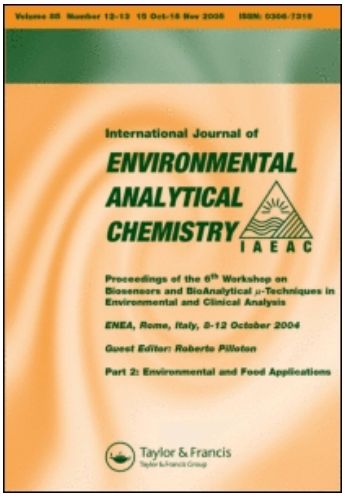

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CONGENER-SPECIFIC DETERMINATION OF CHLOROBIPHENYLS IN BIOLOGICAL TISSUES USING AN AROCLOR-BASED SECONDARY CALIBRATION STANDARD

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A secondary calibration standard was developed from a mixture of Aroclors 1242, 1248, 1254 and 1260. The composition of this standard ($\leq$130 components) was established by high resolution gas chromatographic retention time and mass spectral data supplemented by coinjection of individual congeners. Hydrogen flame ionization detection (FID) and electron impact mass spectrometry (MS) were used for independent quantitation of the mixture. Total chlorobiphenyl concentrations measured in the Aroclor mixture were in both cases within 7% of the gravimetrically determined concentration. However, discrepancies were found between results obtained with the two methods for individual chromatographic peaks. These discrepancies were largely restricted to minor constituents of the Aroclor mixture. Variations for major peaks are attributable to deviations of individual congener response factors from those representative of the corresponding isomer group.

The secondary calibration standard was used to determine chlorobiphenyl concentrations in tissues of two marine organisms by high resolution gas chromatography with electron capture detection. Estimated limits of detection and quantitation for total chlorobiphenyls were 2.1 and 2.9 ng/wet g, respectively. The precision (RSD) of the method for total chlorobiphenyls in soft-shelled clam (*Mya arenaria*) and lobster (*Homarus americanus*) was estimated at 4.5% and 11.4%, respectively. Quantitation of individual Aroclors yielded average concentrations ranging from 86 to 91% of the gravimetrically determined amounts. Advantages and limitations of the secondary calibration standard approach are discussed in light of recent advances in the analytical chemistry of chlorobiphenyls.

KEY WORDS: PCB, calibration standard, chlorobiphenyls.

INTRODUCTION

Owing to their toxicity and widespread distribution in the global ecosystem, chlorinated biphenyls have attracted the attention of environmental chemists, toxicologists and regulatory agencies for more than two decades. Although worldwide production of these compounds has largely ceased, their persistence in the environment should insure the sustained interest of scientists and government agencies for some time to come. Unfortunately, our understanding of the behavior

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of chlorinated biphenyls and their potential for biological effects is still incomplete. This is due primarily to difficulties with the analytical chemistry of this class of halocarbons.

One cause of these difficulties is the sheer complexity of chlorobiphenyl assemblages typically found in environmental samples. Substitution of chlorine on the biphenyl nucleus can give rise to 209 distinct compounds, or congeners. Commercial mixtures, first introduced in 1929, were prepared by direct chlorination of biphenyl followed by fractional distillation of the resultant product.[1] The most widely used mixtures (e.g. Arochlor, Clophen, Kanechlor, etc. . . .) have been reported to contain from 30 to 120 individual components.[2-6] Because environmental samples frequently contain residues of more than one of these products, the total number of congeners in such samples often exceeds 50.[7-9]

Early attempts at quantitating chlorobiphenyls were based on visual pattern matching and peak area comparisons between samples and commercial formulations using packed column gas chromatography.[10] These methods required judgments on the part of the analyst and major simplifying assumptions. The inadequacies of this approach soon became apparent with the advent of high resolution capillary gas chromatography.[2,3] Because of their wide range in physicochemical properties,[11-13] chlorobiphenyls may undergo fractionation due to differential phase partitioning.[14-16] Moreover, it is now well established that individual congeners vary in their rates of bioaccumulation,[17,18] biotransformation[19-21] and photodegradation.[22,23] Consequently, environmental samples bearing residues of industrial mixtures often exhibit compositions that are markedly different from those of the original formulations. Finally, chlorobiphenyls having altogether different compositions than the synthetic mixtures are introduced to the environment, albeit in small amounts, from chemical process streams and during incineration.[24] Thus, natural processes can lead to alteration of the commercial mixtures whereas contributions from other non-synthetic sources can serve to further complicate the patterns. For samples of this type, comparison of peak areas in sample chromatograms with those of Aroclor standards is semi-quantitative, at best.

The logical solution to this problem would seem to be the separation of individual congeners and direct quantitation using authentic standards. As early as 1971 the distinct analytical advantages of this congener-specific approach were recognized,[3] and in succeeding years a sustained effort was made by various investigators to develop methods for the identification and quantitation of individual chlorobiphenyls.[25-28] As recently as 1984, the synthesis of all 209 congeners was first reported.[29] In spite of significant advances in the gas chromatographic analysis of these compounds during the last five years (cf., references in 24), baseline separation of all 209 congeners by one high resolution column remains elusive, and the number of commercially available pure congeners, although growing, is still limited. Failure to resolve isomeric chlorobiphenyls can lead to significant bias when electron capture detection or negative ion chemical ionization are used because of the well known disproportionate response of these detectors.[24] In short, currently available methods, although greatly improved, remain imperfect.

Analytical considerations aside, there is ample incentive to refine and perfect congener-specific measurements. For example, in addition to offering the possibility of greater accuracy in the measurement of total chlorobiphenyl concentrations, particularly for altered Aroclor compositions, the congener-specific approach provides valuable information on the abundance of individual compounds. Insofar as chlorobiphenyl distributions reflect the action of natural processes (e.g., sorption, volatilization, transmembrane transport, microbial activity and photodecomposition), this information can be used to draw inferences about the mechanisms involved.[14,30-32] Thus, it is a potentially powerful tool for testing the validity of transport and fate models currently being developed for a variety of ecosystems.[33,34] Although field data are still somewhat sparse, chlorobiphenyls are also known to vary widely in their toxicities. By providing concentrations of individual compounds, the congener-specific approach will permit more meaningful toxicological interpretations of environmental distributions to be made. Ultimately, this should lead to significant advances in our understanding of structure-activity relationships.[7]

The purpose of this paper is to describe the development of a comprehensive secondary calibration standard for congener-specific determination of chlorobiphenyls in environmental samples. Other investigators have proposed mixtures of Aroclors as secondary standards.[25,28,35,36] This work differs from those previous in taking advantage of recent compilations of retention time data as well as the use of two independent methods for standard quantitation. The goal here is to describe our experiences with this standard, evaluate its performance and, in particular, present its advantages and limitations as contrasted with other currently available techniques.

EXPERIMENTAL SECTION

Materials

Individual chlorobiphenyls usd in the Primary Calibration Standard (PCS), as internal standards and as recovery surrogates were purchased from Ultra Scientific and Analabs (>99% purity). Solutions of each compound were prepared in glass redistilled hexane and evaluated by high resolution gas chromatography using flame ionization and electron capture detection to assure purities >99%.[37]

Aroclor lots used in the Aroclor Mixture (AM) were obtained from the U.S. Food and Drug Administration. This mixture was prepared by adding approximately equivalent weights of each of four Aroclors (1242, 1248, 1254 and 1260) to redistilled hexane and diluting to appropriate concentrations for detection by flame ionization and mass spectrometry. The selection of these Aroclors for use in a secondary calibration standard was based on the fact that together these formulations contain all but a few of the chlorobiphenyls typically observed in environmental samples. Efforts to characterize the Aroclor lots comprising the AM have previously been reported.[38,39]

Sample preparation

Details of the procedures used to isolate chlorobiphenyl fractions from biological tissues are given separately.[37] Briefly, lobster (*Homarus americanus*) and soft-shelled clam (*Mya arenaria*) were collected from locations in Boston Harbor, Massachusetts. Muscle (lobster) and the whole soft tissues (clams) were excised under clean room conditions and stored in glass containers at −20 °C until just prior to extraction. After thawing, tissue samples were homogenized. An aliquot (3–5 g) of homogenate was then transferred to a 100 ml centrifuge tube and spiked with a recovery surrogate solution consisting of congeners 30, 155, 198 (for congener designations see ref. 2).

Twenty-five grams of anhydrous Na_2SO_4 and 35 ml of dichloromethane were added to the centrifuge tube. The mixture was blended vigorously with a Tekmar Tissumizer, after which the contents were centrifuged and the supernatant was transferred to a flask. The extraction procedure was repeated twice, successive extracts being combined for concentration by rotary evaporation at temperatures <30 °C. Aliquots were taken for microgravimetric analysis of lipid concentration, after which a volume corresponding to 10 mg of lipid was removed for adsorption chromatography.

Preparative separation of a chlorobiphenyl fraction was performed on a 1.0×30 cm column packed with alumina over silica gel (1:2, v/v), each deactivated 5% with water. Details of the adsorbent activation procedures and development of the elution scheme are provided elsewhere.[37] After elution, the chlorobiphenyls were concentrated by rotary evaporation, transferred to a vial and evaporated to dryness under a stream of dry nitrogen. The residue was immediately taken up in a solution of the internal standard (congener 169 in hexane) for instrumental analysis.

Instrumental Analysis

Gas chromatography A Varian 6000 gas chromatograph equipped with a hydrogen flame ionization detector and a split-splitless injector of the design described by Grob[40] was used for purposes of peak identification and quantitation of the AM. Analyses of chlorobiphenyl fractions isolated from biological tissues were performed with a Varian 6000 equipped with an on-column capillary injector (Varian Model 1095/11095) and an electron capture detector. Analytical separations were carried out on a 30-meter DB-5 fused silica capillary column, 0.25 mm i.d., film thickness −0.25 meters (J. & W. Scientific). Chromatographic data were acquired on either a Spectraphysics 4270 electronic integrator or a Nelson Analytical 3000 chromatography data system equipped with a Nelson 763SB intelligent interface.

Prior to initiating this study, we determined the gas chromatographic conditions most suitable for optimizing peak resolution and analysis time for the AM (splitless injection). These chromatographic conditions were used in the quantita-

tive and qualitative analysis of the AM (cf. Figure 1, ref. 37). In later work it was decided that on-column capillary injection yielded more reproducible quantitative results, little or no discrimination with respect to the boiling points of the analytes and/or peak distortion.[41–46] Consequently, this injection technique was employed in our evaluation of method performance.[37]

For quantitation of chlorobiphenyls in biological samples, the electron capture detector was calibrated using the secondary calibration standard (internal standard method, congener 169). In this instance all resolvable peaks were included as individual analytes in the linear regression analysis.[37] The concentration range over which the linear model applied was found to be $\leqq 0.064$ to 1.28 ng/μliter (total chlorobiphenyls). The final volumes of chlorobiphenyl fractions were routinely adjusted such that all analytes fell within the linear calibration range. Single point calibrations were conducted daily.

Gas chromatography/mass spectrometry A Finnigan 4510B quadrupole mass spectrometer interfaced to a Finnigan 9611 gas chromatograph and a Data General Nova 4C computer was used for qualitative and quantitative analysis of the AM. We calibrated the mass spectrometer with the PCS by time programmed limited mass scanning (TPLMS) in a manner similar to that described by Westerberg *et al.*[47] At any given time during a run, mass ranges corresponding to the molecular ion clusters of three levels of chlorination were monitored.[37] As elution of the chlorobiphenyls proceeded, the lowest mass range was deleted, and the mass range corresponding to the next highest level of chlorination was added. Previous analysis of the AM using full scan electron impact mass spectrometry permitted us to established the elution ranges of chlorobiphenyls of different chlorination levels. Peak areas of the three most abundant ions in each molecular ion cluster were summed for use in construction of the calibration curve. The TPLMS approach allowed us to enhance detection of minor peaks, maintain major peaks within the linear capacity of the capillary column and acquire mass spectral data for use in confirmation of chlorination level[48] and/or separate quantitation of coeluting chlorobiphenyls of differing chlorine content.

RESULTS AND DISCUSSION

Qualitative Analysis of the Aroclor Mixture

A high resolution gas chromatogram (FID) of the Aroclor Mixture is shown in Figure 1. Peak assignements were made with the following data:

1) Electron impact mass spectrometric data derived from GC/MS analysis of the AM in the full and limited mass scan modes to establish the degree (or degrees) of chlorination of individual peaks. Mass spectral data were also used to infer partial structures where *o,o'*-substitution was involved[49] thereby permitting the elimination or inclusion of specific congeners from consideration.

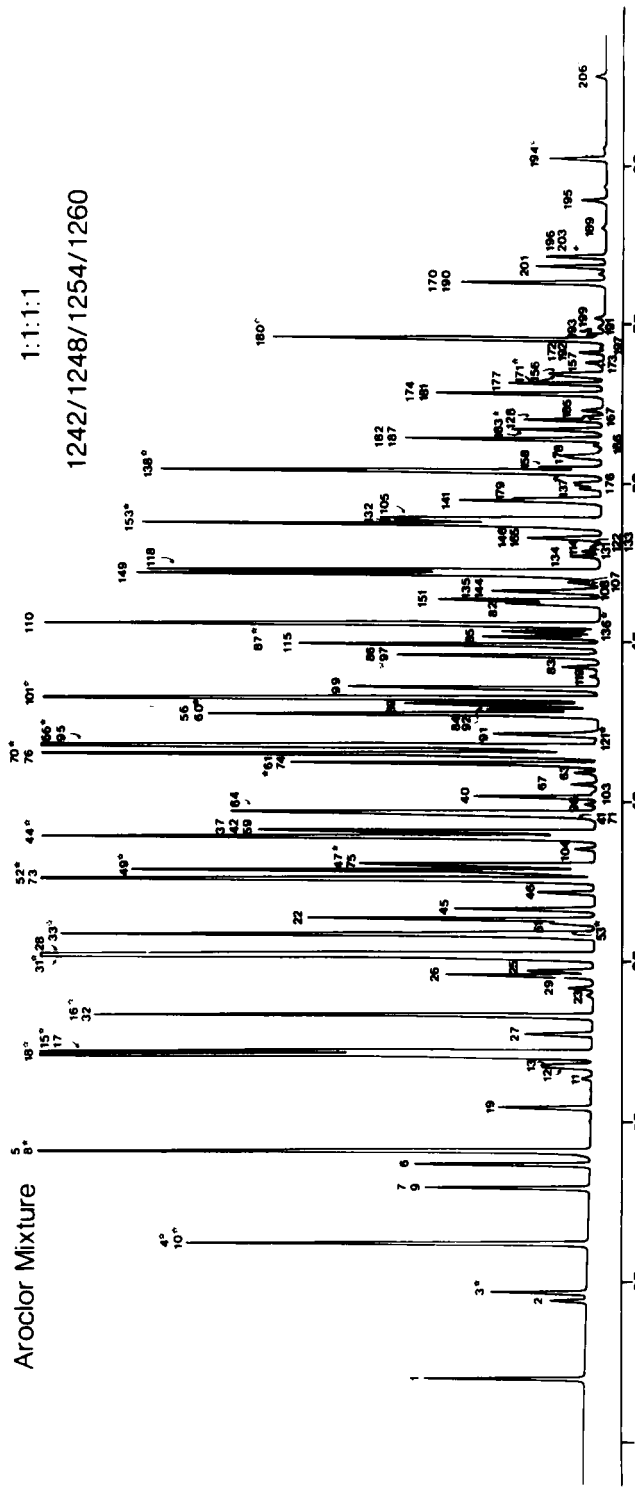

Figure 1 High resolution gas chromatogram (FID) of the Aroclor Mixture. Numbers above peaks are congener designations according to Ballschmiter and Zell.[2] Splitless injection at 40 °C (5 min hold) with temperature programming to 100 °C at 15 °C/min, at 3 °C/min to 285 °C (held isothermal).

2) Coinjection of authentic reference compounds with the AM on a fused silica capillary column.

3) Comparison of retention time data obtained for peaks in the AM with those reported by Mullin *et al.*[29] (discussion of procedures to follow). Several temperature programming rates were employed to assist in determining the number of possible coeluting chlorobiphenyls in multicomponent peaks.

4) Comparison with identifications given in the published literature in which individual Aroclors or other industrial chlorobiphenyl formulations were analyzed on similar capillary columns.[2,6,7,28,50]

Because the mass spectra of most isomeric chlorobiphenyls are virtually identical, mass spectrometry alone could not be used to unambiguously establish the identity of a given peak. In this study, retention time data in conjunction with mass spectral information were considered as *minimal* criteria for making tentative assignments. Frequently, more data were available for assisting in peak identification. However, the number and quality of such independent corroborative data were highly variable. In view of these difficulties, it was necessary to establish a ranking for the certainty of peak identifications. Levels of confidence attributed to assignments were determined as follows:

1. *Highest Probability.* Either (a) no possible coeluting congeners were predicted on the basis of retention time data (criteria to be described) and mass spectral confirmation of chlorination level was obtained, (b) the component was unequivocally confirmed by coinjection, or (c) in cases where two possible coeluting congeners of differing chlorine substitution were involved, mass spectral confirmation of only one chlorination level was determined.

2. *High Probability.* Assignments were based on similarity of retention time data with data published in the literature by several investigators who used both retention time data and coinjection as criteria. GC/MS confirmation of chlorination level and conformance with predicted retention time data based on Mullin *et al.*[29] were attained.

3. *Probability.* Retention time criteria based on data of Mullin *et al.*[29] were met, but the peak could contain more than one component. Literature data were insufficient to draw further conclusions, and mass spectral data equivocal (i.e. possible coeluting isomeric components have similar mass spectra).

4. *Tentative Assignment.* Assignments were based solely on relative retention time data of Mullin *et al.*[29] and mass spectral confirmation of chlorination level. No additional literature data were available to support or refute assignment.

A key element of the identifications we have made is the use of retention time data from Mullin *et al.*[29] To minimize errors in our assignments due to differences in chromatographic conditions, we chose to analyze the AM on a capillary column whose stationary phase (DB-5 vs. SE-54) was similar to that used by Mullin *et al.*[29] However, the instrumental conditions, column length and capillary inside diameter were different. Consequently, it was important to verify that the retention time data of Mullin *et al.*[29] could, in fact, be applied to our chromatographic runs.

The approach we used was to develop a Primary Calibration Standard (PCS) consisting of individual chlorobiphenyls whose properties and retention characteristics spanned those of the 209 possible congeners. Additionally, compounds in the PCS had to be significant constituents of one or more of the Aroclors comprising the AM.[3-5,10,51] The PCS was coinjected with the AM to establish the identities of peaks corresponding to the individual congeners in the PCS. These were then designated as *reference peaks* for purposes of comparing retention times from our analysis of the AM with relative retention times given by Mullin *et al.*[29]

It was first necessary to confirm that the retention time data generated in our laboratory for these pure compounds were, in fact, comparable with those of Mullin *et al.*[29] This was evaluated by using the three first eluting reference peaks to calculate the "fractional retention time" of the second eluting reference peak as given below:

$$\frac{RT_2 - RT_1}{RT_3 - RT_1} = K \tag{1}$$

where:

K = fractional retention time,

or

RT_i = retention times of the reference peaks ($i = 1, 2$ and 3).

This factor was then applied to the difference in relative retention times of the same congeners as given by Mullin *et al.*[29]:

$$K \cdot (RRT_3 - RRT_1) + RRT_1 = RRT_2 \tag{2}$$

where:

RRT'_2 = predicted relative retention time of reference peak 2 in the AM,

RRT_1 = relative retention time (Mullin *et al.*[29]) of the chlorobiphenyl corresponding to the first reference peak, and

RRT_3 = as above for the third reference peak.

By advancing incrementally through the reference peaks, we were able to calculate predicted relative retention times for all but the first and last eluting chlorobiphenyls of the PCS mixture. Comparison of the predicted relative retention times for constituents of the PCS with those given by Mullin *et al.*[29] showed that agreement was, in all but one case (congener 137), with 0.3% (Table 1). (The reason for the discrepancy observed for congener 137 is, as yet, unresolved.) This close agreement provided a basis for making reliable tentative assignments for peaks eluting between reference peaks in the AM. These assignments, the level of confidence associated with them and the difference between predicted and measured relative retention times are given in Table 2.

The criterion used for determining whether a specific congener could be considered as a possible constituent of a given peak was that the difference

Table 1 Comparison of predicted relative retention times of chlorobiphenyl reference peaks in the primary calibration standard with data of Mullin *et al.*[29]

Cong. no.	Cl_x	*Relative retention times*		*Difference*[d] ($\times 10^4$)
		Predicted (RRT2')	*Mullin et al.*[29]	
3	1	NC[a]	0.1975	—
4	1	0.2244	0.2245	1
8	2	0.2791	0.2783	8
15	2	ND[a]	0.3387	—
18	3	0.3380	0.3378	2
31	3	0.4023	0.4024	1
33	3	0.4163	0.4163	0
53	4	0.4186	0.4187	1
52	4	0.4556	0.4557	1
49	4	0.461[c]	0.461[c]	—
47	4	0.4639	0.4639	0
70	4	0.5407	0.5407	0
66	4	0.5450	0.5447	3
121	5	0.5528	0.5518	10
155	6	0.5664	0.5666	2
101	5	0.5817	0.5816	1
97	5	0.61[c]	0.61[c]	—
87	5	0.6176	0.6175	1
136[b]	6	0.6257	0.6257	0
153	6	0.7031	0.7036	5
137	6	0.7290	0.7329	39
138	6	0.7405	0.7403	2
183	7	0.772[c]	0.772[c]	—
171	7	0.8093	0.8089	4
194	8	0.962[c]	0.962[c]	—
206	9	1.0104	1.0103	1
209	10	NA[a]	1.0496	—

[a]NC = not calculated. First and last eluting peaks in the primary calibration standard. ND = not determined.

[b]Not a primary calibration standard component. Standard evaluated later.

[c]Data by Mullin *et al.*[29] has fewer than four significant figures.

[d]Absolute value of difference between predicted relative retention time and relative retention time of Mullin *et al.*[29]

Note: Representation of each chlorination level as follows: Cl_1-33%, Cl_2-17%, Cl_3-12%, Cl_4-14%, Cl_5-9%, Cl_6-12%, Cl_7-8%, Cl_8-8%, Cl_9-33%, Cl_{10}-100%.

between the predicted relative retention time and that reported by Mullin *et al.*[29] be less than or equal to 0.002. This represents a difference of 0.8 to 0.2% from the first to last eluting congeners, respectively. Peaks exceeding this tolerance were visually discernible as partially resolved components (a shoulder; $Rs \approx 0.5$) even though the electronic integrator was usually incapable of detecting two components. On the other hand, this criterion was not so stringent as to eliminate congeners that might be present in small concentrations. In no case did the difference between predicted relative retention times and those of Mullin *et al.*[29]

Table 2 Identification of chlorobiphenyls in the Aroclor Mixture

Cong. no.[a]	*Substitution pattern*	Cl_x[b]	*Confidence level*[c]	*Difference*[d] ($\times 10^4$)	*Interpol. interval*[e]
1	2-	1	1	—	—
2	3-	1	1	—	—
3	4-	1	1	—	—
10	2,6-	2	3	1	3,8
4	2,2'-	2	2	3	3,8
7	2,4-	2	3	1	10,8
9	2,5-	2	3	1–	10,8
6	2,3'-	2	1	5	10,8
8	2,4'-	2	3	8	4,18
5	2,3-	2	3	6	8,18
19	2,2',6-	3	1	9	8,18
11	3,3'-	2	1	2	8,18
12	3,4-	2	1	12	8,18
13	3,4'-	2	1	3	8,18
18	2,2',5-	3	1	2	8,31
15	4,4'-	2	1	12	8,16
17	2,2',4-	3	1	2	15,16
27	2,3',6-	3	4	9	15,16
16	2,2',3-	3	2	2	15,31
32	2,4',6-	3	3	9	15,31
23	2,3,5-	3	2	4–13	16,31
29	2,4,5-	3	1	4–13	16,31
26	2,3',5-	3	1	17	16,31
25	2,3',4-	3	1	11	16,31
31	2,4',5-	3	1	1	18,33
28	2,4,4'-	3	1	1	31,33
33	2',3,4-	3	1	0	31,53
53	2,2',5,6'-	4	1	1	33,49
51	2,2',4,6'-	4	1	12	53,52
22	2,3,4'-	3	1	13	53,52
45	2,2',3,6-	4	1	16	53,52
46	2,2',3,6'-	4	1	11–21	53,52
73	2,3',5',6-	4	3	3	33,49
52	2,2',5,5'-	4	3	0	33,49
49	2,2',4,5'-	4	1	0	52,47
47	2,2',4,4'-	4	1	0	49,70
75	2,4,4',6-	4	2	4	47,44
104	2,2',4,6,6'-	5	4	3	47,44
44	2,2',3,5'-	4	1	5	47,61
37	3,4,4'-	3	1	4	47,61
59	2,3,3',6-	4	2	3–8	47,61
42	2,2',3,4'-	4	2	3–12	47,61
71	2,3',4',6-	4	3	5	44,61
41	2,2',3,4-	4	3	2–10	44,61
64	2,3,4',6-	4	2	0	44,61
96	2,2',3,6,6'-	5	1	1	44,61
40	2,2'3,3'-	4	1	6	44,61
103	2,2',4,5',6-	5	1	8	44,61
67	2,3',4,5-	4	2	20	44,61
63	2,3,4',5-	4	4	12–20	44,61

Table 2 (continued)

Cong. no.[a]	*Substitution pattern*	Cl_x[b]	*Confidence level*[c]	*Difference*[d] ($\times 10^4$)	*Interpol. interval*[e]
61	2,3,4,5-	4	2	10	44, 70
74	2,4,4',5-	4	4	0	61, 70
70	2,3',4',5-	4	3	0	47, 66
76	2',3,4,5-	4	3	1	70, 66
66	2,3',4,4'-	4	1	3	70, 121
95	2,2',3,5',6-	5	1	14	66, 101
121	2,3',4,5',6-	5	1	7	70, 136
91	2,2',3,4',6-	5	4	10	66, 101
56	2,3,3',4'-	4	3	14	66, 101
60	2,3,4,4'-	4	3	14	66, 101
92	2,2',3,5,5'-	5	1	18	66,101
84	2,2',3,3',6-	5	1	16	66,101
89	2,2',3,4,6'-	5	4	14	66, 101
101	2,2',4,5,5'-	5	1	1	97, 155
99	2,2',4,4',5-	5	1	3–6	101, 87
119	2,3',4,4',6-	5	1	3	101, 87
83	2,2',3,3',5-	5	1	1	101, 87
86	2,2',3,4,5-	5	2	4	101, 87
97	2,2',3',4,5-	5	2	4–50	87, 101
115	2,3,4,4',6-	5	3	5	87, 136
87	2,2',3,4,5'-	5	1	1	97, 136
85	2,2',3,4,4'-	5	4	1	87, 136
136	2,2',3,3',6,6'-	6	1	0	87, 153
110	2,3,3',4',6-	5	1	4	136, 153
82	2,2',3,3',4-	5	1	10	136, 153
151	2,2',3,5,5',6-	6	1	13	136, 153
135	2,2',3,3',5,6'-	6	3	12	136, 153
144	2,2',3,4,5',6-	6	3	12	136, 153
108	2,3,3',4,5'-	5	4	14	136, 153
107	2,3,3',4',5-	5	4	7	136, 153
149	2,2',3,4',5',6-	6	1	0	136, 153
118	2,3',4,4',5-	5	1	5	136, 153
134	2,2',3,3',5,6-	6	4	14	136, 153
114	2,3,4,4',5-	5	4	18	136, 153
131	2,2',3,3',4,6-	6	4	17	136, 153
122	2',3,3',4,5-	5	1	9–17	136, 153
133	2,2',3,3',5,5'-	6	1	12	136, 153
165	2,3,3',5,5',6-	6	4	16–25	136, 153
146	2,2',3,4',5,5'-	6	2	14	136, 153
153	2,2',4,4',5,5'-	6	1	5	136, 138
132	2,2',3,3',4,6'-	6	2	23	153, 138
105	2,3,3',4,4'-	5	2	24	153, 138
141	2,2',3,4,5,5'-	6	1	6	153, 138
179	2,2',3,3',5,6,6'-	7	1	7	153, 138
137	2,2',3,4,4',5-	6	1	38	153, 138
176	2,2',3,3',4,6,6'-	7	4	15	153, 138
138	2,2',3,4,4',5'-	6	1	2	153, 171
158	2,3,3',4,4',6-	6	2	5	138, 183
126	2,2',3,3',4,5-	6	4	10	138, 183
178	2,2',3,3',5,5',6-	7	2	15	138, 183

Table 2 (continued)

Cong. no.[a]	*Substitution pattern*	Cl_x[b]	*Confidence level*[c]	*Difference*[d] ($\times 10^4$)	*Interpol. interval*[e]
166	2,3,4,4',5,6-	6	4	31	138, 183
182	2,2',3,4,4',5,6'-	7	3	5	138, 183
187	2,2',3,4',5,5',6-	7	3	6	138, 183
183	2,2',3,4,4',5',6-	7	1	1–9	153, 171
128	2,2',3,3',4,4'-	6	1	12	138, 171
167	2,3',4,4',5,5'-	6	2	20	138, 171
185	2,2',3,4,5,5',6-	7	2	8	138, 171
174	2,2',3,3',4,5,6'-	7	3	13	138, 171
181	2,2',3,4,4',5,6-	7	3	16	138, 171
177	2,2',3,3',4',5,6-	7	1	5	138, 171
171	2,2',3,3',4,4',6-	7	1	4	183, 194
156	2,3,3',4,4',5-	6	2	4	171, 180
173	2,2',3,3',4,5,6-	7	4	13	171, 180
157	2,3,3',4,4',5'-	6	1	2	171, 180
192	2,3,3',4,5,5',6-	7	3	5	171, 180
172	2,2',3,3',4,5,5'-	7	3	14	171, 180
197	2,2',3,3',4,4',6,6'-	8	4	2	171, 180
180	2,2',3,4,4',5,5'-	7	1	0	171, 194
193	2,3,3',4',5,5',6-	7	1	1	171, 194
191	2,3,3',4,4',5',6-	7	1	1	171, 194
199	2,2',3,3',4,5,6,6'-	8	1	13	171, 194
170	2,2',3,3',4,4',5-	7	2	10–20	171, 194
190	2,3,3',4,4',5,6-	7	3	10–20	171, 194
201	2,2',3,3',4',5,5',6-	8	2	8	171, 194
196	2,2',3,3',4,4',5',6-	8	3	0	171, 194
203	2,2',3,4,4',5,5',6-	8	3	0	171, 194
189	2,3,3',4,4',5,5'-	7	2	7	171, 194
195	2,2',3,3',4,4',5,6-	8	2	6	171, 194
194	2,2',3,3',4,4',5,5'-	8	1	0	171, 206
206	2,2',3,3',4,4',5,5',6-	9	1	0	194, 209

[a]Congener numbers based on system of Ballschmiter and Zell.[2] Italicized numbers indicate coinjected compounds.

[b]Number of chlorine atoms substituted on the biphenyl nucleus.

[c]Confidence levels assigned as described in the text: 1 = highest probability; 2 = high probability; 3 = probability; 4 = tentative assignment. See text for definitions.

[d]Absolute value of difference between predicted relative retention time and relative retention time of Mulin *et al.*[29] See equations 1 and 2. Ranges given for congeners whose relative retention times[29] were reported with fewer than four significant figures.

[e]Interpolation interval: congeners used as reference peaks in equations 1 and 2.

exceed 0.5%. This limit is well within the variation of the retention time measurements.

The AM contains 96 peaks comprising as many as 130 individual chlorobiphenyls under the chromatographic conditions employed in this study. There are 34 possible multicomponent peaks whose constituent chlorobiphenyls cannot be detected by electronic integration. Of these, 11 consist of congeners having different levels of chlorination as verified by mass spectrometry; the others are mixtures of isomers. In view of the uncertainties associated with assignments made for constituents of many of the multicomponents peaks, we have designated them at level four in our ranking scheme.

Quantitative Analysis of the Aroclor Mixture

Because of the variable response of the electron capture detector to isomeric chlorobiphenyls,[24,52] we decided to quantitate the AM using two detectors whose response was less variable. It was reasoned that determination of a *representative response factor* for each chlorination level would permit accurate quantitation of the AM assuming that the degree of chlorination of each peak was known. Flame ionization was selected because detection results primarily from production of carbon-containing cations and electrons in the hydrogen flame. Differences in substitution of chlorine atoms on the biphenyl nucleus should, thus, have only second order effects on the FID response of chlorobiphenyl isomers. Previous studies have demonstrated that the average coefficient of variation in response factors within a group of isomers ranges from 1.6 to 9.0%.[53-55] Similarly, reports have documented the relatively low variability in response to isomeric chlorobiphenyls of mass spectrometers operated in the electron impact mode.[56,57]

For the FID calibration, solutions of the PCS were prepared over a concentration range of 2–20 ng/μliter/component. These solutions were analyzed 2–6 times each by high resolution gas chromatography (splitless injection). Injection of PCS solutions was performed in alternation with AM solutions at three concentrations. The reason for using three AM dilutions was to insure that all peaks in the Aroclor Mixture could be analyzed at concentrations within the linear calibration range of the detector.[37] Quantitation was performed by the external standard method.

In the case of the mass spectrometer, the approach was identical to that used in the FID calibration except that calibration was by the internal standard method. 4,4′-difluorobiphenyl was added to five dilutions of the PCS and three dilutions of the AM such that the internal standard concentration in all solutions was identical. All solutions were run in triplicate, and the PCS concentrations ranged from 1–20 ng/μliter/component. As before, AM solutions at various dilutions were run alternately with the PCS solutions to permit quantitation of all peaks within the linear calibration range.[37]

Linear regression analysis of the calibration data was performed to obtain response factors for each level of chlorination.[37] The relative standard deviation (RSD) for FID response factors within individual levels of chlorination varied

from 1.4 to 24.8% and averaged 9.1%. This compares favorably with data reported in the literature.[53-55] In no case did the RSD of the slope for an individual isomer group (pooled data) exceed 3.6%. There was noticeably greater variation in the GC/MS isomer group (relative) response factors than was observed for the GC/FID calibration. Coefficients of variation for the relative response factors within such groups ranged from 5.4 to 35.5% with a mean of 17.6%. This is similar to, although somewhat higher than, comparable data reported in the literature.[56,57] With one exception, the RSDs of the slopes of individual isomer groups (pooled data) were less than 6%.

Table 3 summarizes the results of the AM quantitations by GC/FID and GC/MS. For multicomponent peaks containing congeners of more than one level of chlorination, procedures were used to accurately quantitate the total peak concentration. In the case of GC/MS quantitation of such peaks, this simply involved integration of the appropriate ions and application of the corresponding representative response factors. Occasionally, better separation of such peaks was achieved by GC/FID. If a discernible shoulder was observed (but not integrated), the total integrated area was proportioned according to the peak heights of the shoulder and the major peak. The corresponding isomer group response factors were then used to calculate the concentrations of the two components, with total peak concentration calculated as the summation of the two component concentrations. A third situation that occurred rarely was the complete coelution (GC/FID) of two congeners having differing chlorination levels. These GC/FID peaks were simply quantitated using the average value of the response factors for the two chlorination levels. The weight percentages of all such multicomponent peaks for GC/FID and GC/MS analyses are 15.3% and 12.9%, respectively.

As a first step toward assessing the accuracy of our results, we compared the summed concentrations of the AM peaks with the total chlorobiphenyl concentration derived from gravimetric measurements made during preparation of the mixture. The results (Table 3) show that both quantitations yielded acceptable results as the deviation in either case is less than 7%. This signifies that either the concentrations attributed to individual peaks in the AM are accurate or that, on average, errors associated with application of representative isomer group response factors tend to cancel out.

Closer inspection of the data reveals that the peak quantitations based on GC/FID and GC/MS calibrations do not always agree, particularly for the minor AM components. Confirmation of this observation is obtained by comparing the quantitative results for peaks each of which comprise >1% by weight of the AM (Table 4). When summed, the concentrations of these peaks represent 72% and 83% of the total AM chlorobiphenyl concentration by GC/FID and GC/MS quantitations, respectively. The mean concentration ratio (i.e. FID:MS) for these major components is 1.11 with a coefficient of variation for the ratio of 35%. Thus, the largest discrepancies between the two methods of quantitation are clearly attributable to AM constituents in lower abundance.

We also quantitated major peaks corresponding to congeners present in both the AM and the PCS using individual congener response factors (Table 5). The rationale for doing so was that concentrations calculated from group response

Table 3 Comparison of AM quantitation results by GC/FID and GC/MS

Cong. no.[a]	*Weight %*		*Cong. no.*[a]	*Weight %*	
	GC/FID	*GC/MS*		*GC/FID*	*GC/MS*
1	0.75	0.19	135, 144	1.04	0.53
2	0.24	0.003	108, 107	1.53	0.18
3	0.44	0.05	149	2.79	1.80
4, 10	1.45	0.91	118]	2.15	1.90
7, 9	0.70	0.30	134	0.53	0.13
6	0.60	0.57	114	0.31	0.08
8, 5	2.44	2.78	131	—	0.02
19	0.35	0.20	122	—	0.02
12	0.32	0.09	146, 165	0.55	0.41
13	0.31	—	153]	2.76	5.08
18]	3.55	2.93	132]	0.96	
15]	1.55	0.63	105	1.10	1.22
17]		0.97	141]	0.50	0.94
27	0.55	0.21	179	0.36	0.62
16, 32	1.82	1.74			
23	—	0.004	176]	0.45	0.06
29	0.25	0.02	137]	0.44	0.11
26	0.50	0.62	138	3.36	4.12
25	0.54	0.22	158	0.43	0.25
31]	2.34	4.50	126	0.42	0.09
28]	2.74	4.12	178	0.61	0.18
33]	2.04	2.50	166	—	0.006
53]	0.38	0.09	187, 182	1.30	1.29
51]	0.41	0.02	183	0.80	0.38
22]	1.81	1.19	128]	0.34	0.20
45	0.53	0.30	167]	0.09	0.09
46	0.59	0.14	185	0.41	0.09
52, 73	3.75	4.11	174, 181	1.08	1.18
49	1.99	1.90	177	0.82	0.69
47]	0.67	1.32	171]	0.41	0.12
75]	0.70		202]		0.003
104	0.25	0.02	156]	0.49	0.27
44	2.62	2.43	173	0.06	ND[b]
37]		0.36	157	0.17	0.05
59]	1.40	0.75	192, 172	0.40	0.11
42]			180	2.08	2.64
71, 41]	0.80	2.74	193	0.29	0.11
64	1.28		191	—	0.008
96	—	0.01	199	0.22	0.04
40	0.44	0.37	170, 190	0.95	1.08
103	—	0.001	201	0.74	0.84
67	0.27	0.07	196, 203	0.88	0.84
63	0.24	0.11	189	—	0.001
61, 74	1.20	1.62	195	0.56	0.16
70, 76	2.75	4.61	194	0.71	0.74
66]	1.92	2.75	206	0.30	0.01
95]	2.50	2.91			
121	—	0.005			
91	0.43	0.48			
56, 60	1.67	2.29			

Table 3 (continued)

Cong. no.[a]	*Weight %*		*Cong. no.*[a]	*Weight %*	
	GC/FID	*GC/MS*		*GC/FID*	*GC/MS*
92,84	0.52	0.56			
89	0.89	0.98			
101	3.32	4.01			
99	1.06	1.37			
119	0.09	0.04			
83	0.48	0.12			
86,97	0.93	0.97			
87,115	1.53	1.74			
85	0.74	0.65			
136	0.71	0.76			
110	3.08	4.72			
82	0.58	0.30			
151	1.23	0.86			
Σ CBs by GC/FID = 2,671 ng/μliter;					
Σ CBs by GC/MS = 2,390 ng/μliter;					
Σ CBs by gravimetry = 2,571 ng/μl.					

[a]Brackets to the right of congener numbers indicate partially resolved components for which peak areas and/or heights could be measured. Commas indicate coeluting congeners.
[b]Not detected.

factors could be biased to the extent that the individual congener response factors deviate from the isomer group response factor. We had previously established that the deviations of individual congener response factors from the group response factor could be as great as 48% and 33% for GC/FID and GC/MS, respectively.[37] There is no reason to assume that such deviations should be of the same sign or magnitude for the two methods of detection. Thus, very large discrepancies in quantitative results could occur if the deviation of relative response factors for the two methods was large and of the opposite sign. All other things being equal, the concentrations calculated from individual congener response factors should be accurate regardless of the method of detection.

Many of the quantitations based on individual congeners in Table 5 show good agreement (within 10%). Significant discrepancies appear for peaks quantitated as congeners 15, and 153. The differences may be attributed to the fact that these peaks (as well as 7 others in this group) coelute with chlorobiphenyls other than the ones being used for quantitation. It is inappropriate to assume that the response factors of these congeners are the same as those of coeluting chlorobiphenyls. The fact that agreement is found for many of the multicomponent peaks of the AM (Table 5) suggest that either these peaks are dominated by the congeners whose response factors were used in quantitation, that the response factors of the other components were similar, or both. In any event, the only peaks for which a direct comparison can be made (i.e. single component peaks) are 49, 101 and 138. In these cases agreement is found (< 12% difference). Although the

Table 4 Concentrations of major AM component peaks: GC/FID *vs.* GC/MS[a]

Congener no.	Cl_x	*Concentration (ng/μliter)*		*GC/FID:GC/MS*
		GC/FID	*GC/MS*	
4/10	2/2	38.8	21.8	1.78
8/5	2/2	65.1	66.5	0.98
18	3	94.9	70.0	1.36
15/17	2/3	41.3	38.2	1.08
16/32	3/3	48.6	41.5	1.17
31	3	62.5	107.6	0.58
28	3	73.3	98.4	0.74
33	3	54.5	59.7	0.91
22	3	48.4	28.5	1.70
52/73	4/4	100.2	98.1	1.02
49	4	53.2	45.3	1.17
44	4	70.0	58.0	1.21
37/59/42	3/4/4	37.4	26.6	1.40
41/64/71	4/4/4	55.4	65.4	0.85
61/74	4/4	32.0	38.7	0.83
70/76	4/4	73.4	110.1	0.67
66/95	4/5	51.4/66.8	65.7/69.6	0.78/0.96
56/60	4/4	44.6	54.7	0.82
101	5	88.6	95.9	0.92
99	5	28.2	32.7	0.86
87/115	5/5	40.9	41.5	0.98
110	5	82.3	112.8	0.73
151	6	33.0	20.7	1.59
135/144	6/6	27.7	12.6	2.28
149	6	74.6	42.9	1.74
118	5	57.5	45.4	1.27
153/132	6/6	99.4	121.5	0.82
105	5	29.2	29.3	1.00
138	6	89.7	98.4	0.91
158	6	11.4	6.0	1.88
187/182	7/7	34.7	30.8	1.13
174/181	7/7	29.0	28.3	1.02
180	7	55.6	63.2	0.88
170/190	7/7	25.3	25.8	0.98

[a]All components present at levels greater than 1% by weight of the AM by at least one of the two methods of quantitation.

data are limited, they suggest that the main cause for the discrepancies of major peak quantitations is, in fact, the difference between the group response factors and those of the congeners in a given peak.

Composition of the Secondary Calibration Standard

The complete secondary calibration standard (SCS) is a mixture of the AM, recovery surrogates and an internal standard. The calibration standard was

Table 5 Comparison of quantitative results obtained for major AM components using individual congener response factors: GC/FID *vs.* GC/MS

Congener no.[a]	*Quantitation congener*	*Concentration (ng/μliter)*		*GC/FID:GC/MS*
		GC/FID	*GC/MS*	
4/10	4	37.1	32.5	1.14
8/5	8	62.1	54.5	1.14
15/17/18?	15	44.6	12.0	3.72
18/15	18	74.9	89.3	0.84
52/73	52	95.2	116.8	0.82
49	49	50.2	54.3	0.92
70/76	70	78.9	93.2	0.85
66/95	66	54.7	55.3	0.99
101	101	87.4	99.1	0.89
87/115	87	41.4	47.0	0.88
153/132/105	153	73.6	106.9	0.69
138	138	92.4	103.7	0.89

[a]Question marks to right of congeners indicate uncertainty concerning the presence of the congener in the peak.

prepared after quantitation of the AM and evaluation of the internal and recovery standard candidates had been completed. The criteria used in selecting the internal standard were:

1) The compounds had to be commercially available at high purity.

2) The compounds had to be chlorobiphenyls.

3) The compounds had to be baseline resolved from all other chorobiphenyls present in the AM as well as potential interferences such as phthalates and DDT metabolites.

4) The recovery surrogates had to span the chlorination range of the AM.

5) The compounds had to demonstrate good peak shape under the chormatographic conditions used in these analyses.

On the basis of retention data of Mullin *et al.*,[29] we identified seven chlorobiphenyls (congeners 14, 30, 69, 154, 155, 169 and 198) as candidates for evaluation. After completing high resolution gas chromatographic analysis of the pure compounds and coinjecting them with the AM, we selected three as recovery surrogates (nos. 30, 155 and 198) and one as internal standard (no. 169). The secondary standard was developed for quantitation of chlorobiphenyls using the electron capture detector. Because of the lower variation in isomeric response observed during quantitation of the AM[37] by the flame ionization detector and the greater apparent accuracy of the FID-measured total chlorobiphenyl concentration (Table 3), we use the FID-based quantitations in our application of the SCS to analysis of environmental samples.

Table 6 Precision of GC/ECD analyses of lobster and clam tissues for chlorinated biphenyls

Tissue	*Total chlorobiphenyl concentration (ng/wet g)*					*Mean*	*RSD (%)*
replicates	*1*	*2*	*3*	*4*	*5*		
Lobster (muscle)	54.3	46.7	39.6	44.7	46.4	46.4	11.4
Clam (soft parts)	74.0	74.3	68.5	—	—	72.3	4.5

Performance evaluation

Limits of detection, quantitation Limits of detection and quantitation were determined by replicate analysis of processed blanks. Analysis of the data involved integration of peaks identified by the Nelson chromatography data system as components of the secondary calibration standard. Quantitations relative to the secondary calibration standard were performed by the internal standard method (total chlorobiphenyl concentration—0.64 ng/μliter) with computation of limits of detection and quantitation according to criteria established by the ACS Committee on Environmental Improvement.[58] Values for the LOD and LOQ estimates for each of the chromatographic peaks are given separately.[37] The corresponding values for total chlorobiphenyl concentrations are 2.07 and 2.94 ng/wet g, respectively.

Precision Method precision was determined by replicate analysis of clam and lobster tissues. A summary of the results of these studies is given in Table 6, whereas data for individual peaks are reported separately.[37] No correction for recovery has been made because of the difficulty of applying the relevant factors to individual peaks. Measurements made on the clam replicates indicate an average relative standard deviation for individual peak concentrations of 10.6% whereas the RSD for total chlorobiphenyl concentrations (i.e. summation of individual peaks) is 4.5%. The lobster results show a mean RSD for individual peak concentrations of 14% with an RSD of 11.4% for the total chlorobiphenyl concentrations. The poorer precision obtained for the lobster samples probably reflects the lower concentrations found in these samples.

Accuracy Without the availability of standard reference materials (tissues) characterized at the congener-specific level, it was impossible to directly evaluate the accuracy of this method. As an alternative, we carried out repetitive analyses of solutions of individual Aroclors using the secondary calibration standard for quantitation as before. The results of these measurements are given in Table 7. The average measured concentrations of Aroclors 1242, 1248, 1254 and 1260 are each within 14% of the levels determined gravimetrically when the standards were prepared.

Table 7 Results of GC/ECD analyses of individual Aroclor mixtures

Aroclor	*Total chlorobiphenyl concentration (ng/μ liter)*			*Mean %*	*Std. dev.*
	Gravimetric	*GC/ECD*	*%*[a]		
1242	2.14	2.15	100.5		
		1.72	80.6	88.4	8.7
		1.80	84.1		
1248	1.30	1.25	96.1		
		0.99	76.2	86.5	8.1
		1.13	87.2		
1254	1.38	1.21	88.8		
		1.16	84.1	86.0	1.5
		1.18	86.0		
1260	0.565	0.524	92.8		
		0.506	89.6	91.2	1.3
		0.515	91.2		

[a] $\% = [\text{Conc.}_{ECD}/\text{Conc.}_{Grav.}] \cdot 100$.

CONCLUSIONS

This paper describes methodology for the congener-specific determination of chlorobiphenyls in biological tissues by high resolution gas chromatography/electron capture detection. In recent years a number of approaches have been proposed for the identification and measurement of chlorobiphenyls. The principal differences between these methods are the standards used for qualitative and quantitative analysis, chromatographic conditions of analysis and the means of detection. Each method offers advantages and suffers from specific limitations.

Concerning the choice of calibration standards, there are presently three options: (1) primary standards (i.e. pure congeners); (2) secondary standards based on Aroclor (or other) mixtures; and (3) surrogates. The acquisition of a satisfactory collection of pure congeners as primary standards is highly desirable. The cost for some laboratories, however, may be prohibitive, and the limited availability of these compounds ultimately places constraints on qualitative analysis that can only be overcome by direct synthesis. Aroclors or other commercial mixtures, on the other hand, are available at no cost and provide most if not all of the congeners commonly observed in environmental samples. The cost and effort of developing a secondary standard is not insignificant, and this, again, represents a barrier to implementation of congener-specific procedures.

Recently, Cooper *et al.*[26] described a novel approach based on the use of "surrogates" consisting of 31 individual congeners whose relative response factors (ECD) were found to be representative of subsets of 203 congeners by cluster analysis. This would seem to be a cost effective alternative until one considers that identification of sample peaks, nevertheless, requires the availability of appropriate reference compounds (cf. ref. 59). Moreover, comparison of RRF data of Mullin *et*

al.[29] and Cooper *et al.*[26] indicate that isomeric variations in response appear to be instrument-specific (see below). Thus, each electron capture detector would require separate calibration with individual congeners and subsequent clustering of the data for identification of suitable surrogates.

Because chromatographic separation of the 209 chlorobiphenyl congeners on a single column is not presently possible, all currently available approaches are subject to error as discussed above. While the selectivity of specific stationary phases may provide improved separation of certain closely eluting congeners,[24,35,60] the efficiency of single high resolution columns is approaching practical, if not theoretical, limits. Increased efficiency over that used here, while feasible, can only be obtained at the cost of increased analysis time, reduced linear capacity and/or decreased sensitivity.

Duinker *et al.*[50] recently described the application of multidimensional gas chromatography for baseline separation of congeners normally coeluting on nonpolar stationary phases such as SE-54. The focus of their report was on specific peaks believed to contain the most toxic chlorobiphenyls. If such an approach could be extended to all multicomponent peaks and automated without greatly increasing analysis time, a solution to the problem of chlorobiphenyl identification and measurement would be at hand. In the meantime, data of Cooper *et al.*,[26] Mullin *et al.*[29] and Duinker *et al.*[50] provide an opportunity to estimate the magnitude of bias that might be incurred through the use of individual congeners for quantitation of multicomponent peaks. Table 8 gives ECD relative response factors from the literature for congeners present in multicomponent peaks of the AM under the chromatographic conditions described in this paper. Also presented are compositional data of Duinker *et al.*[50] and similar results obtained in this study for certain multicomponent peaks in the AM.

One can estimate the *maximum potential error* by calculating the difference in RRFs of components having the highest and lowest values as a percentage of the latter. This is a "worst case" scenario whereby one congener is used for quantitation while the other is actually dominant in the sample peak. It is clear that the potential errors are variable and in some cases extremely large (e.g. peak containing congeners 15, 17; Table 8). Most interesting, is the fact that ECDs from two different laboratories appear to vary in their response to individual congeners such that the maximum potential errors may differ in magnitude and occasionally sign.

The compositional data can be combined with RRF data to make more realistic error estimates for specific peaks (Table 8). (Here the peak compositions reflect Aroclor 1242 and the AM, not samples.) The magnitude of the estimated errors are quite variable and are dependent on the congener selected for quantitation. As before, individual ECDs seem to be subject to errors of different sign and magnitude. These results strongly underscore the need for improved separation of complex chlorobiphenyl mixtures, particularly since many of the largest peaks in environmental samples are likely to contain more than one component.

Mass spectrometry offers some advantages in this area.[57,59] For peaks containing congeners of differing chlorine content, quantitation of the individual components is possible. (The same, unfortunately, is not true for peaks containing

Table 8 Estimated errors associated with quantitation of multicomponent peaks using individual congener response factors

Cong. no.	*RRF-ECD*[a]		*Peak comp.*[b]		*Max. pot. error*[c]		*Est. error*[d]	
	1	*2*	*(37)*	*(50)*	*1*	*2*	*1*	*2*
37	0.58	0.258		12.6	36.6	176.0	28.0	146.0
42	0.792	0.683		75.7			−6.0	−7.2
59	0.6	0.712		11.8			24.3	−10.0
15	0.107	0.178	39.4		285.0	170.0	173.0	103.0
17	0.412	0.480	60.6				−29.2	−25.0
16	0.447	0.373			60.8	−1.7		
52	0.418	0.714			38.9	−10.5		
73	0.5805	0.646						
41	0.5469	0.592			29.7	116.0		
64	0.607	0.827						
71	0.468	0.384						
61	1.2227	1.010			82.2	22.1		
74	0.671	0.827						
70	0.658	0.726			13.5	24.0		
76	0.5795	0.586						
66	0.646	0.567	48.6		45.8	−6.7	−16.2	−3.4
95	0.443	0.605	51.4				22.3	−4.0
56	0.829	0.807			22.6	−18.2		
60	1.0164	0.683						
87	1.021	1.071			11.0	−33.9		
115	1.1328	0.799						
135	0.7031	0.639			24.6	22.5		
144	0.8764	0.783						
153	0.688	0.623	74.2		6.1	12.9	1.6	3.3
132	0.7303	0.703	25.8				4.3	−8.4
187	1.122	1.106			0.5	−24.2		
182	1.1272	0.890						
174	0.806	0.744			99.1	17.7		
181	1.6046	0.876						
170	0.75	0.589			74.7	97.0		
190	1.31	1.160						

[a]RRF-ECD = relative response factors relative to octachloronaphthalene. Data taken from: (1) Mullin *et al.*[29] and (2) Cooper *et al.*[26]

[b]Peak compositions determined by Eganhouse *et al.*[37] using GC/MS and Duinker *et al.*[50] by multidimensional gas chromatography.

[c]Maximum potential error calculated as difference between highest and lowest relative response factors as a percentage of the lowest RRF based on data of: (1) Mullin *et al.*[29] and (2) Cooper *et al.*[26] Sign indicates juxtaposition of congener RRFs.

[d]Error estimated by computation of peak concentration using individual congener RRFs; data from (1) Mullin *et al.*[29] and (2) Cooper *et al.*[26]

isomers). Quantitation of *isomer groups* is facilitated by selection of pure congeners (for instrument calibration) whose response factors are close to the mean of the isomeric group form which they come.[38,56,57] For purposes of determining isomer group and total chlorobiphenyl concentrations, this provides an affordable and potentially accurate method of quantitation as long as the isomers present in

samples have response factors that are normally distributed about the mean value. If congener-specific analyses are desired, however, information on the level of chlorination must ultimately be combined with congener identification procedures. This, again, requires the availability of appropriate standards.

Even in the selected ion monitoring, or limited mass scan modes, electron impact mass spectrometry (EI-MS) may lack the sensitivity needed for certain analyses. While negative ion chemical ionization (NICI-MS) affords greater sensitivity, the variability in isometric response appears to be as great as electron capture detection.[24] Moreover, chemical ionization does not lend itself readily to routine use, and both EI-MS and NICI-MS techniques require a GC/MS.

The method presented here has been shown to be sensitive and reliable. With computerized acquisition and reprocessing capabilities, this method provides quantitative data at the congener-specific, isomer group and total chlorobiphenyl levels using instrumentation that is widely available. Development of a satisfactory standard for congener-specific analyses involves a significant investment on the part of any laboratory attempting to implement such procedures, regardless of the approach taken. Additionally, use of the electron capture detector requires isolation of a pure chlorobiphenyl fraction and/or independent corroboration of peak identity. All currently available congener-specific methods utilizing a single chromatographic column may suffer bias of unknown magnitude and sign whenever environmental samples are analyzed because of incomplete separation of specific chlorobiphenyls. The development of practical procedures for the resolution of all 209 congeners is, therefore, strongly encouraged.

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