon measured serum level and amount present in spike standard solution (Table I).
Corrected for blank content.

In the present system, data for quantitation of 61 and 33 peaks, excluding IS and surrogates, are obtained from DB-5 and Apiezon, respectively (Table II). Approximately one-third of these peaks can be unambiguously confirmed on the second column as a unique analyte. For the remaining congeners, the system can only confirm that a peak that contains the congener of interest and/or one or two other analytes is present. This ambiguity is common to all reported dual-column PCB analysis methods in their present state of development. It is acceptable only because of the highly reproducible retention times of individual PCBs on capillary GC columns and the resultant high confidence in peak identity based on data from the primary quantitation column alone.

Some estimate of the effect of lack of unambiguous dual-column confirmation for certain congeners can be made by looking at the overall database occurrence (see below) of less prevalent congeners that coelute with major congeners/analytes on the confirmation column. These might exhibit unexpectedly high prevalence if the lack of unequivocal confirmation results in a significant spurious detection rate. Examples of such congeners are IUPAC 24+27 (coelution with HCB on Apiezon), 32+16 (coelution with HCB), and 141 (coelution with 153) with DB-5 as the primary column, and IUPAC 77 (coelution with 110 on DB-5) and 190 (coelution with 170) with Apiezon as the primary column. In each of these cases, an examination of prevalence data indicates that while the major coeluting congener is present at relatively high levels and at ≥ 97% detection rate, the congeners in question are detected much less often and at lower overall levels. These data suggest that false detection of such congeners is not a significant concern in the present method.

A more challenging issue involves possible spurious detection of uncommon congeners that coelute with other uncommon congeners on the confirmation column. For example, IUPAC 25 and 45 elute cleanly on DB-5 but coelute on Apiezon. If only one of these low prevalence congeners is detected on DB-5, then the existence of the coeluting peak on Apiezon is good confirmation of its true presence in the sample. If both are present on DB-5, than the existence of the coeluting peak on Apiezon only confirms that at least one is present. It has been our experience that the latter situation seldom occurs, and all detections of uncommon congeners undergo additional QA/QC review before final data release.

QA/QC

The QA/QC program in use in the laboratory is summarized in Figure 1 and involves three separate stages, each with specific review activities and decision points. Overall performance and data quality is assured by a combination of quantitative and qualitative limits for calibrations, IS areas, surrogate recoveries, cross-column comparisons, method blanks, QC check standards, and QC sample (spike) recoveries. Out-of-limit results require review and/or rejection. Many of these parameters are monitored over time by the use of cumulative data spreadsheets and QC charts. All laboratory staff have specific roles and responsibilities in the QA/QC program.

Laboratory performance results related to calibration, method blanks, and check standards have been discussed here previously. The magnitude and variance of IS areas are instrument-dependent and appear to be quite sensitive to physical parameters such as positioning of the column ends in the injection port and detector and condition of the autosampler syringe. Consequently, IS warning and control limits are established separately for each instrument and are redetermined following maintenance operations that might influence IS areas. In addition, overall variance in IS areas in the present study was found to decrease following a change from IS prepared as needed by laboratory staff to one commercially prepared in batches (data not shown). Very few rejections/reanalyses because of out-of-control IS areas were necessary during the course of this investigation.

Other dual-column PCB quantitation studies have reported cross-column comparisons of data for selected congeners (26). The results of cumulative analysis of QC data on comparative levels of certain congeners determined on both DB-5 and Apiezon columns are shown in Table IV. A total of 33 congeners/analytes can be quantitated using data from either column, including the five major congeners listed in Table IV. The relative differences in the levels of these congeners in QC check standards calculated from each column are quite low, ranging from 0.2 to 0.7%. Relative differences are higher for serum samples, ranging from approximately 2 to 11%. This level of variability is not unexpected for PCB analyses in the high parts-per-trillion range and is similar to other reported data (26). As discussed in Methods, samples that exceed a 15% relative between-column difference for any of the major congeners listed are flagged for further review. In general, exceedence of this limit has been found to be due to improper peak integration on one or both columns, which is corrected by re-editing the chromatogram.

Recoveries of surrogate standards (IUPAC 125 and 192) over a 13-month period are summarized in Figure 5. During the initial four months of analysis, excessively high surrogate recoveries were encountered for a number of sample batches. During this period, surrogate standard solutions were prepared by laboratory staff from neat materials as needed and diluted to desired levels prior to use. A switch to a commercially prepared mixture of the surrogates at the working concentration and modifications in surrogate solution storage procedures resulted in a substantial decrease in recovery variance and incidence of high recoveries. Mean recoveries for IUPAC 125 and 192 over the nine-month period following implementation of the new surrogate solution were 92.5 and 90.5% respectively. Upper and lower control limits of 130% and 60%, respectively, were established based upon these data. Samples with out-of-limit surrogate recoveries are flagged for additional review and possible reanalysis. Surrogate recovery data are for QC purposes only; sample data are not corrected for recovery.

QC sample (spike) recovery data for major PCB congeners/analytes are summarized in Table V. The blank calf serum contained low but detectable levels of IUPAC 90+101, 110, 118, 153, and 164+163+138. In addition, significant levels of *p,p'*-DDE+85 were present in serum blanks. Overall PCB recoveries were 95% and 83% for the low (5.68 ppb) and high (11.79 ppb) QC spike samples, respectively. Approximately 34% and 17% of congeners were below detection limits in the low and high level spike samples. Recoveries for individual congeners tended to be more variable for the low as compared to the high spike samples. In addition, low concentration congeners (i.e., spike levels at or near the MDL) generally exhibited higher variance

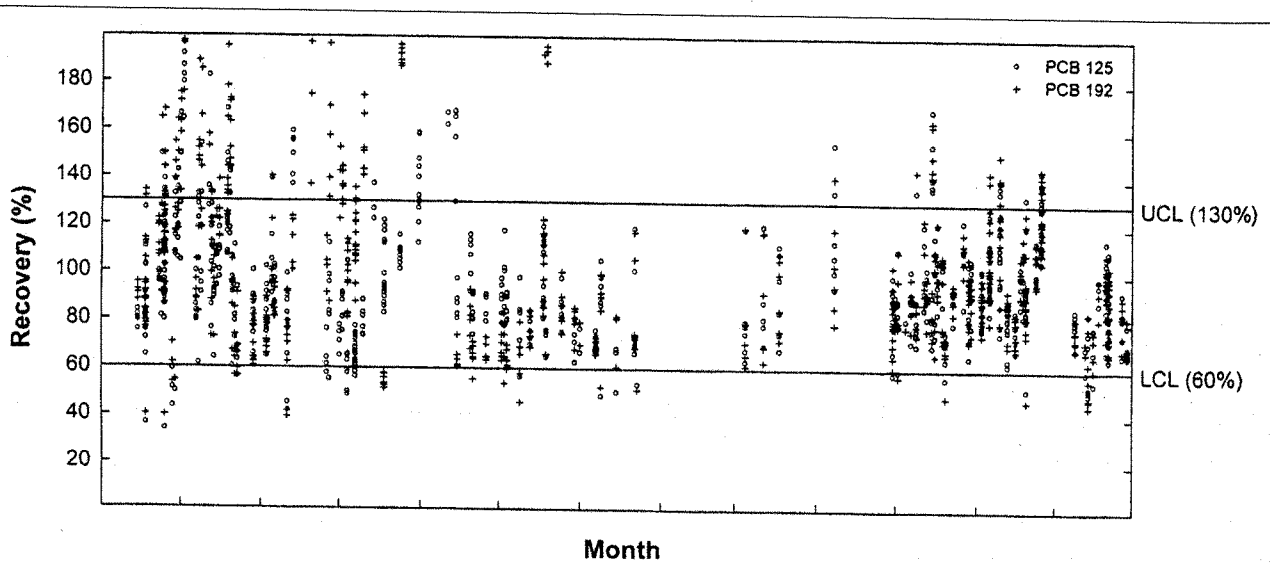

Figure 5. Cumulative chart of surrogate standard (IUPAC 125 and 192) recovery from individual serum samples over a 13-month analysis period. Upper control limit (UCL) and lower control limit (LCL) on recovery are set to 130% and 60%, respectively, for QC purposes.

and a greater proportion of excessively high or low mean recoveries than major congeners (data not shown). Recovery of IUPAC 170 was also more variable than most congeners. Mean recoveries of more prevalent congeners were generally 80–120%, with the exception of IUPAC 1, 4+2, and 8, which were considerably lower, and IUPAC 87, which exhibited high mean recovery in both low and high spike samples (Table VI). No trends in QC spike recovery data which might reflect changes in overall laboratory performance were noted over the 14-month analysis period.

MDLs (based on a 5-g serum sample) for the present method generated prior to the start of the analytical period are also shown in Table I. The majority of MDLs are in the 20–40 ppt range. Reported MDLs for human serum PCB analysis vary widely, with some similar to those of the present method (13,14,38) and others substantially higher (23,45). This variability is likely due to the method used to generate MDL data rather than real differences in analytical sensitivity. Most published reports of serum PCB analysis appear to have employed either blank serum or a single-level spiked serum as the matrix for determining MDLs. These approaches are not ideal for methods that quantitate large numbers of analytes over large concentration ranges (39). Use of blank serum results in valid MDLs only for the minority of congeners that are generally detectable in all samples. Use of single concentration spikes, as in certain USEPA procedures (40), ignores changes in variance often associated with absolute concentration levels of analytes. Some authors have reported that the variance of PCB congener determinations generally increases with concentration (46), a phenomenon that can result in calculation of excessively high MDLs if only single spike sample matrices are employed. The relatively low MDLs in the present method reflect the use of the more appropriate multiple spike level procedure coupled with chromatography software that can reliably detect and quantitate very small peaks.

Quantitation of PCBs in Akwesasne Mohawk adults

The present PCB analytical method supports three individual human health projects as part of the University at Albany's Superfund Basic Research Program. Ultimately, a total of 800 serum samples from individual Akwesasne Mohawk adults and 400 Mohawk children (aged 10–17 years) will be analyzed using this method. A preliminary assessment of indi-

Table VI. Level and Prevalence of Individual Congeners in Adult Serum Samples

	All samples*			Upper 5% total PCB†		
Analyte‡	median§	high"	% ≥ 1 × MDL	% ≥ 3 × MDL	median§	> 50% prevalence#
***p,p'*-DDE+85**	1.99	21.93	100	99	9.24	+
153	0.42	4.04	100	99	1.89	+
164+163+138	0.36	4.17	100	99	1.59	+
180	0.30	2.60	100	97	1.35	+
118	0.19	2.46	100	94	1.08	+
74	0.14	2.52	99	82	1.18	+
99	0.14	2.32	96	87	0.78	+
187	0.11	1.51	98	77	0.59	+
170	0.08	0.76	96	60	0.34	+
90+101	0.07	0.51	98	75	0.08	+
mirex	0.07	1.25	83	60	0.42	+
HCB	0.06	0.26	99	61	0.15	+
110	0.06	0.51	97	59	0.06	+
199	0.06	0.68	95	79	0.38	+
194	0.06	1.56	88	51	0.32	+
156	0.06	0.57	80	57	0.36	+
87	0.05	0.28	96	41	0.07	+
52	0.05	0.24	93	44	0.05	+
146	0.05	0.84	93	48	0.32	+
105	0.05	0.83	91	43	0.26	+
183	0.05	0.55	79	63	0.17	+
28	0.05	0.46	74	48	0.10	+
95	0.03	0.29	92	20	0.04	+
177	0.03	0.43	90	52	0.16	+
66	0.03	0.63	81	21	0.10	+
203	0.03	0.38	80	30	0.18	+
44	0.03	0.14	80	24	0.02	+
123+149	0.03	0.24	76	24	0.07	+
70	0.03	0.19	76	16	0.02	+
206	0.03	3.24	68	30	0.18	+
114	0.03	0.39	61	22	0.14	+
47+59	0.03	0.28	60	32	0.05	+
172	0.03	0.31	59	16	0.11	+
196	0.02	0.21	83	43	0.10	+
84	0.02	0.13	79	<10	0.02	+
201	0.02	2.69	73	21	0.11	+
151	0.02	0.08	68	<10	0.03	+
92	0.02	0.09	67	<10	0.02	+
18	0.02	0.09	66	<10	0.02	+
190	0.02	0.16	66	<10	0.11	+
130	0.02	0.24	65	21	0.06	+
31	0.02	0.19	59	27	0.03	+
141	0.02	0.13	55	<10	0.02	+
137	0.02	0.26	54	13	0.11	+
56	0.02	0.08	52	<10	0.02	+
128	0.02	0.35	51	<10	0.02	+
174	0.01	0.47	56	15	0.02	+
158	0.01	0.13	56	<10	0.02	+
8	nd	0.19	47	<10	0.02	+

* Calculated from all adult serum samples ($N = 282$). Data sorted by median and % ≥ 1 × MDL.
† Calculated from the highest 5% of total PCB samples ($N = 14$).
‡ PCB IUPAC# or pesticide; analytes grouped as major and prevalent (bold text), minor and prevalent (normal text), or sporadic (italic text).
§ Median value of sample data (values <MDL set to zero); nd - median is <MDL.
" Highest value in data set; nd - highest value is <MDL.
"+" indicates congener is present in more than half of the highest total PCB samples.

Table VI. (continued) Level and Prevalence of Individual Congeners in Adult Serum Samples

	All samples*			Upper 5% total PCB†		
Analyte‡	median§	high"	% ≥ 1 × MDL	% ≥ 3 × MDL	median§	> 50% prevalence#
195	nd	0.16	46	<10	0.06	+
49	nd	0.22	45	17	nd	
33	nd	0.36	45	16	0.02	+
132	nd	0.17	40	<10	nd	
29	nd	0.20	39	15	0.02	+
171	nd	0.22	37	10	0.08	+
42	nd	0.03	36	<10	nd	
71	nd	0.28	36	<10	nd	
97	nd	0.15	34	<10	nd	
144	nd	0.25	32	<10	nd	
136	nd	0.16	30	<10	0.04	+
77	nd	0.19	29	<10	nd	
24+27	nd	0.19	28	<10	nd	
19	nd	0.09	27	<10	nd	
179	nd	0.06	26	<10	0.02	+
53	nd	0.09	26	<10	nd	
32+16	nd	0.12	26	<10	0.02	+
40	nd	0.29	24	<10	nd	
134	nd	0.17	24	<10	nd	
26	nd	0.13	22	<10	0.05	+
15	nd	0.13	22	<10	nd	
17	nd	0.10	18	<10	nd	
83	nd	0.05	17	<10	nd	
25	nd	0.03	16	<10	nd	
4+2	nd	0.06	15	<10	nd	
22	nd	0.16	15	<10	nd	
7	nd	0.05	15	<10	nd	
10	nd	0.05	15	<10	nd	
46	nd	0.06	15	<10	nd	
176	nd	0.11	13	<10	nd	
13	nd	0.08	13	<10	nd	
64	nd	0.06	11	<10	nd	
200	nd	0.69	11	<10	nd	
6	nd	0.09	<10	<10	nd	
63	nd	0.01	<10	<10	nd	
91	nd	0.19	<10	<10	nd	
9	nd	0.06	<10	<10	nd	
129	nd	0.03	<10	<10	nd	
147+109	nd	0.11	<10	<10	nd	
3	nd	0.05	<10	<10	nd	
67	nd	0.51	<10	<10	nd	
51	nd	0.13	<10	<10	nd	
45	nd	0.10	<10	<10	nd	
185	nd	0.11	<10	<10	nd	
1	nd	nd	<10	<10	nd	
total PCB	3.63	28.48			13.59	

* Calculated from all adult serum samples ($N = 282$). Data sorted by median and % ≥ 1 × MDL.
† Calculated from the highest 5% of total PCB samples ($N = 14$).
‡ PCB IUPAC# or pesticide; analytes grouped as major and prevalent (bold text), minor and prevalent (normal text), or sporadic (italic text).
§ Median value of sample data (values <MDL set to zero); nd - median is <MDL.
" Highest value in data set; nd - highest value is <MDL.
"+" indicates congener is present in more than half of the highest total PCB samples.

vidual PCB congener and pesticide data for an initial group of 282 samples from adults is presented in Table VI. Median and highest measured concentration, in addition to prevalence (percentage of samples with levels ≥ MDL), are listed for each analyte over the entire data set (median total PCB 3.63 ppb). The percentage of samples with levels at least three times the MDL, a parameter roughly equivalent to a "quantitation limit" in certain analytical protocols, is also listed for each analyte. Median concentrations and an indication of whether the congener was detected in at least half of the samples are also given for the 14 individuals (upper 5%) with the highest total PCB levels (median total PCB, 13.59 ppb). Finally, these data are used to group each analyte into one of three general categories, that is, prevalent and present at relatively high levels, prevalent and present at relatively low levels, and not prevalent (sporadic).

Five analytes were detected in all samples tested and at relatively high levels. Of these, *p,p'*-DDE+85 was the analyte detected at the highest median levels (1.99 ppb over the full data set, 9.24 ppb in the highest total PCB samples). IUPAC 153, 163+164+138, 180, and 118 were also detected in all samples tested at relatively high levels. Using arbitrary thresholds of 0.05 ppb for the median and a > 50% detection frequency, 17 additional analytes (15 PCB congeners/pairs, mirex, and HCB; shown in bold in Table VI) can be classified as major and prevalent. Most of these analytes were also present in the majority of samples at levels above a "quantitation limit" of three times the MDL. These results are consistent with previous reports by numerous groups indicating that these congeners/pesticides are commonly found in human serum samples (6).

A second group of 26 congeners/pairs (shown in normal text in Table VI) can be classified as low level but prevalent. These exhibited median concentration values of ≤ 0.03 ppb in serum but were present at a > 50% detection frequency in both the full cohort and in the subset of samples with the highest total PCB levels. However, almost all were present in fewer than half of the samples at concentrations greater than three times the MDL, further indicating their generally low serum level. Most of these congeners have previously been reported in human serum in at least one published study. Exceptions include IUPAC 95, 114, 137, 158, and 196, which appear to have been reported in human milk and adipose tissue, but not serum.

A third group of 46 congeners/pairs (shown

in italics in Table VI) were detected only at low levels and with low frequency. In all cases, the overall median level of each of these congeners was below the MDL. However, several were detected in more than half of the samples with the highest total PCBs. Many of these "sporadic" or "intermittent" congeners have also been reported as present in other recent congener-specific analyses of human serum (6,13,16). In contrast, the levels and prevalence rates of the final 10-15 congeners listed in Table VI were so low as to make their presence questionable (IUPAC 1 was the only analyte not detected in any sample). Further data are required to determine how many of these represent rare but real, as opposed to spurious, detections. More detailed and comprehensive analysis of individual congener levels, detection rates, and correlations within this population will be presented in future publications.

Acknowledgments

The expert technical assistance of the staff of the First Environment Laboratory, Akwesasne Task Force on the Environment with blood sample collection and processing is gratefully acknowledged. This work was supported under the University at Albany SBRP funded by NIEHS (P42 ES-04913).

References

1. V. Lang. Polychlorinated biphenyls in the environment. *J. Chromatogr.* **595:** 1-43 (1992).
2. ATSDR. *Toxicological Profile for Polychlorinated Biphenyls (Update).* Agency for Toxic Substances and Disease Registry, U.S. Public Health Service, Washington, D.C., 1995.
3. M.D. Erickson. *Analytical Chemistry of PCBs,* 2nd ed. CRC Lewis Publishers, Boca Raton, FL, 1997.
4. H.A. Anderson, C. Falk, L. Hanrahan, J. Olson, V.W. Burse, L. Needham, D. Paschal, D. Patterson, and R.H. Hill. Profiles of Great Lakes critical pollutants: a sentinel analysis of human blood and urine. *Environ. Health Perspect.* **106:** 279–289 (1998).
5. S.H. Safe. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. *Crit. Rev. Toxicol.* **24:** 87–149 (1994).
6. L.G. Hansen. Stepping backward to improve assessment of PCB congener toxicities. *Environ. Health Perspect.* **106:** 171–189 (1998).
7. D.O. Carpenter. Polychlorinated biphenyls and human health. *Int. J. Occup. Med. Environ. Health* **11:** 291–303 (1998).
8. M.S. Wolff, D. Camann, M. Gammon, and S.D. Stellman. Proposed PCB congener groupings for epidemiological studies. *Environ. Health Perspect.* **105:** 13–14 (1997).
9. L.J. Fischer, R.F. Seegal, P.E. Ganey, I.N. Pessah, and P.R.S. Kodavanti. Symposium overview: toxicity of non-coplanar PCBs. *Toxicol. Sci.* **41:** 49–61 (1998).
10. K.C. Jones. Determination of polychlorinated biphenyls in human foodstuffs and tissues: Suggestions for a selective congener analytical approach. *Sci. Total Environ.* **68:** 141–159 (1988).
11. K.M. Wilson-Yang, J.P. Power, E.A. Chilsolm, D.J. Hallet, and A. Schecter. The analysis of human blood for seventy-five PCB congeners by dual column capillary-gas chromatography. *Chemosphere* **25:** 1375–1379 (1992).
12. V.W. Burse, D.F. Groce, S.P. Caudill, M.P. Korver, D.L. Phillips, P.C. McClure, C.R.J. Lapeza, S.L. Head, D.T. Miller, D.J. Buckley, J. Nassif, R.J. Timperi, and P.M. George. Determination of polychlorinated biphenyl levels in the serum of residents and in the homogenates of seafood from the New Bedford, Massachusetts, area: a comparison of exposure sources through pattern recognition techniques. *Sci. Total Environ.* **144:** 153–177 (1994).
13. K.B. Moysich, P. Mendola, E.F. Schisterman, J.L. Freudenheim, C.B. Ambrosone, J.E. Vena, P.G. Shields, P. Kostyniak, H. Greizerstein, S. Graham, and J.R. Marshall. An evaluation of proposed frameworks for grouping polychlorinated biphenyl (PCB) congener data into meaningful analytic units. *Amer. J. Ind. Med.* **35:** 223–231 (1999).
14. E. Devoto, B.J. Fiore, R. Millikan, H.A. Anderson, L. Sheldon, W.C. Sonzogni, and M.P. Longnecker. Correlations among human blood levels of specific PCB congeners and implications for epidemiologic studies. *Amer. J. Ind. Med.* **32:** 606–613 (1997).
15. A.R. Najam, M.P. Korver, C.C. Williams, V.W. Burse, and L.L. Needham. Analysis of a mixture of polychlorinated biphenyls and chlorinated pesticides in human serum by column fractionation and dual-column capillary gas chromatography with electron capture detection. *J. AOAC Int.* **82:** 177–185 (1999).
16. P.J. Kostyniak, C. Stinson, H.B. Greizerstein, J. Vena, G. Buck, and P. Mendola. Relation of Lake Ontario fish consumption, lifetime lactation, and parity to breast milk polychlorobiphenyl and pesticide concentrations. *Environ. Res.* **80:** S166–S174 (1999).
17. V.A. McFarland and J.U. Clarke. Environmental occurrence, abundance, and potential toxicity of polychlorinated biphenyl congeners: considerations for a congener-specific analysis. *Environ. Health Perspect.* **81:** 225–239 (1989).
18. G.M. Frame. A collaborative study of 209 PCB congeners and 6 Aroclors on 20 different HRGC columns 2. Semi-quantitative Aroclor congener distributions. *Fresenius J. Anal. Chem.* **357:** 714–722 (1997).
19. D.G. Patterson, Jr., G.D. Todd, W.E. Turner, V. Maggio, L.R. Alexander, and L.L. Needham. Levels of non-*ortho*-substituted (coplanar), mono- and di-*ortho*-substituted polychlorinated biphenyls, dibenzo-*p*-dioxins, and dibenzofurans in human serum and adipose tissue. *Environ. Health Perspect.* **102:** 195–204 (1994).
20. S.D. Stellman, M.V. Djordjevic, J.E. Muscat, L. Gong, D. Bernstein, M.L. Citron, A. White, M. Kemeny, E. Busch, and A.N. Nafziger. Relative abundance of organochlorine pesticides and polychlorinated biphenyls in adipose tissue and serum of women in Long Island, New York. *Cancer Epidemiol. Biomarkers Prev.* **7:** 489–496 (1998).
21. P. Hess, J. de Boer, W.P. Cofino, P.E.G. Leonards and D.E. Wells. Critical review of the analysis of non- and mono-*ortho*-chlorobiphenyls. *J. Chromatogr.* **703:** 417–465 (1995).
22. J.W. Cochran and G.M. Frame. Recent developments in the high-resolution gas chromatography of polychlorinated biphenyls. *J. Chromatogr.* **843:** 323–368 (1999).
23. J.W. Brock, V.W. Burse, D.L. Ashley, A.R. Najam, V.E. Green, M.P. Korver, M.K. Powell, C.C. Hodge, and L.L. Needham. An improved analysis for chlorinated pesticides and polychlorinated biphenyls (PCBs) in human and bovine sera using solid-phase extraction. *J. Anal. Toxicol.* **20:** 528–536 (1996).
24. H.B. Greizerstein, P. Gigliotti, J. Vena, J. Freudenheim, and P.J. Kostyniak. Standardization of a method for the routine analysis of polychlorinated biphenyl congeners and selected pesticides in human serum and milk. *J. Anal. Toxicol.* **21:** 558–566 (1997).
25. J. Hajslovà, R. Schoula, K. Holadová, and J. Poustka. Analysis of PCBs in biotic matrices by two-dimensional GC–ECD. *Int. J. Environ. Anal. Chem.* **60:** 163–173 (1995).
26. E. Storr-Hansen. Comparative analysis of thirty polychlorinated biphenyl congeners on two capillary columns of different polarity with non-linear multi-level calibration. *J. Chromatogr.* **558:** 375–391 (1991).
27. E. Storr-Hansen. Simultaneous analysis of 32 PCB-congeners on two capillary columns operated in parallel with a glass T-split. *Int. J. Environ. Anal. Chem.* **43:** 253–266 (1991).

28. C.-S. Hong, B. Bush, J. Xiao, and H. Qiao. Toxic potential of non-ortho and mono-ortho coplanar polychlorinated biphenyls in Aroclors, seals, and humans. *Arch. Environ. Contam. Toxicol.* **25:** 118–123 (1993).
29. K.A. Bucholski, J. Begerow, G. Winneke, and L. Dunemann. Determination of polychlorinated biphenyls and chlorinated pesticides in human body fluids and tissues. *J. Chromatogr. A* **754:** 479–485 (1996).
30. B. Bush, M.J. Murphy, S. Connor, J. Snow, and E. Barnard. Improvements in glass capillary gas chromatographic polychlorobiphenyl analysis. *J. Chromatogr. Sci.* **23:** 509–515 (1985).
31. D.E. Schulz, G. Petrick, and J.C. Duinker. Complete characterization of polychlorinated biphenyl congeners in commercial Aroclor and clophen mixtures by multidimensional gas chromatography–electron capture detection. *Environ. Sci. Technol.* **23:** 852–859 (1989).
32. B. Bush and E.L. Barnard. Gas phase infrared spectra of 209 polychlorinated biphenyl congeners using gas chromatography with fourier transform infrared detection: Internal standardization with a ^{13}C-labelled congener. *Arch. Environ. Contam. Toxicol.* **29:** 322–326 (1995).
33. G.M. Frame. Improved procedure for single DB-XLB column GC–MS-SIM quantitation of PCB congener distributions and characterization of two different preparations sold as "Aroclor 1254". *J. High Resol. Chromatogr.* **22:** 533–540 (1999).
34. L.L. Needham, V.W. Burse, M.P. Korver, C.R. Lapeza, J.A. Liddle, D.D. Bayse, and H.A. Price. Design and demonstration of a quality assurance program for labor-intensive analytical systems. *J. Anal. Toxicol.* **7:** 279–282 (1983).
35. J.K. Taylor. *Quality Assurance of Chemical Measurements.* Lewis Publishers, Chelsea, MI, 1987.
36. M. Luotamo and A. Aitio. Quality assurance of isomer-specific analysis of PCBs in serum. *Chemosphere* **34:** 965–973 (1997).
37. W. Horwitz and R. Albert. Reliability of the determinations of polychlorinated contaminants (biphenyls, dioxins, furans). *J. AOAC Int.* **79:** 589–621 (1996).
38. S.S. Atuma and M. Aune. Method for the determination of PCB congeners and chlorinated pesticides in human blood serum. *Bull. Environ. Contam. Toxicol.* **62:** 8–15 (1999).
39. R.D. Gibbons, D.E. Coleman, and R.F. Maddalone. An alternative minimum level definition for analytical quantification. *Environ. Sci. Technol.* **31:** 2071–2077 (1997).
40. USEPA. Definition and procedure for determination of the method detection limit. *Fed. Regist.* **49:** 43430–43431 (1984).
41. E.F. Fitzgerald, D.A. Deres, S.A. Hwang, B. Bush, B.Z. Yang, A. Tarbell, and A. Jacobs. Local fish consumption and serum PCB concentrations among Mohawk men at Akwesasne. *Environ. Res.* **80:** S97–S103 (1999).
42. R.P. Eganhouse, B.R. Gould, D.M. Olaguer, C.S. Phinney, and P.M. Sherblom. Congener-specific determination of chlorobiphenyls in biological tissues using an Aroclor-based secondary calibration standard. *Int. J. Environ. Anal. Chem.* **35:** 175–198 (1989).
43. R. Boonyathumanondh, S. Watanabe, W. Laovakul, and M. Tabucanon. Development of a quantification methodology for polychlorinated-biphenyls by using Kanechlor products as the secondary reference-standard. *Fresenius J. Anal. Chem.* **352:** 261–267 (1995).
44. R.P. Eganhouse and R.W. Gossett. Sources and magnitude of bias associated with determination of polychlorinated biphenyls in environmental samples. *Anal. Chem.* **63:** 2130–2137 (1991).
45. B.J. Fiore, H.A. Anderson, L.P. Hanrahan, L.J. Olson, and W.C. Sonzogni. Sport fish consumption and body burden levels of chlorinated hydrocarbons: A study of Wisconsin anglers. *Arch. Environ. Health* **44:** 82–88 (1989).
46. M.E. Zorn, R.D. Gibbons, and W.C. Sonzogni. Evaluation of approximate methods for calculating the limit of detection and limit of quantification. *Environ. Sci. Technol.* **33:** 2291–2295 (1999).

Manuscript received November 16, 1999;
revision received January 20, 2000.